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EMERGING TRENDS IN BIOTECHNOLOGY: FROM LABORATORY TO INDUSTRY



Emerging Trends in Biotechnology: From Laboratory to Industry

Volume 6, 2026, S1051B2, IIP SERIES



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PREFACE

Biotechnology has emerged as one of the most transformative scientific disciplines of the twenty-first century, reshaping industries ranging from healthcare and agriculture to environmental management, food production, and industrial manufacturing. By integrating advances in molecular biology, genetics, microbiology, bioinformatics, synthetic biology, and bioprocess engineering, biotechnology has bridged the gap between fundamental scientific discovery and practical industrial applications. As the pace of innovation accelerates, the successful translation of laboratory research into commercially viable technologies has become increasingly important for addressing global challenges related to food security, public health, sustainable development, and environmental conservation.

The book, *Emerging Trends in Biotechnology: From Laboratory to Industry*, presents a comprehensive overview of recent advances and emerging technologies that are driving the next generation of biotechnological innovations. It highlights the interdisciplinary nature of biotechnology and emphasizes the critical role of research, innovation, entrepreneurship, and technology transfer in transforming scientific discoveries into industrial solutions. The chapters collectively explore cutting-edge developments in genomics, proteomics, metabolomics, gene editing, synthetic biology, nanobiotechnology, industrial microbiology, biopharmaceutical production, bioinformatics, bioprocess optimization, precision agriculture, environmental biotechnology, and bio-based manufacturing.

A distinctive feature of this volume is its focus on the translation of laboratory-scale innovations into scalable, economically viable, and sustainable industrial applications. Contributors discuss not only scientific breakthroughs but also the challenges associated with commercialization, regulatory frameworks, quality assurance, intellectual property management, biosafety, bioethics, and market adoption. The integration of artificial intelligence, machine learning, automation, and digital technologies into biotechnology has further accelerated research and industrial productivity, opening new avenues for innovation and sustainable economic growth.

The chapters included in this volume have been authored by distinguished researchers, academicians, scientists, and industry professionals who share their expertise, experiences, and perspectives on current developments and future directions in biotechnology. Their contributions provide readers with a balanced understanding of both fundamental principles and practical applications, making the book valuable for a broad audience, including undergraduate and postgraduate students, research scholars, faculty members, industrial practitioners, entrepreneurs, policymakers, and professionals engaged in biotechnology and allied disciplines.

This volume also underscores the importance of interdisciplinary collaboration among academia, research institutions, industries, and government organizations in fostering innovation and promoting sustainable technological advancement. Such collaborations are essential for developing efficient bioprocesses, environmentally friendly products, improved healthcare solutions, and resilient agricultural systems capable of meeting the demands of a rapidly growing global population.

We express our sincere gratitude to all chapter authors for their valuable scholarly contributions, dedication, and timely cooperation in bringing this volume to completion. We

also acknowledge the reviewers whose constructive comments have significantly enhanced the scientific quality and clarity of the chapters. Our heartfelt appreciation extends to the editorial team and the publisher for their continuous guidance, technical support, and commitment throughout the publication process.

We hope that this book will serve as a reliable reference and an inspiring resource for researchers, educators, students, industry professionals, and policymakers. It is our sincere expectation that the knowledge presented in these chapters will stimulate further research, encourage innovation, strengthen academia–industry partnerships, and contribute meaningfully to the advancement of biotechnology for the benefit of society and sustainable global development.

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PART – 1

Emerging Trends in Biotechnology: From Laboratory to Industry

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Series Editorial

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POTENTIAL OF INSECT GUT MICROFLORA IN BIODEGRADATION OF PLASTIC WITH SPECIAL EMPHASIS ON ITS METABOLIC PATHWAYS

Abstract

Now a day, worldwide Plastic becomes an integral part of economy. As plastic is used for so many purposes such as agricultural film formation, fishing nets, building and construction, packaging materials, defence materials, sanitary wares, plastic bottles, household items etc. Hence their production is increasing day by day and emerging as a global industry.

But as the demand of plastics increases it also leads to increased damage to the environment and all other life forms also. It is observed that 80% of marine plastic litter is derived from land based sources. It was also reported that agricultural soil receives more quantity of microplastics than ocean. The larger quantity and long life of plastic leads to different physical, chemical and biological impacts on animals, plants, human being, soil and all the ecosystems. In case of aquatic ecosystem, plastics impede the movement of aquatic animals. The presence of plastic in aquatic ecosystem also leads to its ingestion when animals eat the food to which plastic is attached leading to accumulation of plastics in their gastrointestinal tract and results into blockages, starvation and death also. Plastic pollution also leads to loss of amenity.

As far as the chemical impacts are concerned the toxic chemicals like phthalates have potential of biomagnification in trophic level. While the biological impacts of plastic includes killing of animals through entanglement and engulfment. Plastic also exerts adverse effects on higher as well as lower plants and microbial communities.

By keeping in mind all the above mentioned parameters it is of paramount concern to degrade the plastic from land as well as

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ocean. Traditionally wastewater treatment plants for removing primary and secondary microplastics are used. But the disposal of collected microplastic from effluent treatment plants remains as it is. Also large volume of wastewater being treated the final effluent will also contains large quantity of microplastic which will be discharged into the environment. Another way of removing plastic is injection of microplastics by organisms like insects, insect larvae, annelids, crustaceans etc. But it leads to serious broad range of problems. Hence in the present project proposal we will focus on a better solution for microplastic degradation through biological way.

Now a day there are plenty of plastic industries which produces tons of plastic materials and are used by the people due to its cheapness and convenience. But this plastic material harms the environment very badly. The direct environmental impact includes ingestion, entanglement, ecosystem alteration and indirect environmental impacts includes transfer and movement of individual species from one location to another. It also has economic impacts on tourism, fishing and navigation.

With this, plastic materials also harms significant number of marine species including 86% of all sea turtle species, 44% of all sea bird species and around 43% of all marine mammal species. It was also observed that 55% of the bird species collected from cost of north California, USA contains plastic particles in their guts. Most importantly use of plastic bags and water bottles used to carry food, water, soft drinks, milk and honey etc. leading serious health problems.

Due to all above mentioned impacts of plastics it is of paramount importance to carry out the degradation of plastic through eco friendly technology which does not produces harmful by-products. At present there are numerous studies to degrade the plastic which mainly includes photo

oxidative degradation, thermal degradation, Ozone degradation, catalytic degradation etc. Unfortunately these techniques have some disadvantages like high cost, production of harmful by product, use of chemicals, not suitable for onsite application etc.

Keywords: Plastic pollution, microplastic, environmental effects, insect gut flora, biodegradation, pathways.

I. SUMMERY

Recent statistical data indicates that most of the plastic i.e. around 38% is used for packaging purpose and is continuously increasing by 12% per year. There are hundreds of scientific publications related to microbial degradation of plastics. Still the practical evidence of degradation is lacking. The published literature supports the stronger evidence of microbial plastic degradation but significant degradation under ambient environmental conditions is never demonstrated. With some present literature it was observed that yet at present, nobody invented the creditable microbial approach with on field plastic degradation potential.

Terrestrial plastic including agricultural plastics can found their place into the environment through different ways including as a damaged, degraded and discarded plastic. These plastics consists of mixture of different products like flexible, semi-flexible, and rigid, and most importantly each one have the potential to cause different types of environmental hazards.

The quantity of plastic coming from terrestrial ecosystems including agricultural sources into the environment is mostly unknown; but what is becoming can adversely affects on both ecosystems and human beings also.

It is well known fact that the conventional plastics are mostly resistant to any type of degradation including biodegradation, plastic also persist in the environment for long time and leads to harm for longer time including physical effects (such as entanglement or entrapment); chemical effects (such as the release of additives or combustion products); and biological effects (such as root impediment or tissue/cellular damage) after they have reached into the environment. It was also observed that severity and type of harm caused due to plastics is generally depends on the size. Recent study indicates that the particles of plastic smaller than 5 mm are receiving considerable importance.

It was also found that drinking and tap water also consists of plastic particles. In one study it was found that out of 159 samples of tap water 83% were found to contain plastic particles which are collected from six regions on five different continents. The water samples collected from USA exhibits the highest level of plastic contamination, which is around 94% and is followed by Lebanon and India.

The worst data is collected from Europe as 18 samples collected from seven countries, 72% samples are contaminated. The data suggest that people may be taking microplastic in the range between 3,000 and 4,000 every year only through tap water⁹³. Most of the particles are

of size range between 0.1 to 5 mm, and with quantity of 0 to 57 particles per litre of water, and the average value is 4.34 particles per litre of water.

As far as the human exposure to the microplastic is concerned the most common way is through their diet. It was observed that the microplastics are detected in several food materials mainly including seafood, table salt, sugar, beer, drinking water, canned fish, mussels, and chicken meat. Unfortunately due to very few research related to microplastic, we lack information to assess the true amount of microplastics humans may be exposed to via food. In literature it is also mentioned that total amount of microplastic intake from only salt in China is around at 37 particles per individual annually³⁸⁵. It is also mentioned that a European shellfish consumer intakes around 11,000 microplastic particles annually.

As far as the toxic effects of plastics and its related compounds are concerned, it mainly include negative reproductive and developmental outcomes in the general population associated with BPA exposure. It was also observed that there is links between BPA and cardiovascular disease, type 2 diabetes, and abnormalities in liver enzyme.

Another compound related to plastic is phthalates having adverse effects on the endocrine system (responsible for the regulation of hormones). The adverse effects of phthalates include the interference in foetal development, changes to the reproductive system and metabolic effects such as insulin resistance and obesity, abnormal sexual development and birth defects. Some insects from Lepismatidae family feeds on cellulose containing and plastic materials. Hence it is possible that the gut microflora of these insects may have potential to carry out degradation of plastic and its related compounds.

The key questions which will be considered in this here include which are the insects from family that feeds on plastic materials. Secondly whether gut microflora of plastic feeding insects have the potential to carry out the degradation of plastic or not. Thirdly which are the extracellular enzymes that are responsible for plastic degradation. Another key question includes which is the pathway followed by the microorganisms to carry out plastic degradation.

II. INTRODUCTION

The past few decades have witnessed a huge demand and use of plastic waste materials. It was estimated that each year around 500 billion to 1 trillion plastic bags are used globally. The plastic material is resistant to decomposition which leads to environmental pollution. A plastic material leads to blockage in digestive system of fish, turtles, birds, marine mammals. Recently several cases have been reported related to cow death due to consumption of plastics (Waithaka et al., 2017).

Now a day plastic production is acting as a mother industry to thousands of components and products which are used in our day to day life (Kamboj, 2016).

The dramatic increase in production and use of plastics with lack of degradation technology has focused global attention on huge environmental accumulation and persistent in many countries. It was also observed that some plastic products like Bisphenol A, Phthalates, Polystyrene, Polycarbonate, Polyvinyl chloride, Polyethylene terephthalate etc. acts as a toxic compound which interferes with the hormone functioning, reproductive system, nervous system and some compounds acts as a carcinogenic in nature.

The interest in plastic degradation by using microorganisms was increased when degradation of paraffin by some microorganisms was reported during 1996.

As it was felt that insects are most diverse organism on earth and exhibit a variety of applications which are explored by human being. With the degradation of plastic, it is also of paramount importance to understand degradation mechanism and identification of by-products to ascertain the possible environmental effects. With the effects of plastic pollution on environment we also must consider the effects of other persistent pollutants and additives adsorbed on surface of plastic. It was also noted that there are some microorganisms with diverse community of bacteria, fungi and algae which forms biofilms on plastic material. The microbial community present in the biofilm and their activity is also not studied properly. There are little or no reports are available about significant microbial film for plastic degradation. But not all the microorganisms carry out biodegradation of plastic. However the by-products produced after biodegradation of plastic are also not adequately studied for their possible environmental effects (Kesti & Shivasharana, 2018).

On the basis of data from new report it was observed that microorganisms are opportunistic and they have the ability to adapt in most of the environment. They also have the ability to transform many complex compounds into simpler form through secretion of extracellular enzymes. In case of plastic degradation the extracellular enzymes binds to the polymer which leads to hydrolytic cleavage and causes degradation of polymer and are mineralized to carbon dioxide and water which is later used by microorganisms as a sole source of energy. It is of prime important to isolate and identify the microorganisms from various sources having plastic degradation potential without causing any harm to the environment (Auta et al., 2017). Recently the researchers are focusing on insect gut microflora as a source for the degradation of plastic. The insect gut microflora provides newer opportunity as a solution over plastic pollution. In this technology, the first and most important step is screening of insect guts containing plastic degrading bacteria. Here in the present study we are going to screen the insect gut microflora for the degradation of plastic (Latour et al., 2021).

During 2020 statistical analysis of plastic material disposal indicates about 8 to 40 million metric tons of plastic waste was dumped into the ocean without any type of treatment (Rebello et al., 2021).

Presently no any eco-friendly practically feasible treatment technology is available for degradation of plastic material. Hence Global efforts are urgently required to develop plastic biodegradation technology (Zhe et al., 2021).

Recent statistical data indicates that most of the plastic i.e. around 38% is used for packaging purpose and is continuously increasing by 12% per year (Bakht et al., 2020).

There are hundreds of scientific publications related to microbial degradation of plastics. Still the practical evidence of degradation is lacking. The published literature supports the stronger evidence of microbial plastic degradation but significant degradation under ambient environmental conditions is never demonstrated. With some present literature it was observed that yet at present, nobody invented the creditable microbial approach with on field plastic degradation potential.

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III. CURRENT RESEARCH AND DEVELOPMENT IN PLASTIC DEGRADATION BY USING INSECT GUT MICROFLORA

1. Global work

Brandon et al., 2021, worked on role of microbome of insect *Tenebrio molitor* (yellow mealworms) in degradation of polystyrene through secretions of gut microorganisms. They identified total eight gut microorganisms including *Citrobacter freundii*, *Serratia marcescens*, and *Klebsiella aerogenes* which are associated with polystyrene degradation. The authors also mentioned that the mealworm as well as its gut microbiota contributes in plastic degradation. Here they provides new insights related to insect mediated plastic degradation mechanism.

Mohanani et al., 2020, carried out the work on biodegradation of petroleum derived polymers. In their review they mentioned that microorganisms and enzymes have the potential to degrade polymers. They also wrote that *Pseudomonas* and *Bacillus* have the potential to carry out biodegradation of polyaromatic hydrocarbons. They are also associated with degradation of many plastic polymers. Here they also noted that the gut microbiome of insects have the potential to depolymerize the plastic polymers.

Bakht et al., 2020, isolated novel microorganisms from different landfills and dump sites which can degrade the polyethylene at faster rate. They found that out of 40 samples, 8 samples found to degrade polythene strips in liquid medium. Their further study exhibited that two microbial strains have high potential to degrade polythene.

Thomas et al., 2015, determined the degradation of plastic materials by three different species of microorganisms under both laboratory and field conditions. The result of this study exhibited that *pseudomonas fluorescens* degrades polythene of around 18% and 16% of polythene at 9 and 12 months period respectively while 3.8% of plastics in 12 months under field condition. Under laboratory condition it was found that around 8.06 and 5.63% of polythene and plastics were respectively degraded in a month.

Urbanek et al., 2020, demonstrated that *Tenebrio molitor* (mealworm) consumes polystyrene but they only bite and chew the materials and are not able to degrade. The authors isolated seven classes of microorganisms which are *Gammaproteobacteria*, *Bacilli*, *Clostridia*, *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria* and *Flavobacteria*. Out of these seven classes the most abundant genera includes *Enterobacter*, *Lactococcus* and *Enterococcus*. From the isolated microorganisms three bacterial strains have a potential to utilize the bioplastic as a sole source of carbon.

Jiang et al., 2021, found polystyrene degrading bacterium *Massilia* sp. FS1903 from the gut of *Galleria mellonella* (Lepidoptera: Pyralidae) larvae which are fed with polystyrene foam. After 30 days of incubation period with 0.15 g polystyrene, the weight of the polystyrene film significantly decreased which was around $12.97 \pm 1.05\%$ of weight loss.

2. National work

Kathiresan, 2003, carried the biodegradation of polythene and also plastic cups by using microorganisms which are present in mangrove soil after different incubation period of 2, 4, 6 and 9 months. The result of this study revealed that the degradation of polythene was higher

i.e. 4.21% after 9 months of incubation period while the degradation of plastic cups was found to be only 0.25% after same incubation period. Hence they concluded that the microorganisms present in mangrove soil is a good source for plastic degradation.

Kesti and Shivasharana, 2018, mentioned that to remove the plastic from environment it became necessary to develop new remediation technology. They also stated that there are some physical and chemical methods to degrade the plastic but they have some disadvantages, hence it is better to apply biological methods for plastic degradation. In case of microbial degradation, the authors stated that there are some reports of using microorganisms for plastic degradation but till today practically, there is no any organism was found to be efficient. But recently, the use of insects and their gut microbiome are gaining more important in plastic degradation. Some authors also mentioned that the gut microbiota of insects and their digestive enzymes plays an important role in plastic degradation. But with this they also mentioned that it is important to investigate the molecular characterization and complete pathways of plastic degradation must be studied in detail to end the global issue of plastic pollution.

Sini Francis et al., 2021, reported that plastic degradation by using microorganisms is yet in the primary stage and hence need to focus on isolation and identification of microorganisms having a potential of plastic degradation. They also mentioned that it is of prime important to develop a microbial technology for plastic degradation which can be commercialized and practically feasible to get rid of plastic pollution problem.

Ghosh et al., 2019, mentioned that the use of microorganisms is the better solution for plastic degradation as compared to chemical and physical methods. They mentioned the reason for applying microorganisms for plastic degradation is that due to the degradation pathway there is complete degradation and mineralization of any polymer. With this they also mentioned that the biodegradability of plastic is also depend on the microbial community present in the biofilm, as the different microbial community carry out significant role in modifying physical and chemical properties of plastic.

IV. IMPORTANCE AND LACK OF RESEARCH IN PLASTIC DEGRADATION

Now a day it is witnessed that the global market of plastic is continuously growing. The mass production of plastic was started from 1950s and slowly but continuously its demand for human use is increased and now occupied almost every part of our life. There are so many reasons why the use of plastic is increased. One of the reasons is the intrinsic features and benefits of plastics like light weight, durability, low cost and inertness against most of the chemical, biological and physical factors. Such a characteristic of plastics makes them versatile on one hand while due to these characteristics plastic persists in the environment as it is for longer period of time where they are disposed.

One recent survey estimated that around 8.3 billion tons of plastic was produced and dumped into the environment. The compounds used in plastic production including polyethylene, polypropylene, polystyrene, polyvinylchloride, polyethylene terephthalate and polyurethane etc which acts as toxic material are also dumped into the environment without any treatment. As far as plastic pollution is concerned, ecological disasters are well defined which mainly includes large number of marine animals gets killed due to ingestion of plastic through food materials or getting entangled in fishing nets, growing of pathogens on plastic in the form of biofilm and persistent organic pollutants on plastic materials. With this now a day it was

noted that there is one big problem is emerging related to plastic pollution i.e. microplastics. Due to all these problems it became important to combat the problem of plastic pollution for which 'Beat Plastic Pollution' is the slogan selected as a theme of the World Environment Day during 2018.

Recently most of the researches have focused on identification and screening of the microorganisms for plastic degradation. There are some studies which demonstrated the gut microflora of some insects and their larvae can metabolize the plastic materials. But there is no any report of using on-site practical application for plastic degradation. Another problem related to microbial plastic degradation is during microbial attacks on plastic different types of chemical by-products are released into the environment. Unfortunately there is lack of work related to which chemical by-products are produced during microbial plastic degradation and what are the impacts of these by-products on the environment.

With the use of microorganisms, microbial enzymes are also used to carry out plastic degradation with minimum incubation period. Hence it was felt that the screening of plastic degrading microorganisms and microbial enzymes are emerging as promising technology for plastic degradation. In the present study we will develop novel biotechnology based and on-site applicable technology for plastic degradation.

V. Conclusion

In short, by considering the facts about plastic production rate, their increased use, waste production and toxic environmental as well as human impacts, the time has come to control the plastic pollution. Hence the isolation and genetic identification of plastic degrading microorganisms from insect gut and the metabolic pathways of plastic degradation will be the important solution. The pure enzymes will be isolated in the present research work which can be used for the production of value added products and utilized to enhance the rate of plastic degradation. Hence development of the technology for plastic degradation under optimized laboratory conditions as well as environmental conditions is important.

REFERENCES

- [1] Waithaka, P. N., Gathuru, E. M., Githaiga, B. M., Ochieng, E. O., & Linet, L. T. (2017). Microbial degradation of polythene using actinomycetes isolated from maize rhizosphere, forest and waste dumping sites within Egerton University, Kenya. *International Journal on Emerging Technologies*, 8(1), 05-10.
- [2] Kamboj, M. (2016). Degradation of plastics for clean environment. *International Journal of Advanced Research in Engineering and Applied Sciences*, 5(3), 10-19.
- [3] Kesti, S. S., & Shivasharana, C. T. (2018). The role of insects and microorganisms in plastic biodegradation: A comprehensive review. *Int. J. Sci. Res. Biol. Sci*, 5(6), 75-79.
- [4] Auta, S. H., Emenike, C. U., & Fauziah, S. H. (2017). Screening for polypropylene degradation potential of bacteria isolated from mangrove ecosystems in Peninsular Malaysia. *International Journal of Bioscience, Biochemistry and Bioinformatics*, 7(4), 245-251.
- [5] Latour, S., Noël, G., Serateyn, L., Sare, A. R., Massart, S., Delvigne, F., & Francis, F. (2021). Multi-omics approach reveals new insights into the gut microbiome of *Galleria mellonella* (Lepidoptera: Pyralidae) exposed to polyethylene diet. *BioRxiv*, 2021-06.
- [6] Cf, S. F., Rebello, S., Mathachan Aneesh, E., Sindhu, R., Binod, P., Singh, S., & Pandey, A. (2021). Bioprospecting of gut microflora for plastic biodegradation. *Bioengineered*, 12(1), 1040-1053.
- [7] Zhe, Z., Peng, H., Yang, D., Zhang, G., Zhang, J., & Ju, F. (2021). Polyvinyl Chloride Degradation by Intestinal *Klebsiella* of Pest larvae. *BioRxiv*, 2021-10.
- [8] Bakht, A., Rasool, N., & Iftikhar, S. (2020). Characterization of plastic degrading bacteria isolated from landfill sites. *Int. J. Clin. Microbiol. Biochem. Technol*, 3(1), 030-035.

Chapter 1: POTENTIAL OF INSECT GUT MICROFLORA IN BIODEGRADATION OF PLASTIC
WITH SPECIAL EMPHASIS ON ITS METABOLIC PATHWAYS

- [9] Lear, G., Maday, S. D., Gambarini, V., Northcott, G., Abbel, R., Kingsbury, J. M., ... & Pantos, O. (2022). Microbial abilities to degrade global environmental plastic polymer waste are overstated. *Environmental Research Letters*, 17(4), 043002.
- [10] Brandon, A. M., Garcia, A. M., Khlystov, N. A., Wu, W. M., & Criddle, C. S. (2021). Enhanced bioavailability and microbial biodegradation of polystyrene in an enrichment derived from the gut microbiome of *Tenebrio molitor* (mealworm larvae). *Environmental science & technology*, 55(3), 2027-2036.
- [11] Mohanan, N., Montazer, Z., Sharma, P. K., & Levin, D. B. (2020). Microbial and enzymatic degradation of synthetic plastics. *Frontiers in microbiology*, 11, 580709.
- [12] Thomas, B. T., Olanrewaju-Kehinde, D. S. K., Popoola, O. D., & James, E. S. (2015). Degradation of plastic and polythene materials by some selected microorganisms isolated from soil. *World Appl Sci J*, 33(12), 1888-1891.
- [13] Urbanek, A. K., Rybak, J., Wróbel, M., Leluk, K., & Mironczuk, A. M. (2020). A comprehensive assessment of microbiome diversity in *Tenebrio molitor* fed with polystyrene waste. *Environmental Pollution*, 262, 114281.
- [14] Jiang, S., Su, T., Zhao, J., & Wang, Z. (2021). Isolation, identification, and characterization of polystyrene-degrading bacteria from the gut of *Galleria mellonella* (Lepidoptera: Pyralidae) larvae. *Frontiers in Bioengineering and Biotechnology*, 9, 736062.
- [15] Kathiresan, K. (2003). Polythene and plastics-degrading microbes from the mangrove soil. *Revista de biologia tropical*, 51(3-4), 629-633.
- [16] Ghosh, S., Qureshi, A., & Purohit, H. J. (2019). Microbial degradation of plastics: Biofilms and degradation pathways. *Contaminants in agriculture and environment: health risks and remediation*, 1, 184-199.

NUTRACEUTICAL BIOTECHNOLOGY FOR THE MANAGEMENT OF IRON DEFICIENCY ANEMIA: FROM PHYSIOLOGY TO INDUSTRIAL APPLICATION

Abstract

Iron deficiency anemia (IDA) remains one of the most prevalent nutritional disorders worldwide, affecting human health, productivity, and quality of life across all age groups. Although oral iron remains the most commonly prescribed intervention, its long-term effectiveness is frequently reduced by gastrointestinal discomfort and inconsistent adherence. Nutraceutical biotechnology has emerged as a promising interdisciplinary approach that integrates nutrition science, physiology, zoology, and industrial biotechnology to enhance iron delivery and utilization. This chapter discusses iron physiology, pathophysiology of IDA, limitations of existing treatments, and emerging biotechnological strategies such as fermentation, nano-encapsulation, biofortification, and protein-based chelation. Emphasis is placed on translational aspects from laboratory research to industrial production, regulatory considerations, and future prospects for sustainable anemia management.

Keywords: Iron deficiency anemia; nutraceuticals; biotechnology; iron absorption; bioavailability

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I. INTRODUCTION

Iron deficiency anemia (IDA) remains one of the most prevalent nutritional disorders worldwide, affecting diverse populations across age groups, physiological states, and socioeconomic backgrounds [1,3]. Despite decades of public health interventions, including supplementation programs and food fortification strategies, IDA continues to pose a significant burden, particularly among women of reproductive age, children, and individuals in low- and middle-income countries [2,4]. The persistence of this condition highlights fundamental gaps in the effectiveness, accessibility, and long-term sustainability of existing therapeutic approaches.

Conventional iron supplementation, largely based on inorganic iron salts, has demonstrated limited success in achieving sustained correction of iron deficiency. Gastrointestinal side effects, poor compliance, low bioavailability, and interactions with dietary inhibitors often compromise treatment outcomes [5,6]. Furthermore, parenteral iron therapies, although effective in severe cases, are invasive, costly, and associated with safety concerns, making them unsuitable for widespread preventive use [18]. These challenges underscore the need for alternative strategies that align better with physiological iron regulation and patient acceptability.

In recent years, nutraceuticals have emerged as a promising bridge between nutrition and therapeutics. Nutraceuticals derived from food sources or enhanced through scientific processing offer the potential to deliver bioactive compounds in forms that are both effective and well tolerated [7]. When combined with advances in biotechnology, nutraceutical development has evolved beyond simple supplementation toward targeted modulation of nutrient bioavailability, metabolism, and functional efficacy [10].

Biotechnology provides innovative tools to address key limitations of traditional iron therapies. Techniques such as microbial fermentation, enzymatic modification, biofortification, and nano-encapsulation enable the development of iron formulations with improved absorption, reduced gastrointestinal irritation, and enhanced stability [11–13]. These approaches are particularly relevant in the context of iron, whose absorption is tightly regulated by complex physiological mechanisms and influenced by dietary and microbial factors.

The convergence of iron physiology, nutraceutical science, and biotechnology represents a transformative shift in the management of IDA. Rather than focusing solely on iron quantity, contemporary strategies emphasize iron quality, bioavailability, and biological compatibility [14]. This chapter explores emerging trends in nutraceutical biotechnology for iron deficiency anemia, tracing the journey from laboratory innovation to industrial application, with a focus on physiological relevance, translational feasibility, and future potential.

Beyond its clinical implications, iron deficiency anemia also reflects broader nutritional, agricultural, and socioeconomic challenges. Dietary transitions toward plant-dominant foods, reduced dietary diversity, and reliance on processed staples have altered iron intake patterns globally. Even in regions with food fortification programs, the form of iron used and its interaction with habitual diets often limit effectiveness. These realities reinforce the importance of approaches that integrate nutritional adequacy with biological efficiency. Nutraceutical biotechnology addresses this gap by focusing on how iron behaves within

complex food systems and human physiology, rather than treating deficiency as a purely pharmacological problem [1], [5], [6].

II. GLOBAL BURDEN AND CLINICAL SIGNIFICANCE OF IRON DEFICIENCY ANEMIA

Iron deficiency anemia affects nearly one-third of the world's population, contributing to impaired cognitive development, reduced work capacity, weakened immunity, and increased maternal morbidity [1,2]. In zoological and veterinary contexts, iron deficiency has also been documented in livestock and laboratory animals, influencing growth, reproduction, and experimental outcomes [8].

The economic burden of IDA includes increased healthcare costs and reduced workforce productivity, underscoring the need for long-term, preventive solutions rather than short-term therapeutic interventions [9].

III. IRON PHYSIOLOGY AND HOMEOSTASIS

Iron is an essential micronutrient required for oxygen transport, cellular respiration, DNA synthesis, and immune function [10]. Unlike most micronutrients, Systemic iron balance is maintained primarily through regulated intestinal uptake, since physiological pathways for active iron excretion are absent in humans. [16]. This unique feature makes iron metabolism particularly sensitive to dietary intake, physiological demand, and pathological states [11].

1. Digestion and Intestinal Absorption of Iron

Dietary iron exists primarily in two forms: heme iron, derived from animal-based foods, and non-heme iron, predominantly obtained from plant sources [12]. Heme iron is absorbed efficiently through a relatively direct pathway, whereas non-heme iron absorption is more complex and highly influenced by luminal conditions. In the acidic environment of the stomach, ferric iron (Fe^{3+}) is released from food matrices and subsequently reduced to the ferrous form (Fe^{2+}), which is required for uptake in the proximal small intestine. [13].

Absorption occurs mainly in the duodenum and upper jejunum through specialized transport mechanisms. The divalent metal transporter 1 (DMT-1) facilitates the entry of ferrous iron into enterocytes, while dietary enhancers such as vitamin C promote absorption by maintaining iron in its reduced, soluble form [12]. Conversely, compounds like phytates, polyphenols, and calcium can significantly inhibit non-heme iron uptake, contributing to poor bioavailability in plant-based diets [6,11].

2. Iron Transport, Storage, and Systemic Regulation

Within the enterocyte, iron follows one of two pathways: it may be stored temporarily as ferritin or exported into the circulation through the iron transporter ferroportin. Exported iron is oxidized and bound to transferrin [20], the primary plasma iron carrier, which delivers iron to metabolically active tissues such as the bone marrow for erythropoiesis [14].

Systemic iron balance is regulated by hepcidin, a peptide hormone synthesized by the liver [15]. Hepcidin serves as the central hormonal regulator of iron distribution by controlling the activity of ferroportin and thereby governing iron release into circulation. During

inflammatory or infectious states, elevated hepcidin levels restrict iron absorption and mobilization, producing functional iron deficiency despite adequate or increased iron stores. [22].

3. Pathophysiology of Iron Deficiency Anemia

Iron deficiency anemia develops when iron supply fails to meet the demands of hemoglobin synthesis and red blood cell production. This imbalance may arise from inadequate dietary intake, impaired absorption, increased physiological requirements (such as pregnancy and growth), or chronic blood loss [16]. Over time, depleted iron stores lead to reduced hemoglobin concentration, diminished oxygen-carrying capacity, and compromised tissue oxygenation [16].

The physiological consequences of IDA extend beyond anemia itself. Affected individuals may experience fatigue, impaired cognitive function, weakened immune responses, and reduced work capacity [2,8]. In children, iron deficiency can disrupt neurodevelopment, while in adults it contributes to decreased productivity and quality of life. Importantly, these effects may persist even before overt anemia is clinically evident, emphasizing the need for early and effective intervention.

Understanding the intricate physiology of iron metabolism and the multifactorial nature of IDA is crucial for developing improved therapeutic strategies. Nutraceutical biotechnology seeks to align iron delivery systems with these physiological pathways, offering solutions that enhance absorption, minimize adverse effects, and support long-term correction of iron deficiency.

Recent research has emphasized the dynamic interaction between iron metabolism and the immune system. Iron availability influences host defense mechanisms, while immune activation reciprocally alters iron distribution through cytokine-mediated regulation of hepcidin. This bidirectional relationship explains why iron deficiency frequently coexists with infections and chronic inflammatory conditions. From a therapeutic perspective, this complexity underscores the limitation of high-dose iron strategies and highlights the need for formulations that support gradual, regulated absorption compatible with physiological control systems [7], [8], [10].

IV. LIMITATIONS OF CONVENTIONAL IRON THERAPIES

Despite decades of clinical use, conventional iron supplementation strategies remain inadequate for addressing iron deficiency anemia on a global scale. Oral iron salts and injectable formulations are effective in restoring iron levels in many cases; however, their widespread application is constrained by biological, clinical, and practical limitations. These shortcomings have driven interest in alternative approaches, including nutraceutical biotechnology.

1. Oral Iron Supplementation: Efficacy and Challenges

Oral iron therapy, typically administered in the form of ferrous sulfate, ferrous fumarate, or ferrous gluconate, is the most commonly prescribed treatment for IDA. While these formulations are inexpensive and readily available, their effectiveness is often compromised by poor gastrointestinal tolerance and low bioavailability [5]. A significant proportion of

administered iron remains unabsorbed, particularly in individuals with impaired intestinal function or chronic inflammation.

Unabsorbed iron can interact with the gut mucosa, leading to adverse effects such as nausea, constipation, abdominal discomfort, and diarrhea [6]. These side effects frequently result in poor patient adherence, reducing the therapeutic benefit of long-term supplementation. Iron that remains unabsorbed in the intestinal lumen may participate in oxidative reactions and alter microbial equilibrium, thereby exacerbating gastrointestinal symptoms.

2. Parenteral Iron Therapy: Benefits and Risks

Parenteral iron administration offers an alternative for patients who cannot tolerate or adequately absorb oral iron. Intravenous formulations allow rapid replenishment of iron stores and bypass intestinal absorption barriers. However, this approach is associated with higher costs, the need for medical supervision, and the risk of infusion-related reactions.

Although newer intravenous iron preparations have improved safety profiles, concerns regarding hypersensitivity reactions, iron overload, and altered immune responses persist [18]. In resource-limited settings, the infrastructure required for safe parenteral administration further restricts its feasibility, highlighting the need for orally deliverable yet highly bioavailable iron alternatives.

3. Biological Barriers to Iron Utilization

Beyond formulation-related issues, physiological regulators such as hepcidin significantly influence the success of conventional iron therapies [15]. In conditions characterized by chronic inflammation, elevated hepcidin levels reduce iron absorption and limit mobilization from storage sites. Under conditions of elevated hepcidin activity, higher iron intake does not necessarily enhance erythropoiesis and may instead increase the risk of iron-mediated adverse effects. [17].

Additionally, dietary inhibitors present in staple foods can further reduce iron uptake, particularly in populations relying heavily on plant-based diets. These factors collectively underscore the mismatch between traditional iron supplements and the body's tightly regulated iron metabolism.

4. Need for Innovative Nutraceutical Solutions

The limitations of conventional therapies emphasize the necessity for advanced iron delivery systems that align with physiological absorption pathways and minimize adverse effects. Nutraceutical biotechnology addresses these challenges by integrating bioactive compounds, novel carriers, and food-based matrices to improve iron solubility, stability, and intestinal uptake.

Such approaches aim not only to correct anemia but also to ensure sustained iron availability, improved patient compliance, and broader applicability across diverse populations. As research advances, nutraceutical-based interventions are emerging as promising alternatives capable of bridging the gap between laboratory innovation and real-world clinical needs.

Another critical limitation of conventional iron therapy is its poor adaptability to inter-individual variability. Differences in gastric acidity, intestinal transporter expression, inflammatory status, and microbiota composition result in highly variable absorption outcomes among individuals receiving identical iron doses. This variability contributes to inconsistent clinical responses and complicates population-level interventions. Consequently, there is growing recognition that iron delivery systems must be designed to accommodate biological diversity rather than relying on standardized dosing alone [3], [9], [14].

V. NUTRACEUTICAL BIOTECHNOLOGY: CONCEPT AND SCOPE IN ANEMIA MANAGEMENT

Nutraceutical biotechnology represents an interdisciplinary approach that integrates nutritional science with modern biotechnological tools to develop functional products capable of preventing or managing disease [7]. In the context of iron deficiency anemia, this approach moves beyond traditional supplementation by focusing on the optimization of iron delivery, absorption, and physiological utilization through biologically compatible systems.

Unlike pharmaceutical interventions that rely on isolated active compounds, nutraceuticals are typically derived from food sources or food-grade materials. Biotechnology enhances these products by modifying their composition, structure, or delivery mechanisms to improve functional efficacy [20]. This is particularly relevant for iron, whose bioavailability is influenced by chemical form, food matrix interactions, intestinal transport mechanisms, and systemic regulatory pathways.

1. Principles of Nutraceutical Biotechnology

The core objective of nutraceutical biotechnology is to enhance the biological performance of nutrients while maintaining safety and consumer acceptability. Techniques such as microbial fermentation, enzymatic treatment, and molecular encapsulation are employed to convert inorganic or poorly absorbed forms of iron into biologically favourable complexes. These processes aim to improve solubility, protect iron from dietary inhibitors, and facilitate controlled release in the gastrointestinal tract.

Additionally, biotechnological interventions can incorporate synergistic compounds, including amino acids, organic acids, and phytochemicals, that promote iron absorption or reduce oxidative stress. Such multifunctional formulations align with the physiological requirements of iron metabolism rather than overwhelming regulatory mechanisms with excessive doses.

2. Food-Based Bioengineering Approaches

Food-based bioengineering plays a pivotal role in the development of nutraceutical iron products. Fermentation processes using selected microbial strains can increase iron bioavailability by degrading absorption inhibitors and generating organic iron complexes [21,22]. Biofortification strategies, including plant breeding and microbial enhancement, enable the enrichment of staple foods with iron in naturally assimilable forms.

These approaches are particularly valuable for population-level interventions, as they integrate seamlessly into existing dietary patterns. Unlike conventional supplementation

programs, food-based nutraceutical strategies offer sustained iron delivery without altering consumer behavior or requiring medical supervision.

3. Advantages Over Conventional Supplementation

Nutraceutical biotechnology offers several advantages over traditional iron therapies. Improved tolerability reduces gastrointestinal side effects and enhances long-term compliance. Physiologically aligned absorption minimizes the risk of iron overload and oxidative damage. Furthermore, nutraceutical formulations are often cost-effective and scalable, making them suitable for industrial production and public health implementation [27].

From an industrial perspective, advances in biotechnology have enabled the standardization and quality control of nutraceutical products, ensuring consistency, safety, and regulatory compliance. These developments have facilitated the transition of iron nutraceuticals from laboratory research to commercial markets.

4. Translational and Industrial Relevance

The translational potential of nutraceutical biotechnology lies in its ability to bridge experimental innovation and real-world application. Laboratory-scale processes are increasingly being adapted for industrial manufacturing, supported by advancements in bioprocess optimization, stability testing, and formulation science. Regulatory frameworks governing functional foods and nutraceuticals further support this transition, particularly when products are derived from recognized food sources.

As consumer demand for natural and preventive health solutions continues to grow, nutraceutical biotechnology is positioned as a key driver in the next generation of anemia management strategies. Its emphasis on safety, sustainability, and physiological compatibility makes it a compelling alternative to conventional therapeutic models.

The scope of nutraceutical biotechnology extends beyond product formulation to include process innovation and functional validation. Advances in analytical techniques now allow precise characterization of iron speciation, release kinetics, and cellular uptake in vitro and in vivo. These tools support rational design of nutraceuticals that align with specific absorption pathways. Importantly, biotechnology also enables traceability and reproducibility, addressing quality concerns that historically limited confidence in nutraceutical interventions [15], [18].

VI. BIOTECHNOLOGICAL STRATEGIES FOR ENHANCING IRON BIOAVAILABILITY

Improving iron bioavailability remains the central challenge in the effective management of iron deficiency anemia. Biotechnology offers a diverse toolkit to modify iron at the molecular, cellular, and formulation levels, enabling its efficient absorption and utilization while minimizing adverse effects. These strategies focus on transforming iron into physiologically acceptable forms that align with the body's regulatory mechanisms.

1. Microbial Fermentation-Based Iron Enhancement

Microbial fermentation is one of the most extensively explored biotechnological approaches for improving iron bioavailability. Selected strains of bacteria and yeast can metabolize iron-containing substrates to generate organic iron complexes that are more readily absorbed in the intestine [21]. Fermentation also reduces antinutritional factors such as phytates and polyphenols, which are known to inhibit iron uptake [22].

Fermented iron preparations often exhibit enhanced solubility and stability within the gastrointestinal environment. Additionally, the presence of microbial metabolites may stimulate intestinal transporters involved in iron absorption, thereby improving systemic availability. These properties make fermentation-derived iron nutraceuticals particularly suitable for functional food applications.

2. Iron Chelation and Complexation Technologies

Chelation involves binding iron to organic ligands such as amino acids, peptides, or organic acids to form stable complexes. Biotechnologically produced chelated iron exhibits improved absorption and reduced interaction with dietary inhibitors [25]. Amino acid chelates, for example, mimic naturally occurring iron complexes found in food proteins, facilitating transport across the intestinal epithelium [24].

Protein-based iron carriers generated through enzymatic hydrolysis or recombinant techniques further enhance bioavailability by protecting iron from premature precipitation and oxidative reactions. These complexes also reduce free iron-induced gastrointestinal irritation, improving tolerability.

3. Nano-Encapsulation and Controlled Release Systems

Nanotechnology-driven delivery systems have gained attention for their ability to modulate iron release and absorption [23]. Nano-encapsulation techniques utilize food-grade polymers, lipids, or biopolymers to encapsulate iron particles, shielding them from harsh gastric conditions and releasing them at optimal absorption sites [24].

Controlled release formulations ensure gradual iron availability, reducing mucosal exposure to high iron concentrations. This approach aligns with physiological iron uptake mechanisms and lowers the risk of oxidative stress. From an industrial standpoint, scalable nano-formulation technologies enable consistent product quality and extended shelf life.

4. Synergistic Bioactive Integration

Biotechnological strategies increasingly incorporate synergistic bioactives to enhance iron absorption and utilization. Compounds such as organic acids, bioactive peptides, and plant-derived antioxidants can be co-formulated with iron to improve solubility and reduce inflammation-mediated absorption barriers [20].

These multifunctional nutraceutical formulations address both iron deficiency and associated physiological stress, offering holistic benefits beyond hemoglobin restoration. The integration of synergistic components reflects a systems-based approach to anemia management rather than isolated nutrient replacement.

5. Safety and Regulatory Considerations

Ensuring safety is a critical aspect of biotechnological iron enhancement. Processes are designed to produce food-grade products with controlled iron content and minimal risk of toxicity. Regulatory guidelines governing nutraceuticals emphasize bioavailability, stability, and absence of harmful contaminants, all of which are supported by standardized biotechnological protocols.

As these strategies mature, collaborative efforts between researchers, industry stakeholders, and regulatory bodies are essential to ensure that innovative iron nutraceuticals meet both scientific and public health standards.

Emerging evidence suggests that combining multiple biotechnological strategies within a single formulation may yield synergistic benefits. For example, fermentation-derived iron complexes encapsulated within protein or polysaccharide matrices can enhance both solubility and controlled release. Such hybrid systems reduce exposure of free iron to inhibitory compounds while preserving physiological uptake mechanisms. This layered approach reflects a shift from single-solution designs toward integrated systems biology-informed formulations [19], [20], [23].

VII. NUTRACEUTICAL APPLICATIONS IN PHYSIOLOGICAL AND CLINICAL CONTEXTS

Iron deficiency anemia manifests differently across physiological states and population groups, influenced by variations in iron demand, absorption efficiency, and regulatory mechanisms. Nutraceutical biotechnology allows for the customization of iron interventions to meet these diverse physiological needs, offering targeted solutions that extend beyond uniform supplementation strategies.

1. Iron Requirements Across Life Stages

Iron demand varies significantly during growth, reproduction, and aging. In infants and children, rapid tissue development increases iron requirements, making early-life deficiency particularly detrimental to cognitive and physical development. Nutraceutical formulations designed for pediatric use emphasize mild iron forms with high bioavailability and minimal gastrointestinal irritation, ensuring safety and compliance.

In adolescents and adults, especially women of reproductive age, iron loss due to menstruation and increased demand during pregnancy heightens the risk of anemia. Nutraceutical iron products enriched with absorption-enhancing bioactives provide a sustainable means of maintaining iron balance without the adverse effects associated with high-dose supplements.

2. Iron Deficiency in Inflammatory and Chronic Conditions

Chronic inflammation alters iron homeostasis by increasing hepcidin levels, thereby restricting intestinal absorption and iron mobilization from storage sites. In such conditions, conventional iron supplementation often fails to correct anemia effectively.

Biotechnologically optimized nutraceuticals, designed to bypass inflammatory absorption barriers, offer potential benefits in these contexts [3,15].

Fermentation-derived iron complexes and nano-encapsulated formulations demonstrate improved efficacy under inflammatory conditions by enhancing cellular uptake and minimizing unabsorbed luminal iron. These approaches reduce oxidative stress and support iron utilization even when systemic regulation is compromised.

3. Gastrointestinal Physiology and Microbiome Interactions

The gastrointestinal tract plays a central role in iron absorption and overall iron metabolism. Disruption of gut health can significantly impair iron uptake[19]. Nutraceutical biotechnology addresses this challenge by integrating iron delivery with gut-supportive components, including prebiotic substrates and bioactive peptides.

Such formulations not only enhance iron bioavailability but also support microbial balance, which is increasingly recognized as a determinant of nutrient absorption. By aligning iron supplementation with gastrointestinal physiology, nutraceutical approaches improve both efficacy and tolerability.

VIII.COMPARATIVE INSIGHTS FROM ZOOLOGICAL MODELS

Zoological and animal models have contributed valuable insights into iron metabolism and the efficacy of nutraceutical interventions [26]. Studies in vertebrate models demonstrate that organically complexed iron is absorbed more efficiently and induces fewer adverse effects compared to inorganic salts. These findings support the translational relevance of biotechnologically enhanced iron formulations.

Animal models also facilitate the evaluation of long-term safety, bio-distribution, and physiological adaptation to nutraceutical iron, providing essential data for industrial scale-up and regulatory approval.

IX.SYSTEMS-LEVEL IMPACT OF NUTRACEUTICAL BIOTECHNOLOGY IN IRON DEFICIENCY ANEMIA

The management of iron deficiency anemia increasingly requires a systems-level perspective that accounts for interactions among diet, host physiology, microbiota, and environmental factors. Traditional supplementation strategies often overlook these interactions, leading to variable efficacy and unintended consequences such as gut dysbiosis or oxidative stress. Nutraceutical biotechnology enables the design of iron delivery systems that function within this interconnected biological network. By leveraging food-compatible matrices and biologically derived carriers, iron can be delivered in forms that are recognized and processed efficiently by the body, supporting homeostasis rather than disrupting it [7], [12], [18].

At the intestinal level, growing evidence indicates that the gut microbiome plays a pivotal role in modulating iron availability and absorption. Certain microbial populations compete with the host for luminal iron, while others facilitate iron solubilization and uptake. Biotechnologically engineered nutraceuticals can influence this balance by incorporating prebiotic components or fermentation-derived metabolites that favor beneficial microbial activity. Such formulations not only enhance iron absorption but also contribute to intestinal

health, addressing a key limitation of conventional iron therapies that often exacerbate gastrointestinal discomfort [19], [21], [29].

From a translational standpoint, the integration of nutraceutical biotechnology into anemia management represents a convergence of preventive nutrition and therapeutic intervention. Products developed through this approach occupy a unique position between foods and medicines, allowing flexibility in deployment across clinical, community, and consumer settings. When supported by rigorous scientific validation and standardized manufacturing, these interventions can complement public health programs aimed at reducing anemia prevalence. Their adaptability, safety profile, and alignment with physiological regulation make them particularly suited for long-term use, reinforcing their relevance in both developed and resource-limited contexts [6], [11], [25].

X. CLINICAL TRANSLATION AND CONSUMER ACCEPTANCE

The success of nutraceutical interventions depends not only on biological efficacy but also on consumer acceptance. Products that integrate seamlessly into daily diets and align with cultural food practices are more likely to achieve sustained use. Biotechnology enables the incorporation of iron into familiar food matrices without compromising taste, appearance, or stability.

Clinical studies increasingly support the role of nutraceutical iron in improving hemoglobin levels and iron stores, particularly in populations with mild to moderate deficiency. These outcomes reinforce the potential of nutraceutical biotechnology as a practical and patient-friendly approach to anemia management.

Population-specific nutraceutical design is increasingly recognized as essential for effective anemia management. Pregnant women, older adults, and individuals with gastrointestinal disorders exhibit distinct iron handling characteristics that influence response to supplementation. Biotechnologically optimized nutraceuticals can be tailored to these contexts by adjusting iron form, carrier matrix, and co-nutrient composition. Such targeted strategies improve adherence and outcomes while reducing the risk of adverse effects associated with generalized supplementation [2], [4], [21].

XI. INDUSTRIAL PRODUCTION, FORMULATION, AND SCALE-UP CHALLENGES

The successful translation of nutraceutical biotechnology from laboratory research to industrial application requires careful consideration of production scalability, formulation stability, and regulatory compliance [27]. While numerous biotechnological strategies demonstrate efficacy at the experimental level, their industrial adoption depends on feasibility, cost-effectiveness, and consistency in large-scale manufacturing.

1. Transition from Laboratory to Pilot Scale

Laboratory-scale nutraceutical development often relies on controlled experimental conditions that are difficult to replicate at higher volumes. Parameters such as microbial strain performance, fermentation efficiency, and product yield may vary significantly during scale-up. Optimization of bioprocess variables, including temperature, pH, nutrient composition, and oxygen availability, is essential to maintain product quality and bioactivity.

Pilot-scale studies play a critical role in identifying potential bottlenecks and ensuring reproducibility. These intermediate stages allow refinement of processing protocols before full-scale industrial deployment, reducing economic risk and product variability.

2. Formulation Stability and Shelf Life

Industrial nutraceutical products must remain stable throughout storage, transportation, and consumption. Iron is particularly prone to oxidation and interaction with other food components, which can compromise both efficacy and sensory properties. Biotechnological interventions such as encapsulation and protein binding help protect iron from degradation and prevent undesirable reactions.

Ensuring shelf stability without the use of synthetic preservatives is a major challenge in nutraceutical formulation. Natural stabilizers and food-grade carriers are increasingly employed to maintain bioavailability while meeting consumer demand for clean-label products.

3. Quality Control and Standardization

Consistency in iron content and bioavailability is essential for consumer trust and regulatory approval. Industrial nutraceutical production requires robust quality control systems to monitor raw materials, intermediate products, and final formulations. Analytical techniques are used to assess iron speciation, release kinetics, and biological performance.

Standardization of biotechnological processes ensures batch-to-batch uniformity, a critical requirement for products intended for health management. Advances in process automation and real-time monitoring have significantly improved quality assurance in nutraceutical manufacturing.

4. Regulatory and Safety Considerations

Nutraceutical products must comply with national and international regulatory frameworks governing food safety and health claims. Regulatory requirements vary across regions, but generally emphasize ingredient safety, manufacturing practices, and scientific substantiation of functional benefits.

Biotechnologically enhanced iron nutraceuticals often benefit from being derived from recognized food sources or food-grade microorganisms, simplifying regulatory pathways. However, comprehensive safety assessments, including toxicity and bioavailability studies, remain essential components of product approval.

5. Market Integration and Industrial Viability

The commercial success of nutraceutical iron products depends on their ability to meet market demands while maintaining affordability. Industrial viability is influenced by production costs, supply chain stability, and consumer acceptance. Biotechnology-driven efficiency improvements, such as higher yield fermentation and reduced waste, contribute to cost reduction and sustainability.

Collaboration between academic researchers, biotechnology firms, and food manufacturers accelerates innovation and facilitates market entry. Such partnerships are vital for transforming promising laboratory discoveries into accessible, impactful nutraceutical solutions.

Industrial adoption of nutraceutical iron technologies also depends on supply chain considerations and raw material sustainability. Variability in biological inputs such as microbial strains or plant substrates can influence product consistency if not rigorously controlled. Standard operating procedures, validated starter cultures, and controlled fermentation environments are therefore essential. Advances in bioprocess engineering have improved predictability and scalability, enabling reliable production without compromising biological performance [22], [24].

XII. TABLES, FIGURES, AND CASE-BASED EVIDENCE

Visual representation of complex physiological processes and biotechnological workflows enhances clarity and supports translational understanding. In the field of nutraceutical biotechnology for iron deficiency anemia, tabulated data are particularly valuable for summarizing absorption mechanisms, comparing formulation strategies, and illustrating laboratory-to-industry pipelines.

1. Iron Absorption and Utilization Pathways

Iron absorption is a tightly regulated physiological process involving multiple transporters, enzymes, and systemic signals. A summarized tabular representation of dietary iron forms, absorption sites, transport mechanisms, and regulatory factors provides a concise overview of iron metabolism. Such a table highlights the differential absorption of heme and non-heme iron and underscores the influence of enhancers and inhibitors present in the diet.

By mapping biotechnologically enhanced iron formulations onto these physiological pathways, the table establishes a direct link between nutraceutical design and biological function. This comparative approach demonstrates how modified iron forms align more effectively with natural absorption mechanisms than conventional supplements.

Visual Representation of Complex Physiological Process, Biotechnological Workflows Enhances Clarity and Supports Transitional Understanding in the Field of Nutraceutical Biotechnology For Iron Deficiency Anemia.

Table 1 : Iron Absorption Pathway

Iron Type	Absorption site	Transporter/ Mechanism	Inhibitors
Heme Iron	Duodenum	HCP1	Minimal
Non-Heme Iron	Duodenum	DMT1(Fe ²⁺)	None significant
Fortified Iron	Duodenum	Various (Chelated Forms)	Phytates, Polyphenols, Ca ²⁺ Phytates, Tannins

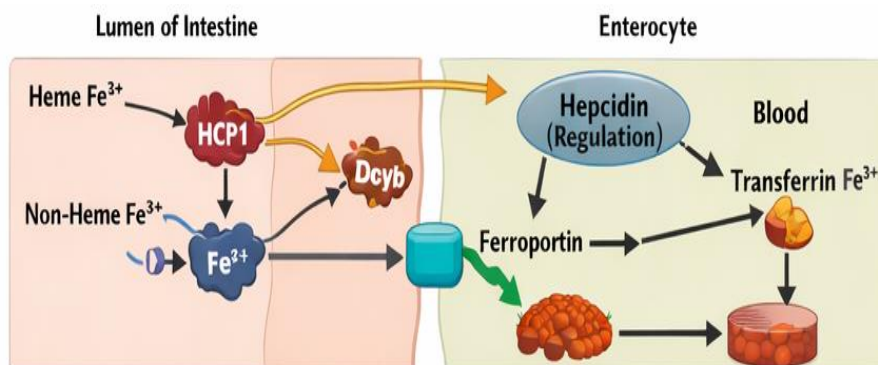


Figure 1: Schematic representation of iron absorption, transport, and regulation.

Figure 1: Schematic Representation of Iron Absorption, Transport and Regulation

1. Biotechnological Workflow from Raw Material to Product

A schematic figure depicting the biotechnological workflow offers insight into the transformation of raw iron sources into finished nutraceutical products. This workflow typically includes raw material selection, microbial or enzymatic processing, formulation optimization, quality control, and packaging.

Such a figure emphasizes the interdisciplinary nature of nutraceutical biotechnology, integrating microbiology, biochemistry, food technology, and industrial engineering. It also illustrates critical control points where bioavailability, safety, and stability are optimized, reinforcing the laboratory-to-industry narrative central to this volume.

2. Comparative Evaluation of Iron Formulations

Tables comparing conventional iron salts with biotechnologically enhanced nutraceutical formulations are effective in summarizing key performance parameters. These may include bioavailability, gastrointestinal tolerance, interaction with dietary components, oxidative potential, and suitability for long-term use.

Comparative summaries allow readers to quickly assess the advantages and limitations of different approaches, supporting evidence-based selection of iron delivery systems. Such tables also provide a foundation for identifying gaps in current technologies and opportunities for future innovation.



Figure 2: Biotechnological workflow for nutraceutical iron production, illustrating stages from raw material selection and microbial processing to formulation, quality control, and industrial packaging.

Table 2: Comparative Evaluation of Iron Formulations

Formulation Type	Bioavailability	GI Tolerance	Interaction with Diet	Industrial Feasibility
Ferrous Sulphate	Moderate	Poor	High	High
Heme Iron	High	Good	Low	Medium
Chelated Iron (AaComplex)	High	Good	Low	Medium
Nano - Encapsulated Iron	Very High	Excellent	Minimal	Medium-high
Fermented - Based Iron	High	Good	Moderate	Medium

3. Case-Based and Experimental Evidence

Experimental studies and pilot clinical evaluations form the evidence base for nutraceutical iron interventions. While detailed datasets may vary across studies, summarized representations of outcomes—such as improvements in hemoglobin levels, ferritin status, or absorption efficiency—enhance interpretability.

Animal and in vitro models are frequently used to demonstrate enhanced absorption and reduced toxicity of biotechnologically modified iron. Integrating these findings into concise tables or illustrative figures strengthens the translational relevance of the chapter and supports claims of improved efficacy.

4. Role of Visual Data in Industrial and Regulatory Contexts

From an industrial perspective, data also serve regulatory and quality assurance purposes. Workflow diagrams facilitate process validation, while standardized data tables support compliance with safety and labeling requirements. Clear visual documentation aids communication between researchers, manufacturers, and regulatory authorities.

Overall, the strategic use of illustrations not only improves readability but also reinforces the scientific rigor and applied relevance of nutraceutical biotechnology research in anemia management.

In addition to enhancing comprehension, they play a critical role in evidence synthesis and decision-making. Comparative visual data allow researchers and manufacturers to evaluate trade-offs between bioavailability, cost, and feasibility. For regulatory bodies, standardized visual summaries facilitate risk assessment and substantiation of health-related claims. Thus, well-designed visual elements function as both scientific tools and translational bridges between research, industry, and policy [16], [25].

XIII.FUTURE PERSPECTIVES AND EMERGING TRENDS

The landscape of nutraceutical biotechnology for iron deficiency anemia is rapidly evolving, driven by advances in molecular biology, food science, and personalized nutrition. Future

strategies aim to move beyond generalized supplementation toward interventions that are precisely tailored to individual physiological, genetic, and dietary profiles.

1. Precision Nutrition and Personalized Formulations

Emerging research emphasizes the importance of inter-individual variability in iron metabolism. Factors such as genetic polymorphisms, gut microbiota composition, age, sex, and underlying health conditions influence iron absorption and utilization. Personalized nutraceuticals, designed using biotechnology-informed approaches, can address these differences by optimizing iron form, dosage, and delivery matrix for specific populations [29].

Integration of omics technologies—genomics, metabolomics, and microbiomics—offers insights into individual iron requirements and responses, enabling the development of adaptive formulations. Such precision-targeted interventions have the potential to improve efficacy, minimize side effects, and reduce the risk of iron overload.

2. Advanced Delivery Systems

The next generation of iron nutraceuticals increasingly relies on sophisticated delivery technologies. Nanocarriers, encapsulation techniques, and bioresponsive matrices allow controlled and site-specific release of iron, improving absorption while reducing gastrointestinal irritation. These systems can also co-deliver synergistic bioactives, such as antioxidants, prebiotics, and vitamins, enhancing both efficacy and safety.

Smart delivery platforms capable of responding to environmental triggers—such as pH changes or enzymatic activity in the intestine—represent a promising frontier in iron supplementation, combining biotechnology with materials science to achieve optimized nutrient release.

3. Integration with Functional Foods and Lifestyle Approaches

Nutraceutical biotechnology is converging with the functional food sector, offering iron-enriched foods that support health as part of daily dietary habits. Fortification of staple foods, incorporation into culturally acceptable food matrices, and combination with gut-friendly components provide holistic solutions for anemia management. Lifestyle-based strategies, including diet diversification and gut microbiome modulation, further enhance the effectiveness of these interventions.

4. Sustainable and Eco-Friendly Production

Environmental sustainability is increasingly relevant in nutraceutical production. Biotechnological approaches, such as microbial iron biofortification, fermentation using renewable substrates, and low-energy nano-encapsulation methods, reduce resource use and waste. These eco-friendly production methods align with global goals for sustainable nutrition while ensuring scalability and industrial viability.

5. Regulatory and Ethical Considerations for Future Innovations

As nutraceutical biotechnology becomes more sophisticated, regulatory frameworks must evolve to ensure safety, efficacy, and transparency. Ethical considerations, including equitable access, population-specific formulations, and labeling accuracy, are essential to guide responsible innovation. Collaborative efforts between researchers, industry, and policymakers will be critical in translating laboratory discoveries into safe and effective products.

6. Potential for Multidisciplinary Research

Future progress in iron nutraceuticals depends on multidisciplinary collaboration. Integration of physiology, microbiology, materials science, clinical research, and food technology will drive the development of next-generation products. Experimental models, clinical trials, and population studies will collectively inform strategies that are biologically effective, consumer-friendly, and commercially viable.

In addition to enhancing comprehension, Well-designed tables and schematic figures serve as effective translational tools by summarizing physiological pathways, comparing formulation strategies, and supporting regulatory communication. Comparative visual data allow researchers and manufacturers to evaluate trade-offs between bioavailability, cost, and feasibility. For regulatory bodies, standardized visual summaries facilitate risk assessment and substantiation of health-related claims. Thus, well-designed visual elements function as both scientific tools and translational bridges between research, industry, and policy [16], [25].

XIV. ADVANCED PHYSIOLOGICAL INTEGRATION

Recent advances in iron research have highlighted the role of cellular iron sensing mechanisms in maintaining systemic balance. Enterocytes, hepatocytes, and macrophages respond dynamically to intracellular iron levels through coordinated signaling pathways that regulate transporter expression and storage proteins. Nutraceutical biotechnology offers the opportunity to design iron formulations that interact favorably with these sensing mechanisms, ensuring uptake occurs in response to physiological demand rather than passive diffusion. Such demand-responsive delivery minimizes excess luminal iron exposure and reduces the risk of oxidative damage, reinforcing the importance of biologically harmonized iron interventions [10], [26], [28].

XV. PUBLIC HEALTH AND TRANSLATIONAL RELEVANCE

From a public health perspective, nutraceutical biotechnology provides a pragmatic alternative to large-scale pharmacological interventions. Iron-enriched functional foods and biologically optimized supplements can be integrated into existing dietary frameworks, improving reach and sustainability. This approach is particularly valuable in low-resource settings where healthcare access is limited and long-term compliance with medicinal supplements is challenging. By aligning industrial feasibility with physiological efficacy, nutraceutical strategies support scalable, culturally adaptable solutions to iron deficiency anemia, strengthening their role in future global nutrition policies [6], [11], [25].

XVI. INTEGRATIVE AND TRANSLATIONAL DIMENSIONS OF NUTRACEUTICAL IRON BIOTECHNOLOGY

The integration of nutraceutical biotechnology into anemia management also necessitates a deeper understanding of host–nutrient interactions at the molecular and systems levels. Iron absorption and utilization are influenced not only by transporter activity but also by intracellular trafficking, mitochondrial demand, and erythroid precursor responsiveness. Emerging evidence suggests that iron delivery systems designed through biotechnological methods can modulate these downstream pathways more effectively than conventional supplements. For instance, organically complexed and nano-structured iron forms demonstrate improved cellular uptake and incorporation into metabolic pools without overwhelming storage pathways. Such formulations may support efficient erythropoiesis while avoiding compensatory hepcidin activation, thereby sustaining therapeutic effectiveness over extended periods [7], [10], [19], [23].

From a translational standpoint, the success of nutraceutical iron interventions depends on harmonizing scientific innovation with regulatory, industrial, and societal considerations. While biotechnology enables precise manipulation of nutrient forms, large-scale implementation requires validation through standardized clinical and functional endpoints. Regulatory agencies increasingly emphasize evidence-based substantiation of health claims, driving the need for well-designed studies that assess bioavailability, safety, and long-term outcomes. Concurrently, consumer perception of nutraceuticals as food-based, natural, and preventive solutions enhances acceptance and adherence. This convergence of biological optimization, regulatory alignment, and consumer trust positions nutraceutical biotechnology as a sustainable model for addressing iron deficiency anemia across diverse populations [6], [15], [21], [25].

XVII. CONCLUSION

Advances in nutraceutical biotechnology have reshaped contemporary strategies for addressing iron deficiency anemia by aligning iron delivery with physiological regulation, bridging the gap between traditional supplementation and modern therapeutic innovation. By integrating insights from physiology, molecular biology, and food science, biotechnologically enhanced iron formulations improve bioavailability, minimize adverse effects, and align with the body’s natural regulatory mechanisms [29].

Laboratory-based innovations, including microbial fermentation, chelation, nano-encapsulation, and bioactive co-integration, have demonstrated enhanced absorption and tolerability in experimental and pilot clinical settings. The successful transition of these innovations from experimental settings to large-scale manufacturing depends on coordinated process optimization, quality assurance, and regulatory alignment, ensuring that nutraceutical products are both effective and safe for diverse populations.

The convergence of precision nutrition, advanced delivery systems, functional food integration, and sustainable production methods represents the future of iron supplementation. Personalized formulations, informed by genetic, microbiome, and dietary data, promise to maximize efficacy and patient adherence, while minimizing the risks of conventional iron therapies.

Ultimately, nutraceutical biotechnology embodies a holistic, science-driven strategy that addresses both biological and practical challenges in anemia management. Continued multidisciplinary research, coupled with responsible industrial and regulatory practices, will be essential to realize the full potential of these interventions, enabling accessible, effective, and sustainable solutions for global iron deficiency anemia.

In summary, the evolution of nutraceutical biotechnology represents a shift from reactive correction of deficiency toward proactive nutritional optimization. By aligning iron delivery with physiological regulation, dietary context, and industrial feasibility, these approaches offer sustainable solutions to a persistent global health challenge. Sustained interdisciplinary collaboration among physiologists, biotechnologists, food scientists, and industry stakeholders will determine the long-term impact of these interventions. [1], [6], [28].

REFERENCES

- [1] N. Abbaspour, R. Hurrell, and R. Kelishadi, "Review on iron and its importance for human health," *Journal of Research in Medical Sciences*, vol. 19, no. 2, pp. 164–174, 2014.
- [2] J. L. Beard, "Iron biology in immune function, muscle metabolism and neuronal functioning," *Journal of Nutrition*, vol. 131, no. 2, pp. 568S–580S, 2001.
- [3] T. H. Bothwell, "Iron requirements in pregnancy and strategies to meet them," *American Journal of Clinical Nutrition*, vol. 72, no. 1, pp. 257S–264S, 2000.
- [4] R. Hurrell and I. Egli, "Iron bioavailability and dietary reference values," *American Journal of Clinical Nutrition*, vol. 91, no. 5, pp. 1461S–1467S, 2010.
- [5] A. López, P. Cacoub, I. C. Macdougall, and L. Peyrin-Biroulet, "Iron deficiency anaemia," *The Lancet*, vol. 387, no. 10021, pp. 907–916, 2016.
- [6] S. Lynch, "Why nutritional iron deficiency persists as a worldwide problem," *Journal of Nutrition*, vol. 141, no. 4, pp. 763S–768S, 2011.
- [7] A. M. Prentice, G. R. Ghattas, and M. Doherty, "Hepcidin and iron regulation," *Blood*, vol. 129, no. 7, pp. 823–833, 2017.
- [8] World Health Organization, *Anaemia*, World Health Organization, Geneva, Switzerland, 2020.
- [9] World Health Organization, *Iron Deficiency Anaemia: Assessment, Prevention and Control*, WHO Press, Geneva, Switzerland, 2001.
- [10] M. B. Zimmermann and R. F. Hurrell, "Nutritional iron deficiency," *The Lancet*, vol. 370, no. 9586, pp. 511–520, 2007.
- [11] N. C. Andrews, "Disorders of iron metabolism," *New England Journal of Medicine*, vol. 341, no. 26, pp. 1986–1995, 1999.
- [12] T. Ganz and E. Nemeth, "Hepcidin and iron homeostasis," *Biochimica et Biophysica Acta*, vol. 1823, no. 9, pp. 1434–1443, 2012.
- [13] T. G. DeLoughery, "Iron deficiency anemia," *Medical Clinics of North America*, vol. 101, no. 2, pp. 319–332, 2017.
- [14] C. Gupta and D. Prakash, "Nutraceuticals for iron deficiency anemia," *Nutrition*, vol. 30, no. 11–12, pp. 126–134, 2014.
- [15] S. Salovaara, A. S. Sandberg, and T. Andlid, "Organic acids influence iron uptake in the human epithelial cell line Caco-2," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 26, pp. 7820–7824, 2003.
- [16] S. Gómez-Ramírez, J. A. Brilli, C. Tarantino, and A. Muñoz, "Intravenous iron in inflammatory bowel disease," *World Journal of Gastroenterology*, vol. 15, no. 37, pp. 4666–4674, 2009.
- [17] M. Hoppe, L. Hulthén, and S. Hallberg, "Iron bioavailability from bread fortified with ferric pyrophosphate," *British Journal of Nutrition*, vol. 102, no. 5, pp. 644–651, 2009.
- [18] R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, Academic Press, New York, USA, 2013.
- [19] P. Rishi, R. Arora, and S. Kaur, "Microbial biotechnology and iron bioavailability," *Applied Microbiology and Biotechnology*, vol. 98, no. 7, pp. 2973–2985, 2014.
- [20] S. Bering, M. Suchdev, and L. Bukhave, "Fermented foods improve iron absorption," *European Journal of Nutrition*, vol. 45, no. 1, pp. 33–39, 2006.
- [21] D. J. McClements, "Nano-delivery systems for nutraceuticals," *Food Science and Nutrition*, vol. 54, no. 6, pp. 109–123, 2015.
- [22] S. Rai, P. Kumar, and A. Verma, "Nanotechnology-based iron delivery systems," *Journal of Controlled Release*, vol. 302, pp. 1–17, 2019.

- [23] EFSA Panel on Dietetic Products, Nutrition and Allergies, “Scientific opinion on dietary reference values for iron,” EFSA Journal, vol. 13, no. 10, Article 4254, 2015.
- [24] Indian Council of Medical Research, Nutrient Requirements and Recommended Dietary Allowances for Indians, ICMR, New Delhi, India, 2020.
- [25] Hoppe, M., and Hulthén, L., “Iron absorption from fortified foods,” British Journal of Nutrition, vol. 102, no. 5, pp. 644–651, 2009.
- [26] Prentice, A. M., “Clinical implications of new insights into hepcidin-mediated iron regulation,” Hematology, vol. 2018, no. 1, pp. 356–362, 2018.
- [27] Hurrell, R. F., and Cook, J. D., “Strategies for improving iron bioavailability in plant-based diets,” American Journal of Clinical Nutrition, vol. 109, no. 4, pp. 1096–1102, 2019.
- [28] Dostal, A., Chassard, C., Hilty, F. M., Zimmermann, M. B., Jaeggi, T., Rossi, S., Lacroix, C. “Iron supplementation promotes gut microbiota metabolic activity but not colonic microbiota composition in human subjects.” American Journal of Clinical Nutrition, vol. 98, no. 3, 2013, pp. 678–689.

ISOLATION, PURIFICATION AND ANTIMICROBIAL EVALUATION OF FERRITIN FROM SOYBEAN (GLYCINE MAX) SEEDS

Abstract

Ferritin is a highly conserved iron-sequestering protein distributed across plants, animals, and microorganisms, where it plays an essential role in regulating intracellular iron levels and limiting oxidative stress [1]. In plants, seed ferritin—commonly referred to as phytoferritin—serves as a major reservoir of bioavailable iron and has gained interest due to its nutritional importance [9]. Soybean (Glycine max) seeds represent a significant source of ferritin-bound iron and were therefore selected for the present investigation. This study describes the extraction and partial purification of ferritin from soybean seeds, followed by an assessment of its antimicrobial activity. Protein extraction involved selective salt precipitation and fractionation, and molecular characterization was performed using SDS-PAGE. Both crude and purified ferritin preparations were evaluated against selected bacterial strains using the agar well diffusion assay. The purified protein displayed an apparent molecular mass of approximately 28 kDa, consistent with reported ferritin subunits. Antimicrobial screening revealed weak inhibitory activity, with only *Escherichia coli* showing marginal sensitivity, while no inhibition was detected against other test organisms. These observations indicate that soybean ferritin primarily functions in iron storage and physiological regulation rather than exhibiting significant antimicrobial properties. The findings contribute to existing knowledge on plant ferritin and highlight its relevance in nutritional and food biotechnology [12,13].

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Keywords: Ferritin; Glycine max; phytoferritin; antimicrobial screening; plant iron protein

I. INTRODUCTION

Iron is a vital micronutrient involved in fundamental biological processes such as cellular respiration, nucleic acid synthesis, and oxygen transport. Despite its necessity, unbound iron can participate in redox reactions that generate reactive oxygen species, resulting in cellular damage. To prevent such toxicity while ensuring iron availability, organisms employ specialized iron-binding proteins, among which ferritin plays a pivotal role [9].

In plant systems, ferritin is primarily localized in plastids and developing seeds, where it functions as an iron reserve during germination and early growth [9]. Plant ferritin, often termed phytoferritin, exhibits structural similarity to animal ferritin but differs in its regulatory control and expression patterns. Leguminous plants, particularly soybean, accumulate substantial quantities of ferritin-bound iron, making them nutritionally valuable crops for combating iron deficiency [17].

Soybean (Glycine max) is widely cultivated due to its high protein content, balanced amino acid profile, and diverse bioactive constituents. Beyond its dietary importance, soybean-derived proteins have been investigated for potential biological functions. In the context of rising antimicrobial resistance, attention has shifted toward plant-based biomolecules as possible alternatives to conventional antimicrobial agents. [4,5]

Although ferritin is not traditionally classified as an antimicrobial protein, its capacity to bind and sequester iron may indirectly influence microbial proliferation. The present study was therefore undertaken to isolate ferritin from soybean seeds, characterize the purified protein, and evaluate its antimicrobial potential against selected microorganisms, thereby examining whether soybean ferritin exhibits biological activity beyond its established role in iron storage [6–8].

II. MATERIALS AND METHODS

1. Sample Preparation

Mature soybean seeds were obtained from a local source and carefully cleaned to eliminate surface impurities. The seeds were briefly rinsed with distilled water, dried under sterile conditions, and ground into a fine powder using a clean grinder. All experimental steps were performed at reduced temperatures to minimize protein denaturation.

2. Ferritin Extraction

Seed powder was homogenized in an extraction buffer containing Tris-HCl and magnesium chloride. The homogenate was centrifuged to separate insoluble material, and the resulting supernatant was collected as the crude protein extract. Controlled heat treatment was applied to selectively denature non-ferritin proteins, exploiting the heat stability of ferritin.

3. Purification by Salt Fractionation

Ferritin was partially purified using ammonium sulfate precipitation [10,11]. The crude extract was gradually saturated with ammonium sulfate under continuous stirring. Precipitated proteins were collected by centrifugation, dissolved in buffer, and dialyzed to remove residual salts, yielding a partially purified ferritin fraction.

4. Protein Characterization

Protein purity and molecular mass were assessed using SDS-PAGE [10,11]. Following electrophoresis, protein bands were visualized by staining and compared with standard molecular weight markers to estimate the size of the isolated ferritin subunits.

5. Antimicrobial Assay

Antimicrobial activity was evaluated using the agar well diffusion technique [12–14]. Selected bacterial cultures, including *Escherichia coli*, were spread evenly on nutrient agar plates. Wells were prepared in the agar and filled with crude and purified ferritin samples. Plates were incubated under appropriate conditions, and zones of inhibition were recorded.

III. RESULTS AND DISCUSSION

1. Ferritin Extraction and Purification

The extraction procedure produced a clear protein solution, indicating effective solubilization of seed proteins. Subsequent heat treatment and ammonium sulfate fractionation reduced the presence of non-specific proteins, resulting in enrichment of the ferritin fraction. Dialysis yielded a stable preparation suitable for analytical and biological evaluation.

2. SDS-PAGE Analysis

Electrophoretic analysis revealed a dominant protein band corresponding to an approximate molecular weight of 28 kDa. This finding is consistent with reported ferritin subunit sizes in plant species. The presence of minor additional bands suggested partial purification rather than complete homogeneity.

3. Antimicrobial Activity

Antimicrobial testing demonstrated limited biological activity. A small zone of inhibition, measuring approximately 8–9 mm, was observed against *Escherichia coli* with the purified ferritin sample. No inhibitory effect was detected against other tested microorganisms. Crude extracts exhibited negligible activity, indicating that the observed effect was specifically associated with the ferritin fraction.

Table 1: Zone of Inhibition Produced by Soybean Ferritin Against Test Microorganisms

Sr. No.	Test Microorganism	Zone of Inhibition (mm) – Crude Extract	Zone of Inhibition (mm) – Purified Ferritin
1	Escherichia coli	Nil	8-9
2	Staphylococcus aureus	Nil	Nil
3	Bacillus subtilis	Nil	Nil
4	Candida albicans	Nil	Nil

Note: Nil indicates absence of detectable inhibition under the experimental conditions.

IV. DISCUSSION

The minimal antimicrobial response observed in this study suggests that ferritin does not function as a potent antimicrobial agent. While iron sequestration can theoretically restrict microbial growth, the effect under the present experimental conditions was weak. These observations support the view that ferritin's primary role lies in iron storage and regulation rather than direct antimicrobial defense.

V. CONCLUSION

Ferritin was successfully extracted and partially purified from soybean (Glycine max) seeds using heat treatment and salt fractionation techniques. SDS-PAGE confirmed the presence of a protein consistent with plant ferritin in terms of molecular mass. Antimicrobial evaluation revealed only marginal inhibitory activity against *Escherichia coli*, with no effect observed against other microorganisms. The results indicate that soybean ferritin does not possess significant antimicrobial properties and should primarily be regarded as an iron-storage protein of nutritional importance. This study enhances understanding of phytoferritin and supports its relevance in food and nutritional biotechnology.

REFERENCES

- [1] Phillipson, J. D., "Phytochemistry and medicinal plants," Phytochemistry, 2001, 56, 237–243.
- [2] World Health Organization, WHO traditional medicine strategy 2002–2005, WHO Press, Geneva, Switzerland, 2002.
- [3] Benowitz, S., "As war on cancer hits 25-year mark, scientists see progress and challenges," The Scientist, 1996, 10, 1–7.
- [4] Cox, P. A., Bioactive compounds from plants, Ciba Foundation Symposium 154, John Wiley & Sons, London, UK, 1990, 40–55.
- [5] Shahidi, G. H., Karimi-Nik, A., "Antibacterial activity of selected Iranian medicinal plants against *Pseudomonas* species," Asian Journal of Plant Sciences, 2004, 3, 61–64.
- [6] Lee, C. K., Kim, H., Moon, K. H., Shin, K. H., "Screening and isolation of antibiotic resistance inhibitors from herbal materials," Archives of Pharmaceutical Research, 1998, 21(1), 62–66.
- [7] Kapoor, L. D., Singh, A., Kapoor, S. L., Srivastava, S. N., "Survey of Indian medicinal plants for bioactive compounds," Lloydia, 1969, 32, 297–302.
- [8] Hasegawa, H., Matsumoto, S., Yamasaki, K., "Reversal of tetracycline resistance in *Staphylococcus aureus* by ginseng prosaponins," Phytotherapy Research, 1995, 9(4), 260–263.
- [9] González-Morales, A., Rodríguez-Casado, A., Pérez-Palacios, T., "Soybean ferritin: isolation, characterization and biological relevance," Journal of Integrative Plant Biology, 2012, 54(1), 45–54.
- [10] Arnon, R. W., "Methods in enzymology," Methods in Enzymology, 1977, 22, 574–578.
- [11] Laemmli, U. K., "Cleavage of structural proteins during the assembly of the head of bacteriophage T4," Nature, 1970, 227, 680–685.
- [12] Atata, R. F., Sani, A., Ajewole, S. M., "Antimicrobial effects of plant stem bark extracts on clinical isolates," Biokemistri, 2003, 15(2), 84–92.

- [13] Bonjar, G. H. S., "Evaluation of antibacterial properties of medicinal plants," Asian Journal of Sciences, 2004, 3(1), 82–86.
- [14] Abu-Shanab, B., Adwan, G., Jarrar, N., Abu-Hijleh, A., Adwan, K., "Antibacterial activity of plant extracts used in Palestinian folk medicine," Turkish Journal of Biology, 2006, 30, 195–198.
- [15] Iwu, M. M., Handbook of African medicinal plants, CRC Press, Boca Raton, FL, USA, 1993.
- [16] Olukoya, D. K., Ndika, N., Odugbemi, T. O., "Antibacterial activity of medicinal plants in Nigeria," Journal of Ethnopharmacology, 1993, 39, 69–72.
- [17] Adams, C. F., Nutritive value of American foods, USDA Handbook No. 456, U.S. Department of Agriculture, Washington DC, USA, 1975, 4–174.

THE TRIPLE HELIX OF SUSTAINABILITY: INTEGRATED PRODUCTION OF BIOFUELS, BIOPLASTICS, AND HIGH-VALUE BIOMATERIALS

Abstract

The Triple Helix of Sustainability represents an integrative and dynamic model that captures the complex interactions among three pivotal sectors: academia, industry, and government. This conceptual framework highlights how these sectors collaborate synergistically to promote sustainable development by leveraging their unique strengths, resources, and expertise. Academia contributes cutting-edge research and theoretical knowledge, industry drives technological innovation and practical application, while government provides regulatory frameworks and policy guidance. Together, they form an interconnected system that facilitates continuous knowledge exchange and co-creation of solutions addressing the multifaceted challenges of sustainability. This model underscores the importance of fostering strong partnerships and communication channels among these spheres to enable the alignment of goals and strategies. By integrating scientific inquiry with market-driven innovation and supportive governance, the Triple Helix framework enhances the capacity to develop sustainable technologies, implement effective policies, and promote social responsibility. It encourages a holistic approach that balances environmental preservation, economic viability, and social equity, recognizing that sustainability demands coordinated efforts across all domains. Furthermore, the Triple Helix of Sustainability promotes adaptive and resilient systems by encouraging iterative feedback loops among academia, industry, and government. This continuous interaction allows for the timely identification of emerging sustainability issues and the rapid development and deployment of innovative responses. The collaborative nature of this model also facilitates capacity building and knowledge diffusion, ensuring that

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sustainable practices are widely adopted and scaled.

Keywords: Biofuels, Bioplastics, High value Biomaterials, Triple helix Sustainability

I. INTRODUCTION

Sustainability has emerged as a paramount global objective, driven by the imperative to reduce carbon emissions, mitigate environmental degradation, and foster circular economic models. The escalating concerns over climate change, resource depletion, and environmental pollution necessitate innovative approaches to resource utilization. Within this context, the concept of the "Triple Helix of Sustainability" encompasses the integrated production of three interrelated bioproduct streams: biofuels, bioplastics, and high-value biomaterials. This integrated approach optimizes the utilization of biomass resources, enhancing resource efficiency and economic feasibility while minimizing waste and environmental impact. This chapter examines recent advancements within this integrated production framework, emphasizing technological innovations, feedstock utilization, process integration, and sustainability evaluations. The holistic valorization of biomass through this triadic system holds significant promise for advancing the circular bioeconomy and achieving global sustainability targets. The concept of the "Triple Helix of Sustainability" represents a groundbreaking and integrative framework that synergistically combines the production of biofuels, bioplastics, and high-value biomaterials within a unified system. This innovative model is designed to harness the full potential of renewable biological resources, fostering a circular bioeconomy that simultaneously addresses environmental, economic, and social dimensions of sustainable development.

By interlinking these three pivotal sectors, the triple helix approach not only mitigates reliance on non-renewable fossil fuels but also promotes waste valorization, resource efficiency, and the generation of diverse bio-based products, thereby contributing to a more resilient and sustainable industrial landscape. At the core of this integrated production system, biofuels emerge as a critical renewable energy source capable of supplanting traditional fossil fuels. Through processes such as fermentation, transesterification, and advanced catalytic conversions, biofuels like bioethanol, biodiesel, and biogas offer substantial reductions in greenhouse gas emissions and air pollutants. This shift supports global climate change mitigation efforts and enhances energy security by diversifying energy portfolios. Moreover, the utilization of agricultural residues, algae, and other non-food biomass as feedstocks exemplifies the sustainable sourcing principles embedded in this model. Parallel to biofuels, bioplastics represent an essential component of the triple helix, offering environmentally friendly alternatives to petrochemical-derived plastics. Produced from renewable feedstocks such as corn starch, sugarcane, and lignocellulosic biomass, bioplastics like polylactic acid (PLA), polyhydroxyalkanoates (PHA), and starch-based polymers exhibit biodegradability or recyclability, thereby tackling the escalating global challenge of plastic pollution. The integration of bioplastic production within the triple helix framework enables the efficient use of biomass and process by-products, enhancing material circularity and reducing landfill dependency. High-value biomaterials constitute the third strand of the triple helix, encompassing a broad spectrum of bio-based chemicals, pharmaceuticals, specialty polymers, and functional materials. These products not only add significant economic value but also diversify the outputs of biorefineries, increasing their overall sustainability and market

competitiveness. Examples include bio-based adhesives, composites, enzymes, and bioactive compounds, which find applications across industries such as healthcare, agriculture, and manufacturing. The valorization of side streams and waste materials into these high-value products exemplifies the circular principles driving the triple helix model.

The true strength of the triple helix of sustainability lies in its systemic integration, where the interdependence of biofuels, bioplastics, and biomaterials fosters enhanced resource utilization and process optimization. Residues from biofuel production can serve as feedstock for bioplastic synthesis, while waste streams from bioplastic manufacturing may be converted into biomaterials or energy, creating a closed-loop system that minimizes environmental impact. This multifunctional biorefinery concept embodies the ideals of green chemistry, process intensification, and circular economy, paving the way for innovative industrial ecosystems that are both economically viable and ecologically responsible. Technological advancements underpinning this integrated approach include cutting-edge enzymatic and microbial bioconversions, metabolic engineering, advanced catalysis, and process integration techniques. These innovations improve product yields, selectivity, and scalability, enabling the efficient transformation of diverse biomass feedstocks into multiple value-added products. For instance, microbial fermentation pathways have been optimized to produce bioethanol and bioplastics simultaneously, while catalytic upgrading processes convert biomass-derived intermediates into specialty chemicals and materials.

Such technological convergence supports the economic feasibility and environmental sustainability of the triple helix framework. By promoting the simultaneous development of biofuels, bioplastics, and high-value biomaterials, the triple helix of sustainability aligns with global policy objectives aimed at climate action, pollution reduction, and sustainable industrial growth. This holistic strategy fosters innovation, creates green jobs, and stimulates regional bioeconomies, thereby contributing to broader socioeconomic development goals. The integrated production model also encourages collaboration among academia, industry, and policymakers, reflecting the multi-stakeholder nature of sustainable development challenges and solutions. In summary, the triple helix of sustainability offers a comprehensive and forward-looking pathway to transform conventional industrial systems into resilient, multifunctional biorefineries. By leveraging the synergies among biofuels, bioplastics, and high-value biomaterials, this framework enhances resource efficiency, reduces environmental footprints, and drives economic diversification within the bio-based economy. Its successful implementation holds the promise of addressing pressing global challenges while fostering sustainable innovation and long-term industrial sustainability.

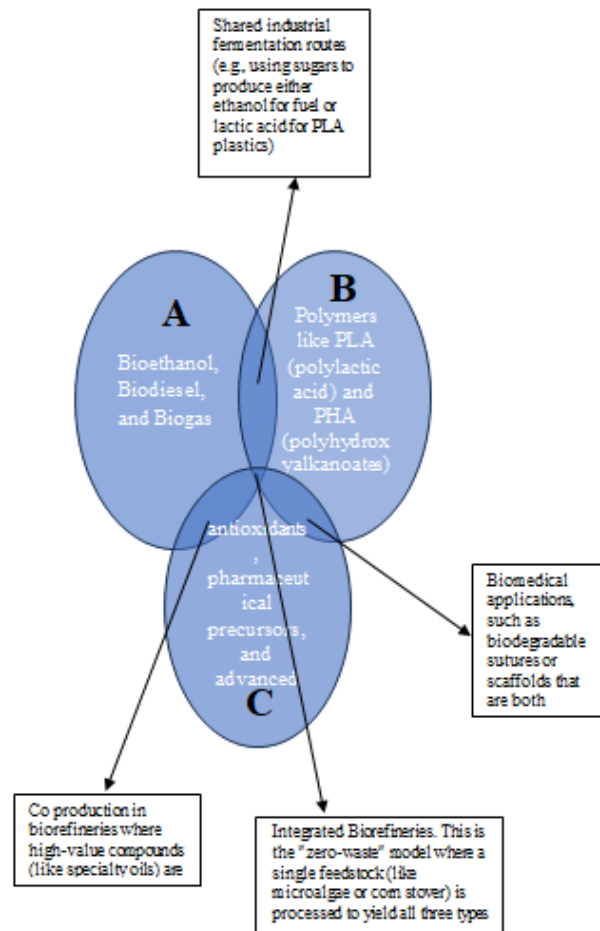


Figure 1: A Venn Diagram Showing the Triple Helix of Sustainability

II. BACKGROUND

1. The Triple Helix Concept

The Triple Helix of Sustainability denotes the synergistic production of:

Biofuels: Renewable energy carriers derived from biomass, including bioethanol, biodiesel, biogas, and advanced biofuels. These fuels serve as sustainable alternatives to fossil fuels, contributing to energy security and carbon emission reduction.

Bioplastics: Biodegradable or bio-based polymers serving as alternatives to conventional plastics. These materials aim to reduce the environmental footprint of plastic waste and promote biodegradability or recyclability.

High-Value Biomaterials: Specialty chemicals, pharmaceuticals, nutraceuticals, and biomolecules extracted or synthesized from biomass. These products leverage the chemical diversity of biomass to produce compounds with high economic and functional value.

This triad supports a circular bioeconomy by valorizing all biomass fractions through cascading utilization, thereby enhancing economic returns and reducing environmental burdens. The concept aligns with principles of green chemistry, waste minimization, and

sustainable industrial development, fostering integrated biorefineries that maximize product output and minimize resource input.



Figure 2: Feedstock Selection and Pretreatment Strategies

2. Feedstock Selection and Pretreatment Strategies

The integrated production framework relies on diverse biomass sources selected based on availability, composition, and sustainability considerations:

Lignocellulosic biomass: Agricultural residues such as corn stover, wheat straw, sugarcane bagasse, forestry residues, and dedicated energy crops like switchgrass and miscanthus. These feedstocks are abundant, non-food competitive, and rich in cellulose, hemicellulose, and lignin fractions. Industrial and municipal organic wastes: Food processing residues, municipal solid waste, and wastewater sludge offer sustainable feedstock options that contribute to waste valorization and circularity.

Algal biomass: Microalgae and macroalgae are characterized by high lipid, carbohydrate, and protein content, rapid growth rates, and the ability to grow on non-arable land or wastewater, thereby circumventing food-vs-fuel conflicts.

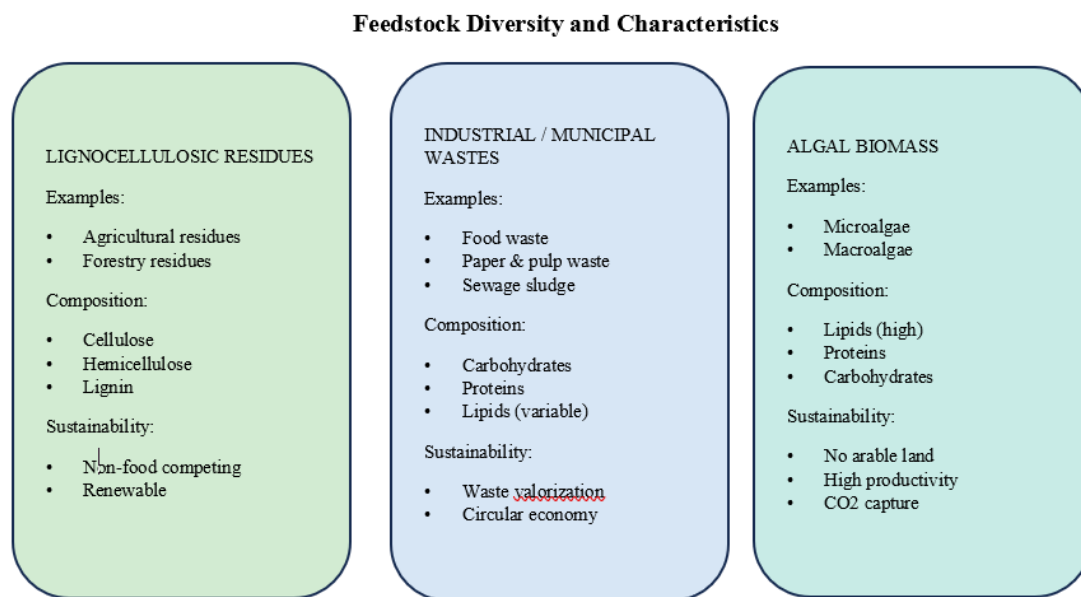


Figure 3: Feedstock Diversity and Characteristics

Table 1: Chemical Composition of Biomass and their Derived Commercial Products

Component	Chemical Nature	Major Products
Cellulose	Structural carbohydrates	Biofuels, biochemicals
Hemicellulose	Mixed sugars	Ethanol, xylitol
Lignin	Aromatic polymer	Bioplastics, energy
Lipids	Energy-dense molecules	Biodiesel, jet fuels
Proteins	Nitrogen-rich compounds	Feed, fertilizers

3. Pretreatment Technologies

Pretreatment is essential for fractionating biomass into fermentable sugars, lignin, and other components, facilitating downstream conversion processes. The choice of pretreatment impacts overall process efficiency, product yield, and environmental footprint.

Physical methods: Mechanical comminution such as milling and extrusion reduce particle size and improve enzyme accessibility.

Chemical methods: Acid hydrolysis (using dilute or concentrated acids), alkaline treatment, organosolv processes, and ionic liquids disrupt lignin-carbohydrate complexes and solubilize hemicellulose.

Biological methods: Enzymatic and microbial treatments employ ligninolytic fungi or enzyme cocktails to selectively degrade lignin and hemicellulose under mild conditions. Recent developments emphasize green, energy-efficient pretreatment techniques, including the use of deep eutectic solvents, which offer low toxicity and recyclability, and novel enzymatic cocktails engineered for enhanced specificity and activity. Integration of pretreatment with downstream processes, such as simultaneous saccharification and fermentation, further improves process economics and sustainability.

III. INTEGRATED BIOREFINERY DESIGN AND PROCESS INTEGRATION

1. Cascading Biorefinery Approach

The cascading biorefinery approach sequentially extracts multiple products from biomass, maximizing valorization and minimizing waste.

Step 1: Extraction of high-value biomaterials such as polyphenols, flavonoids, antioxidants, and other bioactive compounds using solvent extraction, supercritical fluid extraction, or membrane separation techniques. These compounds find applications in pharmaceuticals, nutraceuticals, and cosmetics.

Step 2: Conversion of carbohydrate fractions into biofuels through biochemical (fermentation) or thermochemical (pyrolysis, gasification) routes. This step aims to produce bioethanol, biogas, or drop-in biofuels.

Step 3: Utilization of lignin and residual biomass for bioplastic precursors or energy generation. Lignin valorization is critical to improving overall process economics and sustainability.

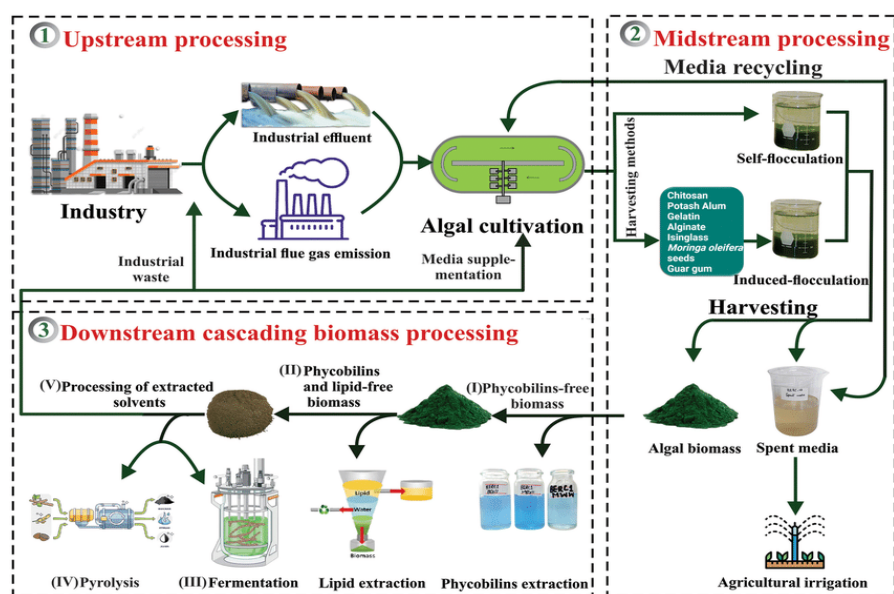


Figure 4: Integrated Biorefinery Design and Process Integration

IV. PROCESS INTENSIFICATION AND INTEGRATION

Process intensification and integration strategies aim to reduce energy consumption, capital expenditure, and waste generation by combining unit operations and optimizing resource flows.

Simultaneous saccharification and fermentation (SSF): This process combines enzymatic hydrolysis of cellulose and fermentation of released sugars into a single reactor, reducing inhibition effects and process time.

Co-production of bioethanol and bioplastics: Fermentation intermediates such as lactic acid can be polymerized into polylactic acid (PLA), enabling integrated production of fuels and materials.

Utilization of lignin-derived aromatics: Advanced catalytic and biochemical methods convert lignin into aromatic monomers for bio-based polymer synthesis, enhancing lignin's value beyond combustion. Integration extends to energy recovery and water recycling within the biorefinery, promoting a closed-loop system that enhances overall sustainability.

Production of Biofuels within the Triple Helix

- **First and Second-Generation Biofuels**
 - **First-generation biofuels:** Derived from food crops such as corn ethanol and biodiesel from vegetable oils. While established, these fuels face criticism due to food security and land-use concerns.
 - **Second-generation biofuels:** Produced from lignocellulosic biomass, offering enhanced sustainability profiles by utilizing non-food feedstocks and agricultural residues.
- **Advanced Biofuels and Novel Pathways**
 - **Biohydrogen:** Generated via dark fermentation and photo-fermentation, biohydrogen offers a clean energy carrier with water as the only combustion product.
 - **Biogas upgrading:** Integration of biomaterial extraction with anaerobic digestion enhances methane yield and biogas quality.
 - **Drop-in biofuels:** Hydrocarbon fuels compatible with existing fuel infrastructure, produced through catalytic pyrolysis, gasification, or microbial conversion pathways. Contemporary research focuses on microbial metabolic engineering to enhance substrate utilization, product specificity, and tolerance to inhibitors. Consolidated bioprocessing (CBP) integrates enzyme production, hydrolysis, and fermentation within a single microorganism or microbial consortium, reducing costs and complexity.

V. TYPES OF BIOPLASTICS

1. Biodegradable Bioplastics

Including polylactic acid (PLA), polyhydroxyalkanoates (PHA), and starch-based plastics, which degrade under environmental conditions, reducing plastic pollution.

2. Bio-Based, Non-Biodegradable Plastics: Such as bio-polyethylene (bio-PE) and bio-polyethylene terephthalate (bio-PET), which are chemically identical to their fossil-derived counterparts but sourced renewably.

3. Bioplastic Synthesis from Biomass Fractions

- **Fermentation-Derived Monomers:** Lactic acid for PLA and 3-hydroxybutyrate for PHA are produced via microbial fermentation of sugars.
- **Lignin Valorization:** Advances in polymer chemistry enable the development of lignin-based polymers and composites with enhanced mechanical and thermal properties.
- **Algal Bioplastics:** Algal polysaccharides and proteins are explored as novel feedstocks for bioplastic production, offering rapid growth and carbon capture benefits. Emerging strategies focus on improving mechanical strength, thermal stability, biodegradability, and scalability to meet diverse application requirements. Innovations also target cost reduction through process optimization and feedstock diversification.

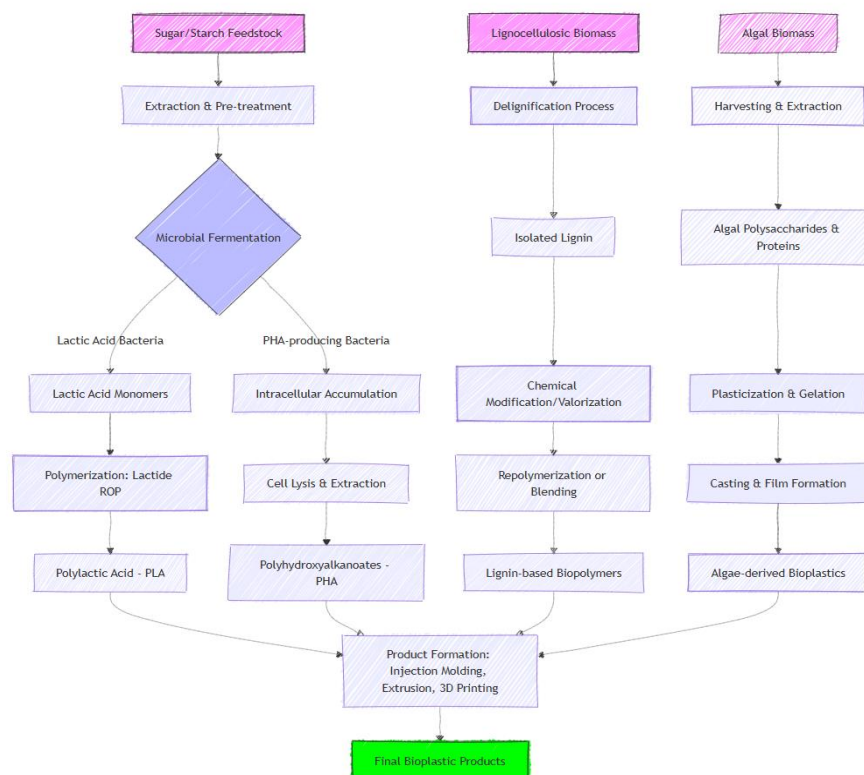


Figure 5: Bioplastics Production and Innovations

4. High-Value Biomaterials: Extraction and Applications

Categories and Sources

- **Phytochemicals:** Antioxidants, flavonoids, terpenoids, and other bioactive compounds with pharmaceutical, nutraceutical, and cosmetic applications.
- **Proteins and peptides:** Functional food ingredients, enzymes, and biopharmaceuticals derived from biomass.
- **Polysaccharides:** Chitosan, alginate, and cellulose derivatives utilized in food, pharmaceutical, and biomedical industries.

VI. EXTRACTION TECHNIQUES

1. Green extraction methods

Ultrasound-assisted extraction, microwave-assisted extraction, and supercritical CO₂ extraction minimize solvent use and energy consumption while maximizing yield and purity.

2. Biotechnological production

Microbial fermentation enables sustainable production of enzymes, biosurfactants, and other biomolecules with tailored functionalities. The integration of these extraction processes within biorefineries enhances economic viability by generating high-value co-products, thereby offsetting costs associated with biofuel and bioplastic production.

3. Sustainability Assessment and Life Cycle Analysis

- **Environmental Impacts:** Integrated biorefineries significantly reduce greenhouse gas emissions compared to fossil-based counterparts, contributing to climate change mitigation. Resource efficiency is enhanced through waste minimization, energy integration, and recycling of process streams.
- **Economic and Social Dimensions:** The development of integrated biorefineries promotes rural and industrial employment, fostering socio-economic development. Market competitiveness is improved through the valorization of multiple products, reducing dependency on single-product markets and enhancing resilience. Life cycle assessment (LCA) methodologies are increasingly employed to quantify environmental impacts, optimize process configurations, and guide policy and investment decisions. Multi-criteria sustainability assessments integrate economic, environmental, and social indicators to facilitate balanced decision-making.

VII. CHALLENGES AND FUTURE PERSPECTIVES

Technical Challenges

Achieving efficient and selective biomass fractionation without degradation remains a key technical hurdle. Scaling integrated biorefinery processes from laboratory to commercial scale requires addressing operational stability, feedstock variability, and process integration complexities. Ensuring that bioplastic properties meet stringent market and regulatory standards for performance and biodegradability is essential for market adoption.

Policy and Market Drivers

Regulatory frameworks that incentivize bio-based products, such as carbon pricing, subsidies, and mandates, are critical to driving market growth. Consumer acceptance, driven by awareness and demand for sustainable materials, influences market dynamics and product development.

Emerging Trends

Synthetic biology enables the design of microbial strains and enzymatic pathways tailored for customized biomaterial production and enhanced process efficiency. Digitalization and artificial intelligence (AI) facilitate process monitoring, optimization, and predictive maintenance, improving operational performance. Circular economy models emphasize waste valorization, resource recovery, and product life extension, aligning with integrated biorefinery principles.

VIII. CONCLUSION

The Triple Helix of Sustainability presents a comprehensive and integrated approach to maximizing biomass valorization through the concurrent production of biofuels, bioplastics, and high-value biomaterials. Advances in feedstock processing, integrated biorefinery design, and product diversification continue to propel this field forward. Sustained innovation, supported by rigorous sustainability assessments and enabling policies, remains crucial to fully realizing the potential of this integrated framework. By fostering economic viability, environmental stewardship, and social benefits, the Triple Helix approach contributes substantially to global sustainability objectives and the transition toward a resilient bioeconomy. In conclusion, the application of the Triple Helix model to the development of **Biofuels, Bioplastics, and High-Value Biomaterials** represents a fundamental shift from a linear, fossil-fuel-based economy to a circular, regenerative **Bioeconomy**. The synergy between academia, industry, and government is not merely a strategy for innovation; it is the essential mechanism for de-risking the transition to sustainable materials and scaling them to global markets.

The following pillars summarize the critical role of this model in achieving long-term sustainability:

1. Bridging the "Valley of Death" in Biomanufacturing

The transition from lab-scale microbial synthesis (University) to commercial-scale biorefineries (Industry) is fraught with high capital risks. The Triple Helix addresses this by:

- **Academia:** Designing high-yield metabolic pathways for 2nd and 3rd generation biofuels and high-value biomaterials.
- **Government:** Providing public sector undertakings (e.g., BIRAC in India or CBE JU in the EU) that offer "seed" funding and pilot facilities.
- **Industry:** Adopting these low-TRL (Technology Readiness Level) innovations and scaling them into competitive market products.

2. Standardizing "Green" and "Circular" Value

A bio-based product is only sustainable if its entire lifecycle is managed. The model ensures:

- **Circular Integration:** Using agricultural waste as feedstock for bioplastics, which then return to the earth as compost, closing the loop.
- **Policy & Certification:** Government-led mandates and "bio-labels" provide the regulatory certainty industry needs to invest in expensive bioplastic production lines over cheaper petroleum alternatives.

3. Economic Diversification through High-Value Biomaterials

Beyond fuels and plastics, the Triple Helix drives the creation of **High-Value Biomaterials** (like bio-based resins, specialty chemicals, and medical biopolymers). This creates a "cascading use" of biomass where the most valuable components are extracted first, ensuring that the bioeconomy is financially self-sustaining and not reliant solely on subsidies.

4. Evolutionary Resilience: The Quintuple Helix

The conclusion of this model is its necessary expansion. For biofuels and bioplastics to be truly sustainable, the **Natural Environment** (the 5th Helix) must be the ultimate benchmark. The collaboration now focuses on ensuring that bio-based production does not compete with food security or lead to land-use changes that increase carbon emissions.

Table 2:

Sphere	Primary Contribution to Bio-Sustainability
University	Breakthroughs in synthetic biology and enzymatic catalysis.
Industry	Supply chain optimization and mass-market distribution of bioproducts.
Government	Carbon taxes, plastic ban, and R&D subsidies to tilt the market.

Final Insight: The success of the Triple Helix in this sector proves that sustainability is a **systemic property**. Biofuels and bioplastics cannot survive in a vacuum; they require a "tri-lateral engine" to transform biological potential into industrial reality.

REFERENCES

- [1] Neupane D. Biofuels from Renewable Sources, a Potential Option for Biodiesel Production. Bioengineering (Basel). 2022 Dec 25;10(1):29.
- [2] Mehmood M.A., Ibrahim M., Rashid U., Nawaz M., Ali S., Hussain A., Gull M. Biomass production for bioenergy using marginal lands. Sustain. Prod. Consum. 2017; 9:3–21.
- [3] Maheshwari P., BelalHaider M., Yusuf M., Klemeš J.J., Bokhari A., Beg M., Al-Othman A., Kumar R., Jaiswal A.K. A review on latest trends in cleaner biodiesel production: Role of feedstock, production methods, and catalysts. J. Clean. Prod. 2022; 335:131588.
- [4] Singh A.R., Singh S.K., Jain S. A review on bioenergy and biofuel production. Mater. Today Proc. 2022; 49:510–516.
- [5] Gupta, V., & Singh, K. P. (2023). The impact of heterogeneous catalyst on biodiesel production; a review. *Materials Today: Proceedings*, 78, 364-371.
- [6] Deora, P. S., Verma, Y., Muhal, R. A., Goswami, C., & Singh, T. (2022). Biofuels: An alternative to conventional fuel and energy source. *Materials Today: Proceedings*, 48, 1178-1184.
- [7] Ansari, K., Goga, G., & Mohan, R. (2022). Utilization of food waste into ethanol and biodiesel. *Materials Today: Proceedings*, 65, 3596-3601.

- [8] Narancic T, Cerrone F, Beagan N, O'Connor KE. Recent Advances in Bioplastics: Application and Biodegradation. *Polymers* (Basel). 2020 Apr 15;12(4):920.
- [9] Ellen Macarthur Foundation. *The New Plastics Economy Rethinking The Future of Plastics* 2016. Ellen Macarthur Foundation; Cowes, UK: 2016.
- [10] World Economic Forum *The New Plastics Economy: Rethinking the future of plastics*. [(accessed on 15 April 2020)]
- [11] Lamb, J. B., Willis, B. L., Fiorenza, E. A., Couch, C. S., Howard, R., Rader, D. N., ... & Harvell, C. D. (2018). Plastic waste associated with disease on coral reefs. *Science*, 359(6374), 460-462.
- [12] Bornscheuer, U. T. (2016). Feeding on plastic. *Science*, 351(6278), 1154-1155.
- [13] Meeker, J. D., Sathyanarayana, S., & Swan, S. H. (2009). Phthalates and other additives in plastics: human exposure and associated health outcomes. *Philosophical transactions of the royal society B: biological sciences*, 364(1526), 2097-2113.
- [14] Ragaert K., Delva L., Van Geem K. Mechanical and chemical recycling of solid plastic waste. *Waste Manag.* 2017; 69:24–58.
- [15] Xin Tan, Guo-Jun Xie, Wen-Bo Nie, De-Feng Xing, Bing-Feng Liu, Jie Ding, Nan-Qi Ren. High value-added biomaterials recovery from granular sludgebased wastewater treatment process. *Resources, Conservation and Recycling* Volume 169, June 2021.
- [16] Ali A, Dias GJ and Hossain SM (2016). High value biomaterials from dairy milk proteins. *Front. Bioeng. Biotechnol. Conference Abstract: 10th World Biomaterials Congress*.
- [17] Cao D, Ding J. Recent advances in regenerative biomaterials. *Regen Biomater.* 2022 Dec 5
- [18] Gao J, Yu X, Wang X, He Y, Ding J. Biomaterial-related cell microenvironment in tissue engineering and regenerative medicine. *Engineering* 2022; 13:31–45.
- [19] Raouf AliAkbari, MohammadHadi Ghasemi, Nariman Neekzad, Elaheh KowsariMehdi Mehrali, Yousef Marfavi. High value add bio-based low-carbon materials: Conversion processes and circular economy. *Journal of Cleaner Production* Volume 293, 15 April 2021.
- [20] Olumoye Ajao, Mariya Marinova, Oumarou Savadogo, Jean Paris. Hemicellulose based integrated forest biorefineries: Implementation strategies. *Industrial Crops and Products* Volume 126, 15 December 2018, Pages 250-260.

BIOPLASTICS: AN ECO-FRIENDLY APPROACH TOWARDS SUSTAINABILITY

Abstract

Bioplastics are derived from natural resources and may be biodegradable and few are non-biodegradable, whereas conventional plastics are fossil fuel based and persist in the environment. Such bioplastics include polylactic acid (PLA), polyhydroxyalkanoates (PHA), cellulose, and starch. Similarly to oil-based plastics, biodegradable biobased plastics can be recycled or incinerated. This type of bioplastic can be microbially degraded. Non-biodegradable bioplastics include biopolyethylene terephthalate (bio-PET), biopolyethylene (bio-PE) and polyol-polyurethane. This mechanism of biodegradation of bioplastics gives rise to different interactions between the product and environment, therefore several components are involved in this process, which decrease or increase degradation. In response to these concerns, bioplastics have emerged as sustainable alternatives, offering reduced dependence on fossil resources and improved environmental compatibility. The global biodegradable plastic market is projected to reach \$6.73 billion by 2025.

Keywords: Bioplastic, Pollution, Environment, Biodegradable, Starch

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I. INTRODUCTION

The extensive use of petroleum-based plastics has led to severe environmental challenges, including persistent pollution, ecosystem degradation, and increased carbon emissions. In response to these concerns, bioplastics have emerged as sustainable alternatives, offering reduced dependence on fossil resources and improved environmental compatibility. At the global level, bioplastics are increasingly recognized as key materials for sustainable development and green technology. Bioplastics are polymeric materials that are either derived wholly or partially from renewable biological resources or are capable of undergoing biodegradation under specific environmental conditions. It is remarkable to note that not all bioplastics are biodegradable, and not all biodegradable plastics are bio-based. The scope of bioplastics includes packaging, agriculture, biomedical applications, and industrial products.

The use of natural polymers such as cellulose, casein, and starch predates the development of synthetic plastics. Early examples include celluloid and cellulose acetate. However, the dominance of petrochemical plastics in the mid-20th century slowed the development of new research in bioplastic. Renewed interest emerged in the late 20th and early 21st centuries due to growing awareness of environmental concerns, advancements in microbial biotechnology particularly in the context of global sustainability policies. Bioplastics represent a promising solution to plastic pollution. Although challenges remain, their environmental benefits and sustainable nature make them a valuable component of future materials science. Research should be focused on cost reduction and enhancing material properties.

II. CLASSIFICATION OF BIOPLASTICS

1. Based on Feedstock Origin

Plant-based bioplastics (corn, sugarcane, potato, cassava)

Microbial-based bioplastics (produced by bacteria and algae)

2. Based on Biodegradability and Compostability

Biodegradable and compostable bioplastics (PLA, PHA)

Bio-based but non-biodegradable plastics (Bio-PE, Bio-PET)

III. Feedstocks for Bioplastics

Bioplastics utilize renewable feedstocks like starch, cellulose, lignocellulosic biomass, vegetable oils, agricultural residues, algae, and microbial substrates. The selection of feedstock significantly influences the economic feasibility and environmental performance of bioplastics.

IV. Production Technologies of Bioplastics

1. Chemical Polymerization Routes

Sugars derived from biomass are fermented to produce monomers such as lactic acid, which are subsequently polymerized through condensation or ring-opening polymerization to form polymers like PLA.

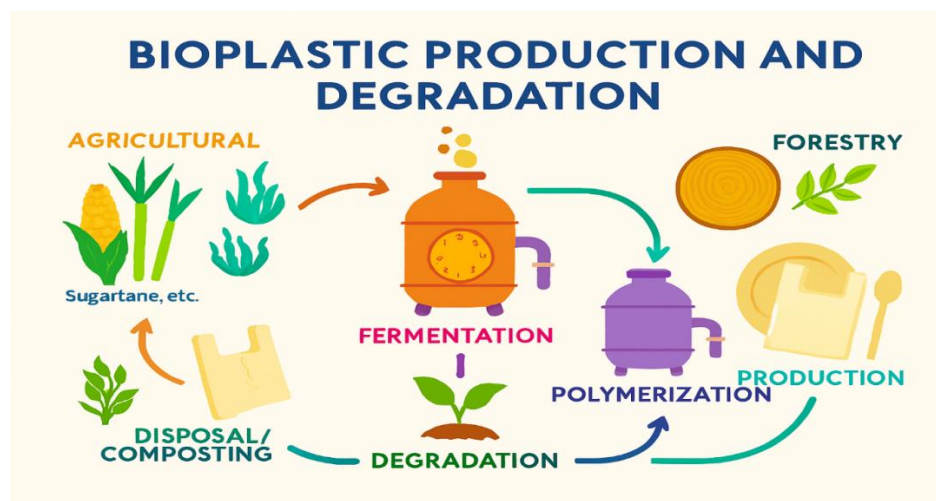


Figure 1: Bioplastic Production and Degradation

2. Microbial and Biotechnological Routes

Certain microorganisms synthesize biopolymers such as PHA as intracellular energy storage materials under nutrient-limited conditions. Advances in genetic engineering have improved yield and material properties.

V. PHYSICO-CHEMICAL AND MECHANICAL PROPERTIES

Bioplastics exhibit properties such as tensile strength, flexibility, thermal stability, and transparency, which vary depending on polymer type and processing conditions. Although generally comparable to conventional plastics, some bioplastics exhibit lower heat resistance and moisture sensitivity.

VI. MAJOR TYPES OF BIOPLASTICS

1. Polylactic Acid (PLA)

PLA is a thermoplastic aliphatic polyester derived by polymerizing lactic acid from renewable resources such as corn starch, tapioca roots, chips or starch, and sugarcane [56]. It is widely used due to its biodegradability, good mechanical strength, and ease of processing.

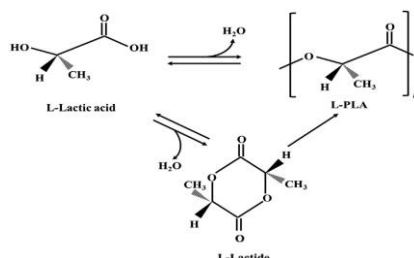


Figure 2: Polylactic Acid (PLA) Hydrolysis

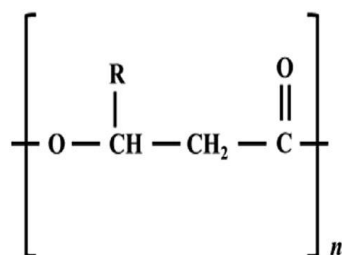


Figure 3: Chemical structure of PHA

2. Polyhydroxyalkanoates (PHA)

PHA are biodegradable and non-toxic polyesters produced by microorganisms. They are biocompatible and helpful in medical and packaging sectors. Polyhydroxyalkanoates (PHAs) are a class of bio-based plastics belonging to the polyhydroxy ester family of 3-, 4-, 5- and 6-hydroxy alkanoic acids.

3. Starch-Based Bioplastics

These bioplastics are produced by blending starch with plasticizers and biodegradable polymers. They are cost-effective but exhibit limited mechanical strength. Seaweeds are excellent for the production of bioplastics. The most commonly used seaweed types in industry contain polysaccharides such as agar, alginate, carrageenan, galactans and starch.

4. Cellulose-Based Bioplastics

Derived from cellulose esters and ethers, these materials offer good mechanical properties and are used in films and coatings. The mango tree leaves were used to extract cellulose through oxidation and bleaching and the results obtained were 29.6% lignin, 36.5% hemicellulose and 33.9% cellulose in mango leaves, in Malaysia.

Table 1: Types of Bioplastics

Type	Source Material	Key Properties	Common Uses
PLA (Polylactic Acid)	Corn starch, sugarcane	Biodegradable, transparent, rigid	Packaging, disposable cutlery
PHAS (Polyhydroxyalkan oates)	Bacteria-fed sugars	Biodegradable, water-resistant	Medical implants, agricultural films
Starch-based Plastics	Potato, corn starch	Compostable, low strength	Bags, food containers
Cellulose-based Plastics	Wood pulp, cotton	Flexible, semi-biodegradable	Film wraps, coatings
Bio-PET/Bio-PE	Sugarcane ethanol	Durable, recyclable (not biodegradable)	Bottles, textiles

VII. BIODEGRADATION MECHANISMS AND COMPOSTABILITY

Biodegradation process of bioplastics involves enzymatic hydrolysis and microbial assimilation, resulting in end products such as water, carbon dioxide, and biomass. Their ability to degrade depends on the type of polymer and the environment they're placed in.

- **PLA (Polylactic Acid):** Breaks down into lactic acid through hydrolysis. Needs high heat and moisture, so it mainly decomposes in industrial composting plants.
- **PHA (Polyhydroxyalkanoates):** Created naturally by microbes. These plastics are highly biodegradable and can break down in soil, water, and compost piles.
- **Starch-based Plastics:** Microorganisms digest starch into sugars, which they then consume. These can degrade even in home compost bins.
- **Cellulose-based Plastics:** Enzymes split cellulose into glucose, which microbes metabolize. They compost well in both home and industrial settings.

Compostability means a material not only biodegrades but also turns into safe, nutrient-rich compost within a set timeframe. It's depending on environmental factors such as humidity, temperature and microbial activity.

- **Industrial composting** → 50–60°C, oxygen, moisture → PLA, slow degraders.
- **Home composting** → lower temp, less control → starch-based, PHA, cellulose.
- **Standards** → EN 13432, ASTM D6400 → 90% breakdown in 6 months, no toxins.

Table 2: Mechanism of Biodegradation and Compostability

Type	Biodegradation	Compostability	Notes
PLA	Needs high temp	Industrial only	Packaging, cutlery
PHA	Soil, marine, compost	Home & industrial	Medical, films
Starch-based	Soil, home compost	Home composting	Bags, wrappers
Cellulose-based	Soil, compost	Home & industrial	Films, coatings

VIII. Environmental Impact and Life Cycle Assessment (LCA)

The Life Cycle Assessment (LCA) evaluates the environmental impacts of bioplastics from raw material extraction to end-of-life disposal. Compared to conventional plastics, bioplastics generally exhibit lower greenhouse gas emissions, though land use and resource competition remain concerns. The following steps are covering whole process-

1. Raw Material Acquisition

- Bioplastics: crops grown, harvested, processed.
- Conventional plastics: crude oil extraction and refining.

2. Production & Manufacturing

- Energy use, chemical processing, emissions.
- Bioplastics often require fermentation and polymerization steps.

3. Distribution & Use

- Transport emissions, durability, and product lifespan.

4. End-of-Life

- Composting, recycling, landfill, or incineration.
- Composting yields nutrient-rich soil; landfills risk methane emissions.

IX. Applications of Bioplastics

Bioplastics are widely used in drug delivery systems, packaging, agriculture (mulch films), biomedical devices, textiles, disposable cutlery and cups and consumer goods. Yet, bioplastics have high production costs, limited infrastructure for composting, scalability issues, and competition with food resources are one of the big challenges limiting widespread adoption. Bioplastics offer reduced carbon footprint and independence from fossil fuels. However, their environmental benefit depends on proper waste management. Compostable bioplastics can close the loop by turning waste into soil nutrients, but only if composting infrastructure is available.

1. Packaging Industry

- **Flexible Films:** PLA films are widely used in snack wrappers and salad bags. For example, several European supermarkets have adopted PLA-based produce packaging to reduce single-use petroplastics.
- **Rigid Containers:** PLA cups and bottles are common in beverage packaging. A notable case is water sold in PLA bottles in Italy, marketed as fully compostable under industrial conditions.
- **Cutlery and Trays:** Starch-based cutlery is used at large events and festivals where composting facilities are available, ensuring rapid breakdown after use.

2. Agriculture

- **Mulch Films:** Biodegradable mulch films made from starch blends are used in strawberry and tomato cultivation. Farmers benefit because the films degrade in soil, eliminating costly removal.
- **Seedling Pots:** PHA-based pots allow seedlings to be planted directly into soil, where the pot decomposes naturally. This reduces transplant shock and labor.
- **Controlled Release Fertilizers:** Bioplastic coatings on fertilizers gradually degrade, releasing nutrients over time and minimizing leaching into groundwater.

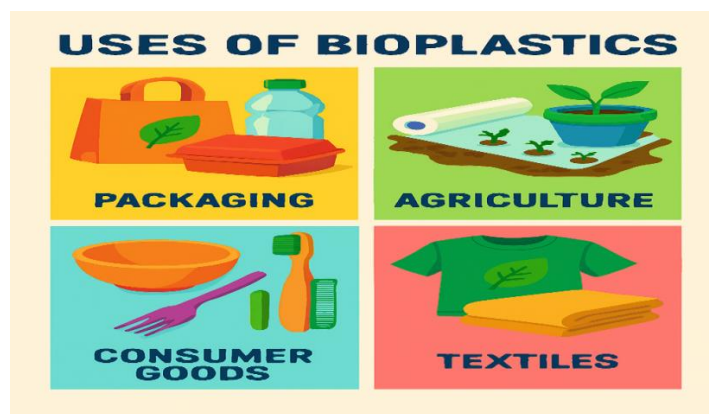


Figure 4: Uses of Bioplastics

3. Medical and Pharmaceutical

- **Absorbable Sutures:** PLA and PGA (polyglycolic acid) sutures are standard in surgery, dissolving safely in the body after healing.
- **Bone Fixation Devices:** PLA screws and pins are used in orthopedic surgery, gradually resorbing as bone heals.
- **Drug Delivery:** PHA microspheres are being developed to deliver chemotherapy drugs in controlled doses, reducing systemic side effects.
- **Tissue Engineering:** PLA scaffolds are used in regenerative medicine to support cell growth, later degrading without toxic residues.

4. Consumer Goods

- **Disposable Items:** PLA straws and starch-based plates are used in eco-friendly restaurants and catering services.
- **Durable Products:** Electronics companies have experimented with PLA casings for laptops and mobile phones, reducing reliance on petroleum plastics.
- **Fashion:** Footwear brands have introduced sneakers with bioplastic midsoles, while textiles made from PLA fibers are marketed as sustainable alternatives to polyester.

5. Automotive and Electronics

- **Interior Components:** PLA and PHA composites are used in dashboards and upholstery by automotive manufacturers in Japan and Germany.
- **Electronics Casings:** PLA housings have been tested in mobile phones and printers, offering lightweight and renewable alternatives.
- **Advantages:** Reduced weight, renewable sourcing, and lower emissions during production compared to petroplastics.

6. Construction and Architecture

- **Insulation Materials:** PLA foams are used in building insulation, offering biodegradability and reduced environmental impact.
- **Coatings:** Bioplastic films are applied to wood and furniture surfaces, providing protective layers that degrade without harmful residues.

- **Composite Materials:** PLA reinforced with natural fibers (like hemp or flax) is used in furniture and lightweight structural panels.

7. Specialty Applications

- **3D Printing:** PLA filaments dominate the consumer 3D printing market due to ease of use and biodegradability.
- **Marine Applications:** PHA is being tested for fishing nets and gear to reduce ocean plastic pollution.
- **Biodegradable Electronics:** Research prototypes include transient sensors and circuits built on bioplastic substrates that dissolve after use.

8. Future Prospects

- Expansion into **high-performance engineering plastics** for aerospace and defence.
- Development of **smart bioplastics** with programmable degradation rates.
- Integration into **circular economy models**, ensuring closed-loop material cycles.

X. ADVANTAGES OF BIOPLASTICS

A plastic made from a biological source sequesters the CO₂ captured by the plant in the photosynthesis process. If the resulting bioplastic degrades back into CO₂ and water this sequestration is reversed. But a permanent bioplastic made to be similar to polyethylene or other conventional plastics stores the CO₂ forever. Even if the plastics recycled many times the CO₂ initially taken from the atmosphere remains sequestered. Lower energy costs in manufacturing. On the other hand, plastics are made from ~4% of the oil that the world uses every year. With oil scarcity the manufacture of plastics becomes increasingly exposed to fluctuating prices. Do not use scarce crude oil. In contrast each kilogram of plastic typically requires 20 kilowatt hours of energy to manufacture more than the amount needed to make the same weight of steel. Almost all this comes from fossil sources.

Reduction in litter and improved compostability from using biodegradable bioplastics. The best understood advantage of biodegradable bioplastics lies in the reduction of permanent litter. Plastic single use shopping bag shares the most obvious example of how plastics can pollute the environment with huge and unsightly persistence. A large fraction of the litter in our oceans is of disposable plastic bags. Cities and countries around the world are taking action against the litter, sometimes by banning non-degradable plastic bags entirely. Potentially a much lower carbon footprint. It should be pointed out that the carbon footprint of a bioplastics crucially dependent on whether the plastic permanently stores the carbon extracted from the air by the growing plant.

XI. CONCLUSION

Bioplastics represent a scientifically and environmentally significant alternative to conventional plastics. To overcome these issues, bioplastics have emerged as an eco-friendly alternative. Bioplastics are designed to reduce dependency on fossil fuels and minimize environmental damage. Continued research, technological innovation, and policy support are essential to realize their full potential in sustainable materials. Bioplastics support the circular economy by promoting renewable resources, reduction of waste, and sustainable material cycles. Integration with recycling and composting systems is essential for maximizing

benefits. Current research focuses on second-generation feedstocks, genetically engineered microorganisms, improved material properties, and hybrid bioplastics to enhance performance and sustainability.

REFERENCES

- [1] Alaswad, M. Dassisti, T. Prescott, A.G. Olabi, Technologies and developments of third generation biofuel production, *Renew. Sustain. Energy Rev.* 51 (2015)1446–1460.
- [2] Chen, S.; Hu, Y.H. Advancements and future directions in waste plastics recycling: From mechanical methods to innovative chemical processes. *Chem. Eng. J.* 2024, 493, 152727. [CrossRef]
- [3] Chen, J.; Wang, X.; Long, Z.; Wang, S.; Zhang, J.; Wang, L. Preparation and performance of thermoplastic starch and microcrystalline cellulose for packaging composites: Extrusion and hot pressing. *Int. J. Biol. Macromol.* 2020, 165, 2295–2302. [CrossRef] [PubMed]
- [4] Erzam, H.; Arrifin, M.K.; Salwa, S.; Aziz, A. Bioplastic from mango leaves. *Int. Innov. Invent. Des. Compet.* 2020, 2020, 288–290.
- [5] Grand view research. Biodegradable Plastics Market Size, Share | Industry Report, 2018-2025; Grand View Research: San Francisco, CA, USA, 2019.
- [6] I.H. Jaafar (Eds.), *Proceedings of the 10th International Research/Expert Conference*, Citeseer, Barcelona, Spain, 2006.
- [7] Iwata, T. Biodegradable and bio-based polymers: Future prospects of eco-friendly plastics. *Angew. Chem. Int. Ed. Engl.* 2015, 54, 3210–3215. [CrossRef]
- [8] L.R. Castilho, D.A. Mitchell, D.M. Freire, Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state fermentation, *Bioresour. Technol.* 100 (23) (2009) 5996–6009.
- [9] L. Semprini, G.D. Hopkins, P.V. Roberts, D. Grbic-Galic, P.L. McCarty, A field evaluation of in-situ biodegradation of chlorinated ethenes: Part 3, studies of competitive inhibition, *Groundwater* 29 (2) (1991) 239–250.
- [10] L.T. Sin, A.R. Rahmat, W.A.W.A. Rahman, *Polylactic Acid: PLA Biopolymer Technology and Applications*, William Andrew Publishing, Norwich, 2012.
- [11] N. Rajendran, S. Puppala, M. Sneha Raj, B. Ruth Angeeleena, C. Rajam, Seaweeds can be a new source for bioplastics, *J. Pharm. Res.* 5 (3) (2012) 1476–1479.
- [12] PlasticsEurope. *Plastics—The Facts*. Available online: <https://www.plasticseurope.org/en> (accessed on 15 April 2020).
- [13] Ragaert, K.; Delva, L.; Van Geem, K. Mechanical and chemical recycling of solid plastic waste. *Waste Manag.* 2017, 69, 24–58. [CrossRef]
- [14] S. Pratt, L.-J. Vandí, D. Gapes, A. Werker, A. Oehmen, B. Laycock, *Polyhydroxyalkanoate (PHA) Bioplastics from Organic Waste*. Biorefinery, Springer, Cham, 2019, pp. 615–638.
- [15] S. Vigneswari, K. Bhubalan, A. Amirul, Design and tailoring of polyhydroxyalkanoate-based biomaterials containing 4-hydroxybutyrate monomer, in: *Biotechnology and Bioinformatics: Advances and Applications for Bioenergy, Bioremediation and Biopharmaceutical Research*, Apple Academic Press, Palm Bay, 2014, p. 281.
- [16] Total life-cycle considerations in product design for sustainability: a framework for comprehensive evaluation, in: I. Jawahir, O. Dillon, K. Rouch, K.J. Joshi, A. Venkatachalam,
- [17] M.A. Huijbregts, W. Gilijamse, A.M. Ragas, L. Reijnders, Evaluating uncertainty in environmental life-cycle assessment. A case study comparing two insulation options for a Dutch one-family dwelling, *Environ. Sci. Technol.* 37 (11) (2003) 2600–2608.
- [18] V. Nagarajan, A.K. Mohanty, M. Misra, Perspective on polylactic acid (PLA) based sustainable materials for durable applications: focus on toughness and heat resistance, *ACS Sustain. Chem. Eng.* 4 (6) (2016) 2899–2916.

EXOSOMES IN CANCER: DIAGNOSTIC, THERAPEUTIC AND TRANSLATIONAL PERSPECTIVES A SYSTEMATIC REVIEW

Abstract

Exosomes are a class of endosome - derived nanosized extracellular vesicles which emerge as crucial drivers of cell to cell communication in cancer. These exosomes are also called as tumor derived exosomes as they transport many bioactive molecules like lipids, proteins, metabolites and nucleic acids and these molecules mirror the molecular basis of their parent profile and play a significant role in tumor initiation, progression, metastasis, immune modulation and therapy resistance. Exosomes helps in excellent stability in biological fluids coupled with intrinsic targeting potential as exosomes are increasingly recognized as clinically relevant platforms for diagnosis and therapy. This review article presents an overview of current research on exosome biogenesis, molecular composition, and biological functions in the tumor microenvironment highlighting diagnostic biomarkers, therapeutic resistance, also engineered exosome-based interventions. In this article the key technology, biological and also regulatory challenges are discussed and future directions required for successful clinical translation are also outlined.

Keywords: Exosomes; Cancer; Tumor microenvironment; Liquid biopsy; Drug delivery; Immunotherapy; Therapy resistance

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I. INTRODUCTION

Cancer continues to represent a major global health burden in the society and has caused a widespread in the mortality rate resulting in the death of millions of people around the world [13] despite many advances in the study of cancer biology like early detection and therapeutic strategies. Even though there are remarkable progress in therapeutic strategies like surgical interventions, chemotherapy, immunotherapy, radiotherapy and targeted therapy, still cancer isn't fully curable because of tumor heterogeneity, immune escape, metastasis and development of resistance in the tumor cells against the therapy [14, 16]. There are many advances in the cancer biology but also many challenges which highlight the importance of gaining deeper mechanistic insights into tumor biology while fostering the development of innovative diagnostic and therapeutic modalities [14].

Across the past two decades these exosomes have emerged as the key components in cancer biology as these exosomes were once the cellular waste disposal vesicles but now over the past 20 years these exosomes have been recognized as the crucial biological mediators that transfer the functional bioactive cargo between the tumor cells and components of cancer microenvironment which includes the stromal cells, endothelial cells and immune components [15]. Exosomes play a very crucial role in regulating complex signaling events which helps in tumor growth and dissemination [16]. Their widespread presence across body fluids and their capacity to mirror the molecular characteristics of tumors render them highly valuable for both fundamental research and clinical oncology [17].

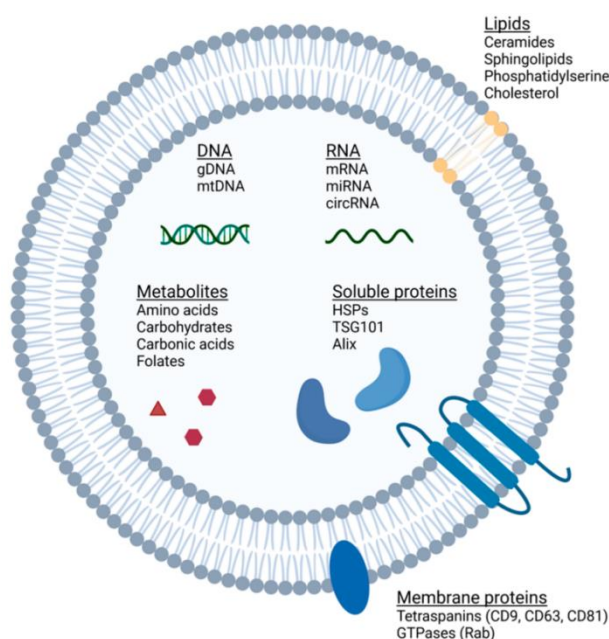


Figure 1: Schematic Representation of an Individual Exosome and its Associated Bioactive Cargo. DOI: 10.3390-life13102033

II. BIOGENESIS AND MOLECULAR CHARACTERISTICS OF EXOSOMES

Exosome biogenesis is explained by Raghu Kalluri *et al* in 2020 in the Science journal. The article reveals that exosome biogenesis begins with the inward budding of the plasma membrane, forming early-sorting endosomes that capture cell-surface and extracellular proteins. These endosomes mature through contributions from the trans-Golgi network and

endoplasmic reticulum, evolving into late-sorting endosomes. Further inward invagination of the endosomal membrane generates multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs). This double-invagination process gives rise to ILVs that are the precursors of exosomes. MVBs either fuse with lysosomes for degradation or merge with the plasma membrane to release ILVs as exosomes measuring ~40–160 nm [25] in diameter [2, 12].

The Exosome biogenesis is highly regulated process which is controlled by both ESCRT (endosomal sorting complex required for transport)-dependent and ESCRT-independent mechanisms. ESCRT machinery components such as TSG101, ALIX, and VPS proteins helps and promote the membrane budding and cargo sorting, as ESCRT-independent exosome promotes the ceramide-driven lipid raft pathways and tetraspanin-enriched micro domain formation. Rab GTPases, including Rab27a/b, Rab11, and Rab35, further plays a crucial role in regulating the MVB transport and then release the exosomes [1, 18, 19, 25].

Molecularly, exosomes are characterized by conserved set of proteins which are called as the canonical markers and these canonical markers includes tetraspanins (CD9, CD63, CD81, CD82), heat shock proteins (HSP60, HSP79, HSP90), SNAREs (v-SNARE and t-SNARE), cytoskeletal proteins, and membrane transport proteins [1, 4, 7, 10, 18, 19, 22, 25]. Beyond these, the exosomes contain highly selective and functional cargo like proteins (tetraspanins, heat shock proteins, MVB formation associated proteins, proteins related to membrane transport and fusion), enzymes (lipolytic enzymes), receptors, transcription factors, lipids (cholesterol, ceramides, sphingolipids, and phosphatidylserine), metabolites (amino acids and their derivatives, carbohydrates, carbonic acids, and folates) and diverse nucleic acids which includes the microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), messenger RNAs (mRNAs), tRNAs, fragmented RNAs and DNA (genomic (gDNA) as wells as mitochondrial (mtDNA)). This selective packaging underlines the biological specificity of exosome-mediated communication in cancer [5, 10, 20, 22, 25].

III.ROLE OF EXOSOMES IN THE TUMOR MICROENVIRONMENT

Tumor-derived exosomes facilitate the remodeling of the pre-metastatic niche (PMN), creating a favorable and permissive environment that supports tumor cell seeding, colonization, and metastatic progression [9]. The tumor microenvironment constitutes of a highly complex dynamic and heterogeneous ecosystem comprises of malignant cells, stromal cells like cancer-associated fibroblasts (CAFs) and endothelial cells, immune cells, soluble factors like cytokines and chemokines and extracellular matrix components [4, 21]. Exosomes play key role in shaping this microenvironment by mediating intercellular crosstalk within TME. Through regulated biogenesis, sorting, secretion, and selective uptake, exosomes influence tumor growth, angiogenesis, metastasis, and drug resistance, making them an important emerging focus of cancer research [22].

Tumor-derived exosomes enhance the angiogenesis by transferring the pro-angiogenic factors which includes the VEGF, angiopoietins, and miRNAs (e.g., miR-210, miR-21) into endothelial cells receptors [5, 22]. Tumor-derived exosomes deliver miRNAs that reprogram fibroblasts and mesenchymal stem cells toward a pro-tumorigenic, CAF-like phenotype, promoting the release of growth factors and matrix-remodeling enzymes that facilitate tumor invasion and progression [22].

According to the article in Biomedical Research and Therapy in 2020 by Truong *et al.*, tumor-derived exosomes play a crucial role in modulating immune cell function and

promoting tumor immune escape. These vesicles influence both innate and adaptive immune cells by altering their activation, differentiation, and effector responses. Exosomes released from cancer cells suppress cytotoxic T lymphocyte activity and induce T-cell apoptosis or exhaustion, thereby weakening anti-tumor immunity. They also promote the expansion of regulatory T cells, which further inhibits immune surveillance. Natural killer cell cytotoxicity is reduced through exosome delivery of immunosuppressive molecules and altered receptor expression. In addition, tumor exosomes impair dendritic cell maturation and antigen-presenting capacity, leading to inefficient T-cell priming. Macrophages are reprogrammed toward an immunosuppressive M2 phenotype that supports tumor growth. Myeloid-derived suppressor cells are expanded and activated, enhancing immune suppression in the tumor microenvironment. Thus, tumor-derived exosomes create an immune-tolerant environment [6, 23].

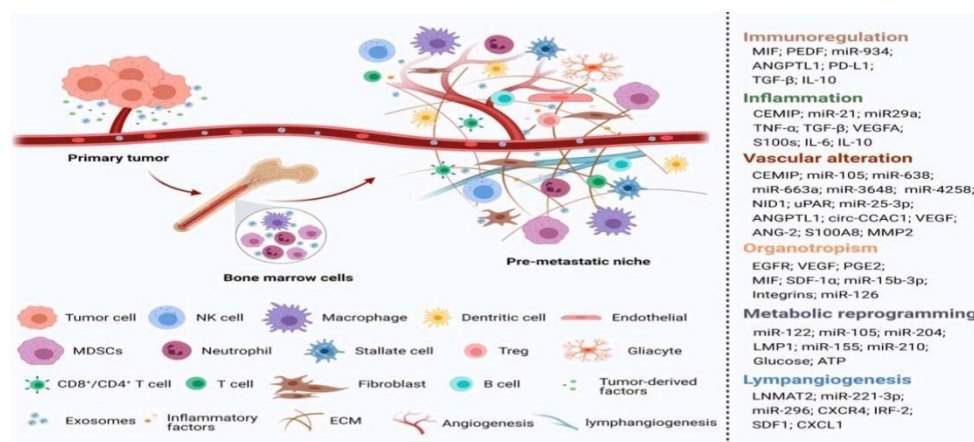


Figure2: The Illustration Highlights the Contribution of Tumor-Derived Exosomes to the Formation and Modulation of the Pre-Metastatic Niche. DOI: 10.3389/Fmolb.2021.703640

IV. EXOSOMAL BIOMARKERS AND LIQUID BIOPSY APPLICATIONS

Liquid biopsy has become an important transformative, cost effective tool for cancer detection, prognosis and treatment monitoring. Among many tools like circulating biomarkers, these exosomes provides several advantages due to their high stability, widespread abundance and tumor-specific cargo. Exosomes can be isolated from from various biofluids like blood, urine, saliva, breast milk, ascetic fluid, amniotic fluid, bile, cerebrospinal fluid and other biofluids, allowing repeated sampling in the progression of clinical translation [6, 10, 24].

Exosomes carry both messenger RNA and microRNA that can be transferred to recipient cells, where they remain biologically active; this transported RNA is therefore referred to as “exosomal shuttle RNA” (esRNA) [3]. Exosomal microRNAs (miRNAs) have emerged as promising biomarkers due to their stability, specificity, and presence in various body fluids. These miRNAs are selectively packaged into exosomes and reflect the physiological or pathological state of their parent cells. In cancer, exosomal miRNA profiles differ significantly between healthy and diseased conditions, enabling their use in early diagnosis and prognosis. They play a critical role in regulating tumor cell survival, apoptosis resistance, and therapeutic response. Because exosomes protect miRNAs from enzymatic degradation,

they remain highly stable in circulation. Altered expression of specific exosomal miRNAs correlates with tumor progression, metastasis, and drug resistance. Their non-invasive detection from blood, urine, or saliva makes them attractive clinical biomarkers. Exosomal miRNAs also facilitate intercellular communication within the tumor microenvironment. Monitoring these miRNAs can help predict treatment outcomes and disease recurrence. Overall, exosomal miRNAs represent powerful tools for precision diagnostics and personalized cancer therapy [26].

V. EXOSOMES AND THERAPY RESISTANCE

Cancer treatment strategies depend on tumor type, stage, and metastatic status and commonly involve surgery, chemotherapy, immunotherapy, targeted therapy, and radiotherapy, often in combination. A major limitation of these approaches is primary or acquired therapy resistance, which accounts for most cancer-related deaths. Increasing evidence shows that **exosomes**, as key mediators of tumor–microenvironment communication, play a central role in the development and horizontal transfer of resistance. In chemotherapy, exosomes promote resistance by exporting drugs, transferring drug-efflux transporters, pro-survival proteins, and resistance-associated miRNAs, thereby reducing intracellular drug levels and inhibiting apoptosis. In immunotherapy and targeted therapy, exosomes can suppress immune responses, act as decoys for monoclonal antibodies and immune checkpoint inhibitors, and transmit resistance via oncogenic proteins and non-coding RNAs. Radiotherapy similarly enhances exosome release, with exosomal cargo promoting DNA repair, cell survival, stemness, and radioresistance, while also contributing to radiation-induced bystander effects and tumor spread. Conversely, mesenchymal stem cell–derived exosomes may enhance therapy sensitivity and support tissue repair after radiation damage. Overall, exosomes emerge as critical regulators of therapy resistance and potential biomarkers and therapeutic targets across cancer treatment modalities [10].

VI. EXOSOMES AS THERAPEUTIC AND DRUG DELIVERY PLATFORMS

Juntang Shao *et al.* in 2020 in International Journal of Nanomedicine has revealed that exosomes are emerging as effective therapeutic and drug delivery platforms due to their natural ability to transport bioactive cargo across cellular and biological barriers. Their antitumor efficacy depends on efficient intracellular delivery, which can be enhanced through strategies such as surface modification with cell-penetrating peptides, promotion of membrane fusion, and facilitation of endosomal escape. Exosomes can enter cells via endocytosis, pinocytosis, or direct membrane fusion, enabling cytoplasmic delivery of therapeutic payloads. Importantly, exosomes can cross restrictive barriers like the blood–brain barrier and the gastrointestinal tract, making them promising carriers for CNS and oral drug delivery. These properties position exosomes as versatile, targeted, and biocompatible nanocarriers for advanced therapeutic applications [7].

Wandrey, M *et al.* in the journal Life in 2023 discloses that exosomes are emerging as powerful drug carriers and therapeutic agents due to their natural nanoparticle-like properties, high biocompatibility, and low side effects. Unlike synthetic nanoparticles, exosomes avoid issues such as protein corona formation and enable efficient delivery of chemotherapeutics, siRNAs, and miRNAs, even in drug-resistant cancers. Their surfaces can be chemically or genetically engineered with peptides, affibodies, or receptor ligands to achieve highly targeted delivery to specific tumor cells. Beyond cargo delivery, exosomes themselves can act as therapeutic agents by inducing immunogenic cell death and activating anti-tumor

immune responses, particularly through dendritic cell–derived exosomes. Although highly promising, most exosome-based strategies remain at the preclinical stage, requiring further development for clinical translation [10].

VII. CHALLENGES, LIMITATIONS AND REGULATORY CONSIDERATIONS

In the article ‘A comprehensive review of challenges and advances in exosome-based drug delivery systems’ in 2024, Sushesh Srivatsa Palakurthi *et al.* has recorded that developing exosome-based drug delivery systems presents several technical and logistical challenges, including poor drug loading, reduced in vivo stability, and rapid clearance from blood circulation. Key limitations involve the lack of robust, economical, and scalable production methods, as well as the difficulty in generating well-characterized, homogeneous exosome batches. Furthermore, common isolation techniques like ultracentrifugation often result in low yields and high impurity levels, which hinder clinical translation. While storage at -80°C is often required for stability, such conditions are impractical for many pharmaceutical products, and comprehensive shelf-life data remains scarce. Regulatory considerations are also paramount, as factors like isolation source, extraction validation, and potential immunogenicity—especially for non-human sources like bovine milk—must be rigorously evaluated. Long-term toxicity assessments are indispensable to ensure safety and clinical efficacy for these complex biological vesicles. Hybrid technology and synthetic mimetics are being explored to overcome these hurdles, yet sufficient evidence is still needed to validate their performance in human subjects [27].

VIII. FUTURE PERSPECTIVES OF EXOSOMES IN CANCER THERAPY

Exosomes are expected to play a major role as **next-generation biomarkers** for early cancer detection, prognosis, and treatment monitoring. Their presence in body fluids makes them ideal candidates for non-invasive liquid biopsies, enabling real-time tracking of tumor progression and therapeutic response. Advances in high-throughput profiling of exosomal RNA, proteins, and lipids are likely to improve diagnostic accuracy and patient stratification. Additionally, standardized isolation and characterization techniques will enhance reproducibility and clinical reliability. These developments could allow personalized treatment decisions based on tumor-derived exosomal signatures [8].

From a therapeutic perspective, exosomes hold strong promise as **targeted drug delivery systems and immunomodulatory agents**. Engineered exosomes may be used to deliver chemotherapeutic drugs, siRNA, miRNA, or immune-activating molecules directly to tumor cells, minimizing off-target toxicity. The document also highlights their potential role in cancer vaccines and immunotherapy by modulating immune responses within the tumor microenvironment. However, future success depends on overcoming challenges related to large-scale production, cargo loading efficiency, and regulatory approval. With continued research, exosome-based therapies could complement or even replace conventional cancer treatments [8]. Ultimately, Exosomes can be engineered to transport a wide range of therapeutic cargos, such as short interfering RNAs, antisense oligonucleotides, chemotherapeutic drugs, and immune modulators, while enabling targeted delivery to specific cells or tissues [11].

IX. CONCLUSION

Exosomes have emerged as pivotal mediators of intercellular communication in cancer, profoundly influencing tumor initiation, progression, metastasis, immune modulation, and therapy resistance [14, 16]. Their unique biogenesis, molecular composition, and selective cargo packaging enable them to mirror the biological state of parent tumor cells, positioning them as valuable diagnostic and prognostic tools [2, 5]. The role of exosomes in shaping the tumor microenvironment, particularly through angiogenesis, immune evasion, and stromal reprogramming, highlights their functional significance in cancer biology [15, 22]. Advances in liquid biopsy technologies have further strengthened the clinical relevance of exosomal biomarkers, especially miRNAs, for non-invasive cancer detection and treatment monitoring [10, 24]. Moreover, exosomes offer promising opportunities as natural, biocompatible drug delivery systems capable of targeted and efficient therapeutic transport [7, 10]. However, challenges related to large-scale production, isolation standardization, cargo loading efficiency, stability, and regulatory approval remain significant barriers to clinical translation [27]. Addressing these limitations through technological innovation and regulatory harmonization is essential. Overall, continued interdisciplinary research is expected to accelerate the successful integration of exosome-based diagnostics and therapeutics into precision oncology [8].

REFERENCES

- [1] Raposo, G., & Stoorvogel, W. (2013). "Extracellular vesicles: Exosomes, microvesicles, and friends". *Journal of Cell Biology*, 200(4), 373–383. doi: 10.1083/jcb.201211138. PMID: 23420871; PMCID: PMC3575529.
- [2] Kalluri R, LeBleu VS. "The biology, function, and biomedical applications of exosomes". *Science*. 2020 Feb 7;367(6478):eaau6977. doi: 10.1126/science.aau6977. PMID: 32029601; PMCID: PMC7717626.
- [3] Valadi, H., et al. (2007). "Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells". *Nature Cell Biology*, 9(6), 654–659. doi: 10.1038/ncb1596. Epub 2007 May 7. PMID: 17486113. [Abstract]
- [4] Peinado, H., et al. (2012). "Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype". *Nature Medicine*, 18(6), 883–891. doi: 10.1038/nm.2753. Erratum in: *Nat Med*. 2016 Dec 6;22(12):1502. doi: 10.1038/nm1216-1502b. PMID: 22635005; PMCID: PMC3645291.
- [5] Becker, A., et al. (2016). "Extracellular vesicles in cancer: Cell-to-cell mediators of metastasis". *Cancer Cell*, 30(6), 836–848.
- [6] Li, W., Li, C., Zhou, T., Liu, X., Liu, X., Li, X., & Chen, D. (2017). "Role of exosomal proteins in cancer diagnosis". *Molecular Cancer*, 16, 145. doi: 10.1186/s12943-017-0706-8. PMID: 28851367; PMCID: PMC5576100.
- [7] Shao, J., Zaro, J., & Shen, Y. (2020). "Advances in exosome-based drug delivery and tumor targeting. From Tissue Distribution to Intracellular Fate". *International Journal of Nanomedicine*, 2020 Nov 24;15, 9355–9371. doi: 10.2147/IJN.S281890. PMID: 33262592; PMCID: PMC7700079.
- [8] Nam, G.-H., Choi, Y., Kim, G. B., Kim, S., Kim, S. A., & Kim, I.-S. (2020). "Emerging prospects of exosomes for cancer treatment: From conventional therapy to immunotherapy". *Advanced Materials*, 2020, 32, 2002440. <https://doi.org/10.1002/adma.202002440>
- [9] Yang, X., Zhang, Y., Zhang, Y., Zhang, S., Qiu, L., Zhuang, Z., Wei, M., Deng, X., Wang, Z., & Han, J. (2021). "The key role of exosomes on the pre-metastatic niche formation in tumors". *Frontiers in Molecular Biosciences*, 8, 703640. doi: 10.3389/fmolb.2021.703640. PMID: 34595207; PMCID: PMC8476876.
- [10] Wandrey, M., Jablonska, J., Stauber, R. H., & Gül, D. (2023). "Exosomes in cancer progression and therapy resistance: Molecular insights and therapeutic opportunities". *Life*, 13, 2033.
- [11] Kalluri, R., & LeBleu, V. S. (2020). "The biology, function, and biomedical applications of exosomes". *Science*, 367(6478), eaau6977. doi: 10.1126/science.aau6977. PMID: 32029601; PMCID: PMC7717626.
- [12] Whiteside, T. L. (2016). "Tumor-derived exosomes and their role in cancer progression". *Advances in Clinical Chemistry*, 74, 103–141. doi: 10.1016/bs.acc.2015.12.005. Epub 2016 Apr 7. PMID: 27117662; PMCID: PMC5382933.

- [13] Ma X, Yu H. "Global burden of cancer". *Yale J Biol Med.* 2006 Dec;79(3-4):85-94. PMID: 17940618; PMCID: PMC1994799.
- [14] Zafar A, Khatoon S, Khan MJ, Abu J, Naeem A. "Advancements and limitations in traditional anti-cancer therapies: a comprehensive review of surgery, chemotherapy, radiation therapy, and hormonal therapy". *Discov Oncol.* 2025 Apr 24;16(1):607. doi: 10.1007/s12672-025-02198-8. PMID: 40272602; PMCID: PMC12021777.
- [15] Da Costa VR, Araldi RP, Vigerelli H, D'Ámelio F, Mendes TB, Gonzaga V, Policíquo B, Colozza-Gama GA, Valverde CW, Kerkis I. "Exosomes in the Tumor Microenvironment: From Biology to Clinical Applications". *Cells.* 2021 Oct 1;10(10):2617. doi: 10.3390/cells10102617. PMID: 34685596; PMCID: PMC8533895.
- [16] Javdani -Mallak A, Mowla SJ, Alibolandi M. "Tumor-derived exosomes and their application in cancer treatment". *J Transl Med.* 2025 Jul 8;23(1):751. doi: 10.1186/s12967-025-06814-7. PMID: 40629355; PMCID: PMC12236016.
- [17] Zhou, Y., Zhang, Y., Gong, H., Luo, S., & Cui, Y. (2021). "The Role of Exosomes and Their Applications in Cancer". *International journal of Molecular Science*, (22), 12204. <https://doi.org/10.3390/ijms222212204>
- [18] Wei H, Chen Q, Lin L, Sha C, Li T, Liu Y, Yin X, Xu Y, Chen L, Gao W, Li Y, Zhu X. "Regulation of exosome production and cargo sorting". *Int J Biol Sci.* 2021 Jan 1;17(1):163-177. doi: 10.7150/ijbs.53671. PMID: 33390841; PMCID: PMC7757038.
- [19] Krylova, S. V., & Feng, D. (2023). "The Machinery of Exosomes: Biogenesis, Release, and Uptake. *International Journal of Molecular Sciences*", 24 (2), 1337. <https://doi.org/10.3390/ijms24021337>
- [20] Zhao, G., Zhou, A., Li, X., Zhu, S., Wang, Y., Zhang, S., & Li, P. (2021). "The Significance of Exosomal RNAs in the Development, Diagnosis, and Treatment of Gastric Cancer". *Genes* 12 (1), 73. <https://doi.org/10.3390/genes12010073>
- [21] Fatima S. "Tumor Microenvironment: A Complex Landscape of Cancer Development and Drug Resistance". *Cureus.* 2025 Apr 11;17(4):e82090. doi: 10.7759/cureus.82090. PMID: 40351953; PMCID: PMC12066109.
- [22] Maheshwari S, Singh AK, Arya RK, Pandey D, Singh A, Datta D. "Exosomes: Emerging Players of Intercellular Communication in Tumor Microenvironment". *Discoveries (Craiova).* 2014 Sep 25;2(3):e26. doi: 10.15190/d.2014.18. PMID: 32309554; PMCID: PMC6941565.
- [23] Truong, N. C., Huynh, T. N., Pham, K. D., & Pham, P. V. (2020). "The role of tumor-derived exosomes in tumor immune escape: A concise review". *Biomedical Research and Therapy*, 7(11), 4132-4137. <https://doi.org/10.15419/bmrat.v7i11.650>
- [24] Abreu RDS, Ferreira DDP, de Araujo NS, Horita S, Tilli TM, Degraive W, Moreira ADS, Waghbi MC. "Liquid biopsy in cancer diagnosis and prognosis: a paradigm shift in precision oncology". *Front Mol Biosci.* 2026 Jan 12; 12:1708518. doi: 10.3389/fmolb.2025.1708518. PMID: 41602544; PMCID: PMC12832364.
- [25] Zhu M, Gao Y, Zhu K, Yuan Y, Bai H, Meng L. "Exosomal miRNA as biomarker in cancer diagnosis and prognosis: A review". *Medicine (Baltimore).* 2024 Oct 18;103(42):e40082. doi: 10.1097/MD.00000000000040082. PMID: 39432619; PMCID: PMC11495718.
- [26] Mohammad Salehi, Mohammad Javad Kamali, Daniyal Arab, Naghme Safaeian, Zahra Ashuori, Moein Maddahi, Narges Latifi, Amir Moein Jahromi, "Exosomal microRNAs in regulation of tumor cells resistance to apoptosis, *Biochemistry and Biophysics Reports*", Volume 37, 2024, 101644, ISSN 2405-5808, <https://doi.org/10.1016/j.bbrep.2024.101644>.
- [27] Sushesh Srivatsa Palakurthi , Brijesh Shah , Sumedha Kapre , Nitin Charbe , Susan Immanuel , Sindhura Pasham , Maharshi Thalla , Ankit Jain and Srinath Palakurthi "A comprehensive review of challenges and advances in exosome-based drug delivery systems" *Nanoscale Adv.*, 2024, **6**, 5803-5826 DOI: 10.1039/D4NA00501E

CHARACTERIZATION OF AGAVE AMERICANA L. BIOLEATHER

Abstract

Leather, a versatile material plays a significant role in various industries, including fashion, automotive and furniture. It is material made from the skin of an animal to make certain fashionable things like jackets, coats, boots and other accessories. The alternative source of leather is called as Bio-leather, which is also known as lab-grown leather or cultured leather. It is more environmentally friendly and cruelty-free option for leather product. *Agave americana* plant which contains more starch and fibre content is used to prepare leather material which is used as alternative for animal leather.. SEM analysis were done to study and understand the morphological properties of bio leather. Physical characteristic test such as colour, thickness, texture, Tensile strength were also studied. The antimicrobial effect was tested on two gram positive bacteria and two gram negative bacteria using zone of inhibition on solid media. These test prove that it can be used as a good alternative source for animal leather

Keywords: bioleather, agave, SEM analysis, fibre, gram positive bacteria.

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I. INTRODUCTION

Leather making is an ancient art that has been practiced for more than 7,000 years. Fresh skins were dried in the sun, softened by pounding in animal fats and brains, and preserved by salting and smoking. Beginning with simple drying and curing techniques, the process of vegetable tanning was developed by the Egyptians and Hebrews about 400 BCE. During the Mid Ages the Arabs preserved the art of leather making and so improved it that morocco and cordovan (from Córdoba, Spain) became highly prized leathers.

Bio-leather is a sustainable biomaterial derived from natural sources such as plants and microorganisms, making it environmentally friendly and biodegradable, thus helping reduce textile waste [1] According to the previous study, the general sources for bio-leather include natural latex, pineapple, mushroom, jellyfish, and bacterial cellulose (BC) [2] This study is focused on BC to develop the bio-leather. In this study, to overcome these aforementioned drawbacks, bio-leather is modified by physical entrapment of proteins, which are naturally derived materials, and are non-toxic and biodegradable [3] Proteins have shown to improve the mechanical properties of the cellulose composite material owing to their structural properties and high molecular binding potential [4]. The key characteristics and features of bio-leather include:

The synthesis of bio-leather from the plant *Agave americana*, is the weed which plays a role in its native ecosystems by providing food and habitat for pollinators, birds, and other wildlife. The plant's deep roots help prevent soil erosion, and its ability to survive in harsh conditions contributes to ecosystem resilience. *Agave americana*, commonly known as the American agave or century plant, is a large and striking succulent native to Mexico but now cultivated worldwide for ornamental, agricultural, and commercial purposes. It is a rosette-forming plant with thick, succulent leaves that are grayish-green to blue-green in colour. The leaves are rigid, fleshy, and have sharp spines along the margins and at the tip.[5] Mature plants can reach impressive sizes, with leaves growing up to 6 to 8 feet (1.8 to 2.4 meters) long and the whole plant spanning several feet in diameter. Despite its common name "century plant," *Agave americana* typically lives for 10 to 30 years before flowering. The plant usually blooms after 10 to 30 years, producing a tall flower spike that can reach up to 30 feet (9 meters) in height. It is native to Mexico and parts of Central America. It has been introduced to other regions with similar climates and is cultivated as an ornamental plant in gardens, landscapes, and botanical collections. The leaves of *Agave americana* contain strong fibres that have been traditionally used by indigenous peoples for making ropes, mats, baskets, and textiles. The fibres are extracted through a process called decortication. [6]

The objective of the experiment on bio leather is to develop a sustainable and cruelty-free alternative to traditional leather by utilizing innovative bio-based materials and production methods. This includes investigating different sources such as plant fibres, bio-based polymers and optimizing processes for material extraction, processing, and finishing. The goal is to achieve bio leather with properties comparable to conventional leather in terms of durability, flexibility, water resistance, and aesthetic appeal. Through this experiment, we aim to contribute to reducing environmental impact, promoting ethical practices, and meeting the growing demand for sustainable materials in various industries.

II. MATERIALS AND METHOD

1. Collection of *Agave Americana*

Agave americana is commonly known as century plant or American aloe. In Tamil Nadu these plant is called as Aanai kattrazhai leaves were collected freshly from the forest area present in Mel Kaalvai, near Thiruporur, Chengalpattu district.

2. Extraction of Starch and Fibre from the Agave Plant

- **Cleaning and washing:** The collected leaves consist of poisonous thorn at the edges of the leaves, remove the thorns with the help of the knife. Then wash the leaves clearly to remove sand particles from the leaves.
- **Peeling:** After washing the leaves, slightly remove the upper layer of the leaves with the help of the knife. Then wash it again with the tap water. The starch was collected during peeling the leaves.
- **Extraction of fibre:** After removing all starch from the leaves the fibres can be clearly appear at the base of the leaves. By squeezing and twisting the base layer of the leaves the fibres can be easily extracted out from the unwanted layer of leaf.
- **Drying:** The extracted fibres are dried in the sunlight for about two days which is about 32°C. It was done to remove all the moisture content and unwanted debris from the fibre.
- **Preparation of kombucha scoby:**
Materials required for scoby:
 - Apple cider vinegar
 - Hot water
 - Black tea bags
 - Sugar
 - Starter scoby
 - Bottle jar
 - Muslin cloth
 - Thread
 - Thermometer

III. SYNTHESIS OF BIO-LEATHER

The ingredients agave fibre and starch, PCL and distilled water at various concentrations were mixed in a blender. Blend it into fine paste and filter it in the muslin cloth to remove excess starch from the fibre. Then spread the filtrate as sheet on the muslin cloth till the excess starch is filtered out from the fibre. Transfer it to the casting plate and allow them to dry for 3 to 4 hours in direct sunlight.

Mix another batch of ingredients such as kombucha scoby and PCL at different concentrations in the blender. Blend all the ingredients into fine liquid and pour it on the casted fibre material and allow them to dry in 30°C.

Fabrication:-

After drying of leather sheets cut them into different shapes as per our wish. Three different samples are made at different concentration of ingredients. Then coat them with the glycerol at different concentration to avoid quick water absorption.

IV. PHYSICAL CHARACTERISTICS TEST

1. Colour

The colour was determined by the visual assessment of produced bio-leather.

2. Thickness of bio-leather

The thickness of the bio-leather can be measured by using screw gauge, and the average value is calculated. The usual thickness for car or furniture leather is about 0.9 to 1.2 millimetres in thickness. The thickness of the leather can be depends on the uses, like leather used in shoes has the different measurement of thickness, whereas the leather used in the production of jacket has less thickness comparing to the shoe leather.

3. Tensile strength

This test method is intended to determine the tensile properties of leather. The tensile strength test is conducted to measure the maximum stress a material can withstand before breaking under tension. First, prepare samples according to standard dimensions. Secure the sample in the testing machine grips, ensuring alignment. Apply a gradual and uniform force until the sample fractures. Record the maximum force and calculate tensile strength using the formula:

$$\text{TENSILE STRENGTH (TS)} = F/S$$

TS= tensile strength

F= the maximum force or load applied to the material during the test

S= the cross-sectional area of the material.

Maintain a controlled environment throughout the procedure to ensure consistent results.

V. RESULT AND DISCUSSION

In this present study, Bio-leather is prepared by using fibre and starch of *Agave americana* plant. Three different concentration of ingredients (fibre, PCL, starch, KBC, glycerol) were used and three different bio- leather were prepared Bio-leather which is about 3.34 mm and 3.82 thickness sample were used for studying various parameters such as SEM analysis and tensile strength

SEM analysis exhibited the surface of the bio-leather produced in in this experiment. It was found to be smooth and contains little cracks which found in some places. These cracks can be occurs due to the incomplete mixing of the raw materials. The image is also shows the good adhesion between the various components such as starch, KBC scoby, PCL and glycerol of the bio-leather.

Tensile strength:Tensile strength is the amount of maximum strength to break the plastic film. Tensile strength modulus is defined as the stress change divided by change in strain within the linear viscoelastic region of the stress/strain curves. Elongation at break is the indication

of the amount of the variation of extreme film length while attaining tensile strength until the film breaks, related to the original length.

Colour and texture: The colour and texture of the Bio-leather can be observed through the naked eye and the texture of the bio-leather can be determined by touching the sample.

Table 1: Samples Made Up of Different Concentration

SAMPLES	FIBRE(g)	STARCH(ml)	PCL(ml)	KBC(g)	GLYCEROL(ml)
Sample-1	12	50	10	80	2
Sample-2	13	70	15	100	3
Sample-3	15	100	20	130	5

Table 2: Different Trails Made out from Different Concentration

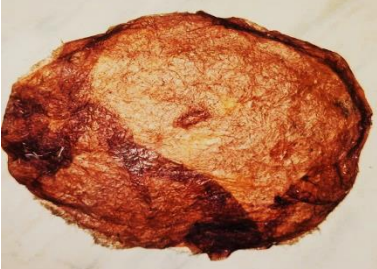
Trail	Images	Composition
T-1		Fibre – 12 g, Starch - 50 ml, KBC – 80 g, PCL – 10 ml, Glycerol - 2ml
T-2		Fibre - 13 g, Starch – 70 ml, KBC – 100 g, PCL – 15 ml, Glycerol – 3 ml
T-3		Fibre – 15 g, Starch – 100 ml, KBC – 130 ml, PCL- 20 ml, Glycerol – 5 ml

Table 3: Colour and Texture of the Three Different Samples of Three Different Measurements of Components

Colour	Texture
Black to dark brown	Hard

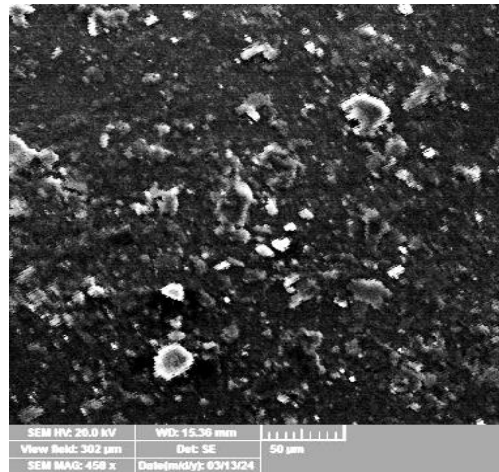


Figure1: Plate 1- Indicates the Presence of Starch Granules which is Ovoid and Less Lighter in Colour

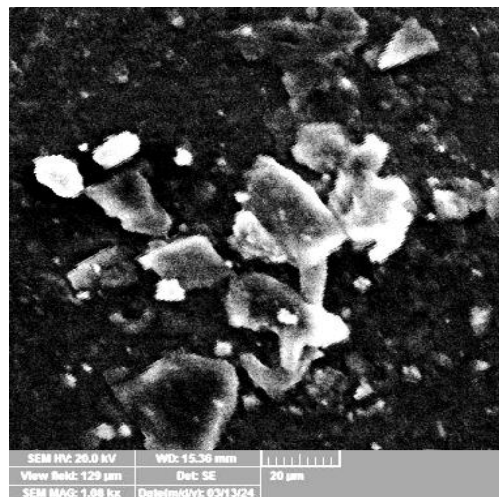


Figure 2: Plate 2-Tensile Strength of 3.82 Mm Thickness and 22.41 Mm Width Bi0-Leather



Figure 3:

VI. CONCLUSION

In this study, we can determine that the bio-leather made out from the starch and fibre of the plant *Agave americana* can efficiently useful in the alternative source of animal leather. It has

been successfully fabricated and characterized by various analysis such as physical and morphological characterization. The morphological characters can be analysed by Scanning Electron Microscope (SEM). It indicates the presence of smooth surface with small brittle structure containing starch granules and KBC granules. Thus the bio-leather can be used as the alternative for animal leather in various fashionable industries such as shoes, belt, bags, seats and leather jackets textile industries. Bio-leather contains the less toxic and degradable materials it can be able to decay faster than the animal leather. It can't cause any pollution or hazardous to the living being as well as environment. It definitely be the eco- friendly product without harming or killing any living organisms.

REFERENCES

- [1] Britannica, The Editors of Encyclopaedia. "leather". Encyclopedia Britannica, 2 Feb. 2024, <https://www.britannica.com/topic/leather>. Accessed 20 March 2024.
- [2] Ward, A. G. (1974). The mechanical properties of leather. *Rheologicaacta*, 13(1), 103-112.
- [3] Bhattacharya, S. (2018). Transforming skin, changing caste: Technical education in leather production in India, 1900–1950. *The Indian Economic & Social History Review*, 55(3), 307-343.
- [4] Garai, J. (2014). Environmental aspects and health risks of leather tanning industry: a study in the Hazaribag area. *Chinese Journal of Population Resources and Environment*, 12(3), 278-282.
- [5] Meyer, M., Dietrich, S., Schulz, H., & Mondschein, A. (2021). Comparison of the technical performance of leather, artificial leather, and trendy alternatives. *Coatings*, 11(2), 226.
- [6] Kim, H., Song, J. E., & Kim, H. R. (2021). Comparative study on the physical entrapment of soy and mushroom proteins on the durability of bacterial cellulose bio-leather. *Cellulose*, 28(5), 3183-3200.
- [7] Black, S. Desserto®: cactus fibre as leather substitute. In *Accelerating Sustainability in Fashion, Clothing and Textiles* (pp. 143-151). Routledge.
- [8] Memon, H., Chaklie, E. B., Yesuf, H. M., & Zhu, C. (2021). Study on effect of leather rigidity and thickness on drapability of sheep garment leather. *Materials*, 14(16), 4553.
- [9] Robinson, C., Topp, N. E., & Beakbane, M. E. (1948). The penetration of leather by water and water vapour. *Discussions of the Faraday Society*, 3, 273-282.
- [10] Kwon, J. W., Choo, Y. Y., Choi, H. H., Cho, J. M., & Ki, G. S. (2004, November). Development of leather quality discrimination system by texture analysis. In *2004 IEEE Region 10 Conference TENCON 2004*. (pp. 327-330). IEEE.
- [11] Thanikaivelan, P., Shelly, D. C., & Ramkumar, S. S. (2006). Gauge length effect on the tensile properties of leather. *Journal of applied polymer science*, 101(2), 1202-1209.

REGULATORY FRAMEWORKS, BIOSAFETY, AND PATENT PROTECTION

Abstract

The rapid advancement of biosciences and biotechnology has significantly transformed agriculture, healthcare, industry, and environmental management. Alongside these developments, concerns related to biosafety, ethical research practices, environmental protection, and intellectual property rights have gained importance. Regulatory frameworks ensure that biotechnological research and applications act in accordance with legal, ethical, and safety standards. Biosafety guidelines protect researchers, the public, and ecosystems from potential biological hazards, while patent protection encourages innovation by safeguarding intellectual property. This chapter provides a comprehensive overview of regulatory mechanisms, biosafety principles, and patent protection systems at national and international levels, with special reference to bioscience and biotechnology applications.

Keywords: Regulatory frameworks; Biosafety; Biotechnology regulations; Biosafety levels; Patent protection; Intellectual property rights; TRIPS; Genetic engineering

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I. INTRODUCTION

The development of modern biosciences greatly transformed the fields of agriculture, medicine and industrial biotechnology. Advances in genetic engineering have enabled precise modification of biological systems for targeted trait improvement in plants and microorganisms [1, 2]. Genome editing technologies, particularly CRISPR–Cas systems, have challenged traditional biological constraints by allowing programmable, site-specific alterations in genomes [3, 4].

Synthetic biology has expanded these capabilities by integrating engineering principles to redesign biological pathways and networks [5, 2]. These advances have led to the development of high-yielding genetically engineered crops with improved productivity, stress tolerance, and nutritional quality [6, 7]. Genetically modified (GM) biological systems are widely used for the production of industrial enzymes, improving efficiency and sustainability in sectors such as biofuels, food processing, and pharmaceuticals [8, 9]. In agriculture, GM and gene-edited crops have enhanced productivity, pest resistance, stress tolerance and nutritional characteristics of food plants for improved global food security to meet climate change [6]. Within medicine, recombinant vaccines, monoclonal antibodies and gene-based therapies have achieved considerable success [10, 11]. In the same way, synthetic biology has extended industrial biotechnology by providing a sustainable production of biofuels, bioplastics and pharmaceuticals through engineered microbe systems which could replace dependence on petrochemicals [2, 9].

However, despite these significant achievements, critical concerns persist regarding biosafety, biosecurity, ethical responsibility, and environmental security on one hand, and intellectual property rights (IPR) on the other. Potential risks such as the accidental release of genetically engineered organisms, unintended ecological effects including gene flow to wild relatives, and biosecurity threats arising from the misuse of advanced biotechnologies require careful evaluation and regulatory oversight [12, 13]. In addition, ethical controversies regarding the property in life forms as well as to access patented biotechnological products and protection of indigenous and traditional knowledge persist in influencing public debate and policy regimes [14, 15].

To tackle these challenges, regulations have been put in place to make sure biotechnology research gets done in a safe controlled environment. National and international governments have established protocols to ensure biotech companies comply with strict standards for safety, impact on the environment and the ethical implications. This includes things like strict lab guidelines that limit the risk of accidents to the people working in the lab, to the public and the wider ecosystem [16]. Patent laws aim to reward innovation while protecting public interest. They ensure access to essential health technologies, especially for economically disadvantaged populations [17, 18]. Together, these systems support the advancement of biotechnology in a responsible and equitable manner, while minimizing potential harm to the environment in an era of rapid technological change [19, 20].

II. REGULATORY FRAMEWORKS IN BIOSCIENCES

1. Concept and Objectives of Regulatory Frameworks

Regulatory frameworks are the set of rules and guidelines that govern bioscience research, development, and the commercialisation of this research. These frameworks aim to make sure

that all the advances in biotechnology are made with human health, the environment, and ethical standards all being kept top priority still allowing scientific progress. Regulatory systems aim to minimize potential hazards associated with genetically modified organisms (GMOs) by establishing clear procedures for risk assessments, risk management, monitoring and regulatory approvals[7, 21].

At the international level, regulation is maintained through multilateral agreements. Organisations that promote biosafety ensure responsible use of biotechnology. The Cartagena Protocol on Biosafety, which came into effect in 2000 under the convention on Biological Diversity, plays a key role in regulating the transboundary movement of GMOs. The Cartagena Protocol places the Precautionary Principle at the core of its regulatory approach, mandating advance risk information for importing countries prior to environmental introduction of genetically modified organisms [20, 22].

Recent scientific and policy debates have highlighted the regulatory challenges posed by new breeding techniques (NBTs). These challenges are particularly significant for gene-edited organisms developed using CRISPR-Cas technologies. Because these organisms often lack foreign DNA and closely resemble natural mutations, determining whether they should be regulated as strictly as conventional GMOs remains a significant regulatory challenge. This development highlights the need for adaptable regulatory approaches that can address emerging technologies while maintaining biosafety standards and sustaining public trust [12, 23].

2. Regulatory Framework in India (overview)

In the Indian context, biosafety governance is guided by clear legal provisions established under the Environment (Protection) Act, 1986. The regulatory framework for ensuring compliance in handling hazardous microorganisms and genetically engineered organisms is provided by the Rules for the Manufacture, Use, Import, Export, and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells, 1989, commonly referred to as the 'Rules, 1989' [24]. Unlike certain countries where a single agency oversees all regulatory aspects, India adopts a multi-tiered approach to biosafety regulation. This system involves multiple agencies collaborating to ensure a comprehensive biosafety evaluation, spanning from the initial stages of research to the eventual release of a product into the environment, which is considered highly effective, as noted by Ahuja (2018) [25].

This hierarchical system involves oversight at institutional, departmental, and national ministerial levels, ensuring a rigorous "check and balance" mechanism. Recently, the regulatory landscape has evolved in response to global scientific advancements. The Department of Biotechnology (DBT) has introduced significant policy reforms, notably the Guidelines for Safety Assessment of Genome-Edited Plants, 2022, to streamline the approval process for genome-edited crops, particularly those in the Site-Directed Nuclease-1 and -2 categories [26]. These reforms aim to exempt certain low-risk gene-edited plants from the stringent GMO regulations, thereby aligning India with global trends that distinguish between transgenic organisms and precision-bred crops, while maintaining robust monitoring mechanisms for higher-risk applications [26, 27]. The interplay between international biosafety commitments, such as the Cartagena Protocol on Biosafety, and national regulatory frameworks is essential for achieving harmonized governance. Figure 1 illustrates this integrated global-domestic framework, showing the functional relationships between international treaties and the Indian regulatory hierarchy.

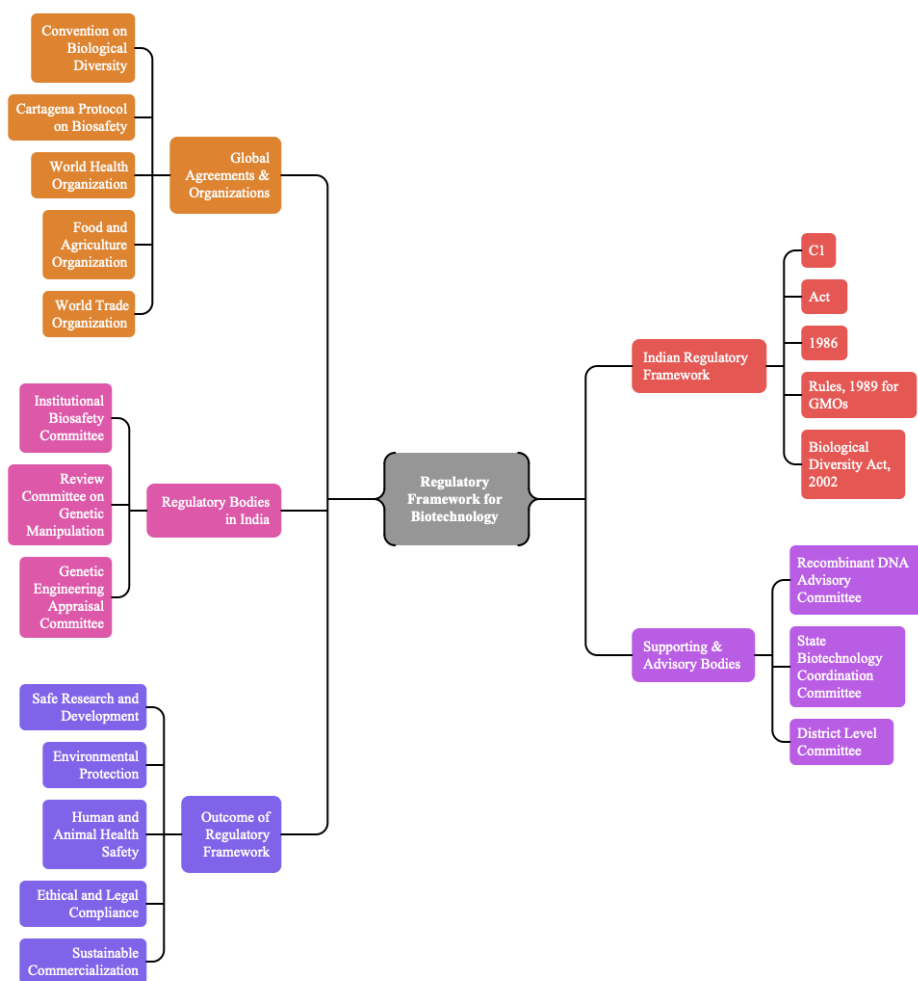


Figure 1: Schematic representation of the international and Indian regulatory framework for biotechnology, highlighting the interface between global biosafety instruments—namely the Convention on Biological Diversity (CBD) and the Cartagena Protocol on Biosafety—and India’s multi-tiered regulatory system overseeing genetically modified organisms and biotechnology research (DBT, 2022; CBD, 2023).

- **Institutional Structure and Functional Roles:** India’s regulatory network operates through a structured system of statutory committees, each with clearly defined responsibilities. These bodies function under the administrative control of the Department of Biotechnology (DBT) and the Ministry of Environment, [28].
 - **Institutional Biosafety Committee (IBSC):** The IBSC is the key player at each research institute. IBSC is the point of contact for day-to-day monitoring of experiments, making sure all the safety guidelines are being stuck to. IBSC bridge the gap between researchers working on the ground and the national regulatory bodies that oversee it all.
 - **Review Committee on Genetic Manipulation (RCGM):** RCGM operating under the DBT, is responsible for overseeing the safety aspects of scientific

research. It authorizes pre-clinical studies and approves small-scale field trials, ensuring that research activities comply with established biosafety standards [29].

- **Genetic Engineering Appraisal Committee (GEAC):** Within the Ministry of Environment, Forest and Climate Change [28], the Genetic Engineering Appraisal Committee (GEAC) serves as the primary regulatory authority with the power to grant approval for the large-scale use of potentially hazardous microorganisms or genetically modified organisms. Critically, the GEAC also has the final authority to determine whether GMOs can be released into the environment or commercialized [28].
- **Recombinant DNA Advisory Committee (RDAC):** RDAC monitors developments in biotechnology at both the national and international levels, helping to identify and formulate new safety regulations as needed. A summary of these regulatory bodies and their respective roles within the system is provided in Table 1."

Table 1: Major Regulatory Bodies Involved in Biotechnology Governance in India

Regulatory Body	Acronym	Primary Administrative Authority	Key Functions and Mandate
Institutional Biosafety Committee	IBSC	Institution/University (Registered with DBT)	Institutional-level monitoring of recombinant DNA research; ensuring onsite biosafety compliance and emergency response planning.
Review Committee on Genetic Manipulation	RCGM	Department of Biotechnology (DBT)	Authorization of import/export of etiological agents; approval and monitoring of pre-clinical studies and small-scale confined field trials (biosafety research level I & II).
Genetic Engineering Appraisal Committee	GEAC	Ministry of Environment, Forest & Climate Change (MoEF&CC)	Apex body for authorization of large-scale field trials, environmental release, and commercial cultivation/marketing of GM products.
Recombinant DNA Advisory Committee	RDAC	Department of Biotechnology (DBT)	Macro-level policy advice; reviewing evolving biotechnology trends to recommend safety regulations and guideline updates.

Note: The table synthesizes the distinct functions of bodies operating within the multi-tier regulatory framework, encompassing oversight from institutional-level monitoring to national-level strategic authorization. Collectively, these institutions form a comprehensive safety mechanism that ensures biotechnological applications comply with environmental protection mandates (EPA, 1986) and established ethical standards.

III. BIOSAFETY

1. Concept of Biosafety

Biosafety involves all measures aimed at protecting human health and the environment from accidental exposure to biological agents [16]. As biologists advance the frontiers of molecular biology, genetic engineering, and synthetic biology, research increasingly involves working with complex and novel biological systems [30]. These may include viruses or genomes assembled from components that have not been combined before [31]. Such studies explore entirely new configurations of biological material, rising important considerations for biosafety and risk assessment [16]. These novel biological systems are often complex and can be difficult to fully predict. As a result, the risks they pose are frequently uncertain [30]. Consequently, biosafety has evolved from a set of fixed rules into a dynamic discipline that adapts to emerging developments and discoveries in biotechnology [16]. It remains a fundamental component of ensuring the safety of scientific research, while also protecting public health and the environment [31].

Advances in biological research have necessitated a re-evaluation of biosafety practices [30]. Biosafety is no longer limited to containment within laboratories; it now also encompasses biosecurity and careful oversight of research with dual-use potential—studies intended for beneficial purposes that could be misapplied for harm [31]. High-containment laboratories, such as BSL-3 and BSL-4 facilities, and Dual-Use Research of Concern (DURC) receive the greatest scrutiny. Contemporary approaches to biosafety emphasize ethical responsibility in addition to technical compliance. International organizations, including the World Health Organization (WHO) and the Organisation for Economic Co-operation and Development (OECD), advocate for fostering a culture of biosafety, which involves continuous risk assessment, thorough training of personnel, and institutional accountability [16]. Modern biosafety practices are thus not merely procedural checklists but integral to responsible scientific conduct.

2. Biosafety Levels: The Hierarchy of Containment

Biosafety Levels, or BSLs, range from BSL-1 up to BSL-4 and provide a global framework for managing laboratory risks [16]. These levels are not arbitrary; they are based on an assessment of the biological agent's pathogenicity, mode of transmission, availability of preventive measures such as vaccines, and the existence of effective treatments in case of infection. As the level increases from BSL-1 to BSL-4, containment requirements become more stringent, including enhanced personal protective equipment, specialized laboratory designs, and controlled work practices. Table 2 provides a detailed summary of these levels, illustrating the escalating safety measures associated with each:

- **Biosafety Level 1 (BSL-1):** BSL-1 serves as the foundational level for biological research. Laboratories at this level work with well-characterized agents that are not known to cause disease in healthy adults, such as standard laboratory strains of *Escherichia coli* [16]. Basic microbiological practices are sufficient, and no specialized equipment or containment barriers are required.
- **Biosafety Level 2 (BSL-2):** BSL-2 laboratories handle biological agents that pose moderate risk, such as *Staphylococcus aureus* or Hepatitis B virus [16]. Access to these labs is restricted to authorized personnel, and work that may generate aerosols

must be conducted within Class II Biological Safety Cabinets. The rationale is that these agents cause diseases that are generally preventable or treatable, and they do not spread easily through the airborne route.

- **Biosafety Level 3 (BSL-3):** BSL-3 laboratories handle high-risk biological agents, such as *Mycobacterium tuberculosis* or SARS-CoV-2, which can be transmitted through the airborne route and may cause severe or potentially lethal infections [33]. These laboratories require advanced engineering controls, including negative air pressure to direct airflow, HEPA filtration of exhaust air, and strict access restrictions to prevent the release of hazardous agents.
- **Biosafety Level 4 (BSL-4):** BSL-4 represents the highest level of biosafety containment. Laboratories at this level work with the most dangerous and exotic pathogens, such as Ebola or Marburg viruses, which are highly transmissible and often fatal, with no available treatment or vaccine [16, 34]. Researchers conduct work either within sealed Class III biosafety cabinets or while wearing positive-pressure, air-supplied suits. These laboratories are designed as fully contained facilities, physically isolated from other areas to prevent any accidental release of pathogens.

Table 2: Biosafety Levels (BSL-1 to BSL-4) and Laboratory Requirements

Biosafety level	Risk category	Type of biological agents	Laboratory features	Safety practices and equipment	Examples of agents
BSL-1	Minimal risk	Non-pathogenic, well-characterized microorganisms	Open bench laboratory; no special containment	Standard microbiological practices; hand washing; routine surface disinfection	Non-pathogenic <i>Escherichia coli</i>
BSL-2	Moderate risk	Agents causing mild to moderate human disease	Restricted access; biological safety cabinet (Class I or II)	Laboratory coat, gloves, face protection; proper waste decontamination	<i>Salmonella</i> spp., <i>Staphylococcus aureus</i>
BSL-3	High risk	Serious or potentially lethal agents transmitted by inhalation	Controlled access; negative air pressure; HEPA-filtered ventilation	Respiratory protection; strict containment and disposal procedures	<i>Mycobacterium tuberculosis</i> , SARS-CoV-2
BSL-4	Extreme risk	Life-threatening agents with no available treatment or vaccine	Isolated facility; maximum containment; dedicated air supply	Positive-pressure suits; full-body protection; rigorous entry and exit protocols	Ebola virus, Marburg virus

Note. Biosafety levels (BSL-1 to BSL-4) indicate increasing degrees of biological risk and corresponding containment requirements. Higher biosafety levels require enhanced laboratory design, stricter safety practices, and specialized equipment to protect personnel and the environment.

Classifying laboratories according to biosafety levels is not merely bureaucratic. It forms the foundation of safe scientific research [16]. system ensures that laboratory practices and containment measures match the risks posed by biological agents. Each experiment is aligned with appropriate safety protocols. This approach helps prevent laboratory-acquired infections. It also reduces the likelihood of accidental release of hazardous organisms into the environment [33].

3. The Strategic and Ethical Imperative of Biosafety

Biosafety is not merely a procedural requirement; it underpins the integrity and reliability of the global bioeconomy [30]. Its importance cannot be overstated. Effective biosafety measures protect laboratory workers, safeguard the environment, and help maintain public trust in scientific research [16].

Biosafety protocols are essential for protecting both people and the environment. They serve as the primary safeguard for laboratory personnel, particularly when handling infectious pathogens, toxins, or novel genetically modified materials. In high-containment laboratories, such as BSL-3 and BSL-4 facilities, these protocols provide the final barrier against potentially fatal laboratory-acquired infections [35].

But it's not just about people. Biosafety measures act like a firewall for the environment. They keep dangerous biological agents from slipping out and causing trouble. Without these safeguards, you risk things like engineered organisms edging out native species or genetic material leaking into wild populations, which can throw entire ecosystems off balance (FAO, 2022; Mueller, 2019).

Biosafety measures protect more than just human health; they also serve as a safeguard for the environment. These protocols prevent hazardous biological agents from escaping and causing ecological harm. Without such measures, genetically engineered organisms could displace native species, or novel genetic material could spread into wild populations, potentially disrupting entire ecosystems [7, 36].

4. Ethical Responsibility and Public Trust

Biosafety is not merely about containment in laboratories; it is closely linked to the ethical conduct of science. It embodies the principle of doing good while avoiding harm. In contemporary research, where scientific complexity is increasing and public debate is often polarized, adherence to robust biosafety standards helps maintain public trust. Demonstrating careful and thorough scientific practices increases confidence in innovations such as genetically modified crops or mRNA vaccines. Conversely, a single major lapse in biosafety can rapidly erode public trust and hinder scientific progress for years [12, 37]. This balance between safety and innovation is reflected in the structured biosafety levels, outlined in Table 2, which provide a clear scientific framework for safely managing increasingly complex technologies.

IV. REGULATORY APPROVAL PROCESS FOR GENETICALLY MODIFIED ORGANISMS (GMOS)

The development of genetically modified organisms (GMOs) follows a comprehensive process, beginning with computational design and sometimes ending with consumer products. This process is governed by stringent, multi-layered regulations. The overarching principle guiding these regulations is the Precautionary Principle, which aims to advance technology while ensuring safety. Regulatory authorities conduct thorough evaluations of each product, assessing biosafety, potential environmental impacts, and socio-economic consequences before market approval [9, 38].

1. Global and National Context

Globally, the regulation of genetically modified organisms (GMOs) begins with international agreements, particularly the Cartagena Protocol on Biosafety, adopted in 2000. This protocol requires countries to provide Advance Informed Agreement (AIA) and conduct comprehensive risk assessments before any Living Modified Organisms (LMOs) are transboundary shipped [20]. All signatory countries must comply with these provisions, they are also expected to adapt the rules to suit their specific agricultural and environmental contexts.

In India, the regulatory framework for genetically modified organisms is science-based and operates through a stepwise process. It is established under the Environment (Protection) Act, 1986, and the Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells, 1989. This system evaluates GMOs at each stage of development. Regulatory responsibilities are distributed among multiple authorities, each with clearly defined roles, while coordination is maintained where necessary [26, 28].

2. The Stepwise Approval Mechanism

- **Institutional Scrutiny (IBSC):** The regulatory process begins at the institutional level with the Institutional Biosafety Committee (IBSC). The IBSC conducts an initial review of the research proposal. It verifies laboratory safety certifications, including the appropriate biosafety level (BSL). The committee also assesses the qualifications and training of the research personnel. At this stage, the IBSC ensures that all preliminary experiments involving recombinant DNA comply with the 2017 Regulations and Guidelines for Recombinant DNA Research and Biocontainment [16, 39].
- **2. Scientific Risk Assessment (RCGM):** After a proposal is approved at the institutional level, it is forwarded to the Review Committee on Genetic Manipulation (RCGM) under the Department of Biotechnology. The RCGM functions as the primary scientific review body. It conducts a detailed evaluation of molecular characterization data, including transgene stability and levels of target protein expression. The committee also examines results from preliminary toxicity studies. Importantly, the RCGM determines whether a project may proceed to Biosafety Research Level-I (BRL-I) trials. These trials involve small-scale, strictly contained field evaluations designed to generate data on plant performance and environmental interactions, while preventing unintended release into the environment [26].

- **Environmental and Commercial Authorization (GEAC):** The final and most critical stage of the regulatory process is overseen by the Genetic Engineering Appraisal Committee (GEAC), which operates under the Ministry of Environment, Forest and Climate Change. The GEAC reviews comprehensive data generated from both Biosafety Research Level-I (BRL-I) and Biosafety Research Level-II (BRL-II) trials. Its assessment encompasses food safety parameters, including allergenicity and toxicity, as well as potential environmental impacts, such as effects on non-target organisms. The committee also considers public consultations and stakeholder feedback where applicable. The GEAC is the sole authority empowered to approve environmental release and commercial cultivation of genetically modified organisms [28].

3. Post-Release Monitoring

Regulatory oversight does not end with the commercial approval of a genetically modified organism. As illustrated in Figure 2, post-release monitoring remains a central component of GMO regulation. This process, often referred to as biovigilance, enables the identification of long-term or unforeseen effects, such as the development of pest resistance to genetically engineered crops (e.g., resistance of pink bollworm to Bt cotton). Post-approval monitoring also ensures continued compliance with stewardship and management requirements. Collectively, these measures contribute to a regulatory system that is predictable, transparent, and legally robust, thereby facilitating sustained progress in biotechnology [7, 41].

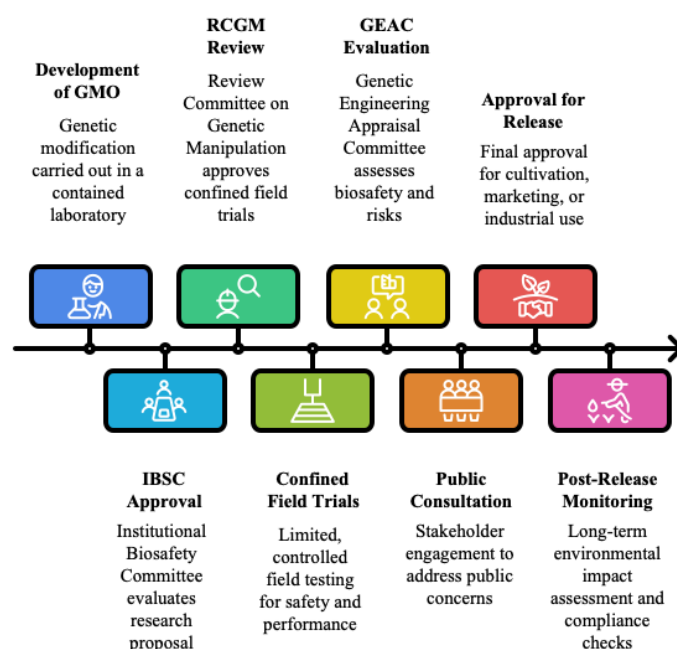


Figure 2: Flowchart depicting the regulatory approval process for genetically modified organisms (GMOs), illustrating the sequential engagement of institutional, national, and environmental regulatory authorities to ensure biosafety and regulatory compliance.

V. PATENT PROTECTION IN BIOSCIENCES

1. Concept of Patents in the Bio-Economy

Patents form a foundational pillar of the bioeconomy. They promote innovation by granting inventors exclusive rights—typically for a period of 20 years—to make, use, and commercialize their inventions. In the biosciences, patents are not merely legal instruments; they serve as critical incentives that help offset the high levels of risk and investment associated with fields such as genetic engineering, recombinant vaccine development, and synthetic biology. Translating scientific discoveries from the laboratory to the marketplace is highly challenging, with most projects failing during development. The approval of a single new drug can require investments exceeding USD 1 billion [42]. Strong intellectual property protection therefore plays a vital role in enabling firms to recover development costs and sustain long-term research and development efforts [18, 19].

2. Patentable and Non-Patentable Biological Inventions: The Legal Divide

Patent law, both globally and in India, draws a clear distinction between inventions and discoveries. The objective is to encourage innovation while safeguarding ethical considerations and environmental interests. In India, patent protection may be granted for genetically modified microorganisms, recombinant DNA vaccines, and novel biotechnological processes, provided they demonstrate technical advancement and industrial applicability. However, Section 3 of the Indian Patents Act explicitly excludes certain subject matter from patentability. Plants and animals, whether in whole or in part, as well as essentially biological processes for their production, are not patentable. These exclusions are intended not only to prevent excessive monopolization but also to protect biodiversity and preserve farmers' traditional rights to save, use, and exchange seeds, which are central to Indian agricultural practices [43].

The Indian patent framework also excludes traditional knowledge from patent protection under Section 3(p) of the Patents Act. As a result, mere discoveries of naturally occurring substances or attempts to appropriate community-held knowledge are not eligible for patenting. These exclusions align India's intellectual property regime with international agreements, including the Convention on Biological Diversity and the Nagoya Protocol on Access and Benefit-Sharing [18, 44]. The system is designed to reward genuine technological innovation rather than simple discoveries or misappropriation of cultural and traditional knowledge.

3. TRIPS Agreement and Biotechnology: Balancing Rights and Welfare

The Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) significantly reshaped the global intellectual property landscape. It established minimum standards that all members of the World Trade Organization are required to follow. However, TRIPS includes an important flexibility under Article 27.3(b). This provision allows member states to exclude plants and animals from patentability, provided that they implement an effective sui generis system for the protection of plant varieties [45].

India has actively exercised the flexibility provided under the TRIPS Agreement. Rather than adopting a conventional Western-style patent regime, it developed a distinct legal framework designed to balance multiple stakeholder interests. The Protection of Plant Varieties and

Farmers' Rights (PPV&FR) Act, enacted in 2001, exemplifies this sui generis system. The Act recognizes and protects the rights of plant breeders while simultaneously providing explicit legal recognition of farmers' rights. Such a balanced approach is uncommon in many Western intellectual property systems, where patent protections often take precedence over farmers' interests [18, 46].

The TRIPS Agreement plays a central role in contemporary global health debates. This was clearly evident during the COVID-19 pandemic, when proposals for TRIPS waivers on mRNA vaccine technologies generated intense international discussion. Proponents argued that strict intellectual property protections constrained vaccine manufacturing and limited access in low- and middle-income countries. In contrast, critics cautioned that weakening patent protection could undermine incentives for innovation and reduce preparedness for future pandemics [47, 48]. These debates extend beyond legal interpretation. They reflect a broader tension between safeguarding innovation and ensuring equitable access to essential technologies. This dynamic is illustrated by the innovation–access continuum presented in Figure 3, which highlights the intersection of patents, ethics, and public health.

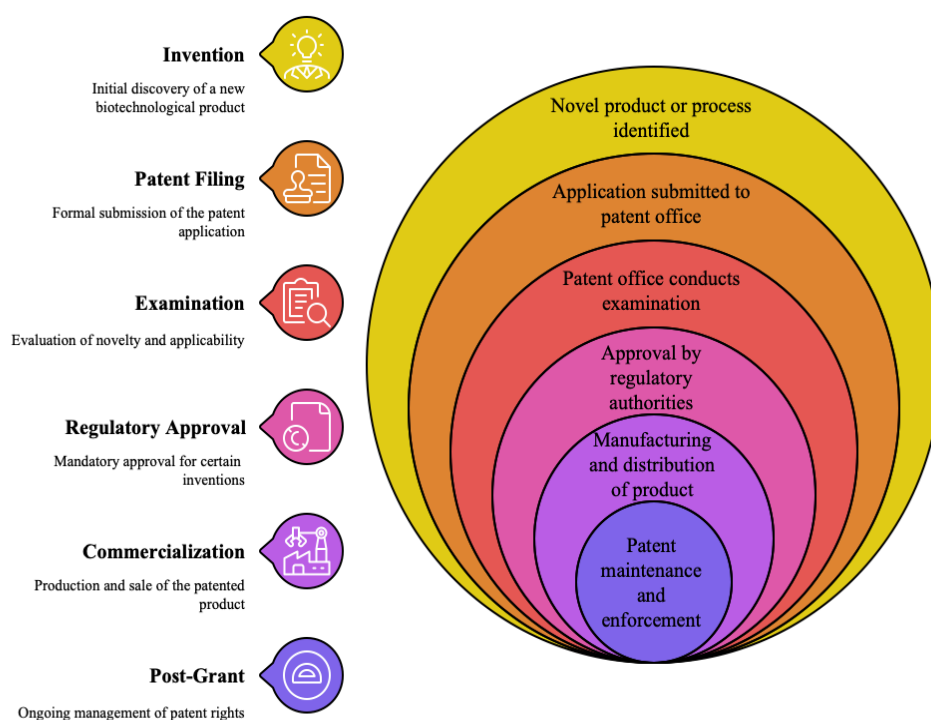


Figure 3: Patent lifecycle in biotechnology, illustrating the sequential stages from invention and patent application through grant, regulatory approval, and eventual commercialization of biotechnological innovations.

4. The Indian Patent System: Safeguards Against Monopoly

The Indian Patents Act, 1970, as amended in 2005, is notable for its strong emphasis on public health protection. Section 3(d) plays a central role in this framework. It prevents the practice of patent evergreening by disallowing patents for new forms of known substances unless they demonstrate a significant enhancement in therapeutic efficacy. This interpretation was firmly upheld by the Supreme Court of India in *Novartis AG v. Union of India* (2013).

In addition, the Act provides for compulsory licensing under Section 84. This provision allows the government to authorize third parties to manufacture a patented product if the patent holder fails to meet public requirements at an affordable price. Compulsory licensing thus serves as a critical mechanism to ensure access to essential medicines while balancing patent rights with public interest [45, 50]. The law also requires inventors to disclose the source and geographical origin of any biological material used in an invention. This disclosure obligation directly links intellectual property law with biodiversity conservation. It promotes transparency and accountability in the use of biological resources. By enforcing this requirement, the legal framework helps prevent the unauthorized appropriation of biological materials and ensures compliance with access and benefit-sharing principles [18, 44].

VI. ETHICAL, ENVIRONMENTAL, AND SOCIAL ISSUES

1. Biopiracy and Benefit Sharing

As genetic resources are commercialized at an increasing pace, concerns about **biopiracy** have grown. Biopiracy occurs when biological resources or associated traditional knowledge are used without permission or benefit sharing. In response, India enacted the **Biological Diversity Act, 2002**.

This law led to the establishment of the **National Biodiversity Authority (NBA)**. The NBA regulates access to biological resources and associated knowledge. It ensures that any commercial use follows the principle of **Access and Benefit Sharing (ABS)**.

Under this framework, if profits are generated from biological resources, a share must be returned to the local and indigenous communities that conserved them. This system aims to promote fairness and equity. It ensures that communities are recognized and rewarded for their role in conserving biodiversity and traditional knowledge [44, 51].

2. Protection of Traditional Knowledge

The **Traditional Knowledge Digital Library (TKDL)** represents a major advance in protecting India's traditional knowledge. India digitized ancient texts from **Ayurveda, Unani, Siddha, and Yoga**. These texts were translated into formats and languages that international patent offices can easily search and understand.

As a result, patent examiners can clearly see that many traditional remedies already exist as **prior art**. This has helped prevent the wrongful granting of patents on long-known Indian practices. Well-known examples include the use of **turmeric for wound healing** and **neem as a fungicide**.

Through the TKDL, India successfully challenged and stopped patent claims at offices such as the **United States Patent and Trademark Office (USPTO)** and the **European Patent Office (EPO)**. In this way, the TKDL acts as a protective shield. It ensures that traditional knowledge is not misappropriated and remains in the public domain [18, 52].

3. Emerging Ethical Challenges

Biotechnology is rapidly advancing into new and complex areas. As a result, ethical and legal questions are becoming more difficult to address. One major concern is **Digital Sequence Information (DSI)**.

DSI challenges the existing framework of the **Nagoya Protocol on Access and Benefit Sharing**. A key question remains unresolved. If a researcher downloads a genetic sequence from an online database and later synthesizes a biological product, does this activity count as “access” to genetic resources? If it does, should benefit-sharing obligations apply?

At present, there is no global consensus on this issue. Ongoing debates highlight the difficulty of ensuring fairness while also supporting open scientific research. Consequently, DSI is considered one of the most significant unresolved challenges in achieving equitable governance of modern biosciences [53, 54].

VII. INTEGRATION OF REGULATION, BIOSAFETY, AND PATENTS: THE TRIAD OF GOVERNANCE

Regulation, biosafety, and patent protection do not operate in isolation. They are closely interconnected and together form the foundation of modern biosciences. This integrated framework is often described as the “**Triad of Governance**.”

The triad guides a biotechnological invention at every stage. It begins at the point of discovery and continues through development, approval, and real-world application. Each component supports the others by ensuring safety, legal clarity, and innovation incentives. Without this coordinated system, managing the complex pathway of biotechnology products would be extremely difficult.

Regulatory systems establish the basic framework for biotechnology. They determine what activities are permitted based on safety, efficacy, and compliance with the law.

Biosafety measures translate these regulations into practice. They operate at the laboratory and field levels to contain risks and prevent accidental harm to humans and the environment. Patent protection supports the entire system. It encourages companies and researchers to invest in costly and high-risk research by offering exclusive rights as a reward for successful innovation. These components do not function independently. They actively reinforce one another.

First, patent law requires inventors to fully disclose their inventions. This transparency provides regulators with detailed technical information needed to evaluate new technologies. Second, regulatory authorities require robust scientific evidence, such as toxicity and safety studies. The same data are often used by inventors to support patent claims and demonstrate novelty and utility.

Third, biosafety practices, including compliance with **Good Laboratory Practice (GLP)**, serve multiple purposes. They protect researchers and the environment. They also ensure that experimental data are credible and acceptable to regulatory agencies, such as the FDA. Together, these interactions create a coherent and reliable system for advancing biotechnology safely and responsibly.

The pace of progress in bioscience is rapid and unprecedented. Advances in genetic engineering, CRISPR technologies, and synthetic biology offer powerful tools to address global challenges. These include food insecurity, public health emergencies, and environmental pollution.

However, such progress also brings significant challenges. Scientific work must be conducted safely. Environmental protection must be ensured. Ethical responsibilities must be upheld. Intellectual property systems must support innovation without excluding vulnerable populations.

This chapter argues that sustainable bio-innovation depends on an integrated approach. Effective regulation, robust biosafety practices, and equitable patent protection must function together. This combined framework forms the foundation for responsible and long-term advancement in the biosciences.

VIII. CONCLUSION

Strong regulatory frameworks serve multiple purposes. They do not merely define rules. They provide governments with the authority to assess risks and enforce compliance. They also ensure that national policies remain aligned with international agreements, such as the **Cartagena Protocol on Biosafety**.

At the same time, biosafety systems act as the first line of protection. These systems are structured around the **Biosafety Level (BSL) classification hierarchy**. They safeguard laboratory personnel and prevent accidental release of hazardous biological agents into the environment [16].

Intellectual property rights play a distinct and essential role in biotechnology governance. Patents do more than protect discoveries. They encourage private investment in biotechnology research and development by offering time-limited exclusivity.

However, India's experience demonstrates the need for safeguards. Legal exclusions for **living entities** and provisions for **compulsory licensing** introduce necessary checks and balances. These measures help maintain access to essential technologies and prevent the unchecked commercialization of biodiversity [17, 18].

1. Future Outlook: Adaptive Governance

Recent scholarship increasingly supports a move toward **adaptive governance** in biotechnology. Emerging technologies such as synthetic biology and gene drives challenge traditional regulatory frameworks. In many cases, these innovations do not fit neatly within existing categories, such as conventional definitions of genetically modified organisms, particularly when no foreign DNA is introduced.

As a result, regulatory systems must remain flexible and responsive. Governance approaches should be science-based and capable of evolving alongside technological advances. At the same time, they must continue to uphold rigorous safety standards to protect human health and the environment [9, 12].

2. Summary

This chapter outlined the governance landscape of modern biosciences. It began by emphasizing the importance of comprehensive regulatory frameworks for managing risks associated with genetically modified organisms and emerging pathogens. India's regulatory system was presented as a practical example of how a decentralized structure can still ensure effective oversight and accountability.

The discussion then examined biosafety in practice. It highlighted that responsible science depends not only on physical containment measures but also on fostering a strong culture of safety within institutions. Both elements are essential and cannot be compromised.

The chapter next explored patent law. This section illustrated the delicate balance between incentivizing innovation and safeguarding the public interest. International mechanisms such as the TRIPS Agreement, along with India's statutory exclusions and safeguards, demonstrate how legal systems attempt to maintain this balance.

Ethical dimensions were also addressed. Issues such as biopiracy, farmers' rights, and benefit sharing underscored the need for biotechnology to achieve social legitimacy in addition to scientific success. Public trust and equitable outcomes emerged as central concerns.

In conclusion, effective biotechnology governance requires more than scientific excellence alone. Sustainable progress depends on the integration of regulation, biosafety, and intellectual property protection. Together, these pillars create a system that is predictable, safe, and equitable, enabling biotechnology to contribute to sustainable development while protecting future generations and the environment.

REFERENCES

- [1] S.N. Cohen, A.C.Y. Chang, H.W. Boyer, and R.B. Helling, "Construction of biologically functional bacterial plasmids in vitro," *Proc. Natl. Acad. Sci. U.S.A.*, vol. 70, no. 11, pp. 3240–3244, 1973.
- [2] J. Nielsen and J.D. Keasling, "Engineering cellular metabolism," *Cell*, vol. 164, pp. 1185–1197, 2016.
- [3] J.A. Doudna and E. Charpentier, "The new frontier of genome engineering with CRISPR–Cas9," *Science*, vol. 346, no. 6213, Art. no. 1258096, 2014.
- [4] Y. Zhang, X. Liu, J. Wang, M. Li, L. Chen, and H. Zhao, "Applications of CRISPR–Cas systems in agriculture and biotechnology," *Trends Biotechnol.*, vol. 39, no. 7, pp. 695–707, 2021.
- [5] D. Endy, "Foundations for engineering biology," *Nature*, vol. 438, no. 7067, pp. 449–453, 2005.
- [6] M. Qaim, "Role of genetically modified crops in sustainable development," *Sci. Adv.*, vol. 6, no. 20, Art. no. eaaz7956, 2020.
- [7] Food and Agriculture Organization, *Biosafety in agricultural biotechnology: Guidelines for safe use of genetically modified organisms*, Rome, Italy, FAO, 2022.
- [8] J.R. Cherry and A.L. Fidantsef, "Directed evolution of industrial enzymes: An update," *Curr. Opin. Biotechnol.*, vol. 14, no. 4, pp. 438–443, 2003.
- [9] Organisation for Economic Co-operation and Development, *Global regulatory approaches to emerging biotechnologies*, Paris, France, OECD Publishing, 2023.
- [10] O.J. Wouters, K.C. Shadlen, M. Salcher-Konrad, A.J. Pollard, H.J. Larson, Y. Teerawattananon, and M. Jit, "Challenges in ensuring global access to COVID-19 vaccines," *Lancet*, vol. 397, no. 10278, pp. 1023–1034, 2021.
- [11] L. Corey, J.R. Mascola, A.S. Fauci, and F.S. Collins, "A strategic approach to COVID-19 vaccine R&D," *Science*, vol. 368, no. 6494, pp. 948–950, 2020.
- [12] J. Kuzma and K. Grieger, "Responsible governance of biotechnology: Maintaining public trust in emerging technologies," *Nat. Biotechnol.*, vol. 38, no. 1, pp. 25–33, 2020.
- [13] *Natl. Acad. Sci., Eng., and Med., Biosecurity and dual-use research in the life sciences*, Washington, DC, USA, Natl. Acad. Press, 2021.

- [14] F.M. Abbott and J.H. Reichman, "Facilitating access to cross-border supplies of patented pharmaceuticals," *J. Int. Econ. Law*, vol. 25, no. 1, pp. 1–28, 2022.
- [15] D.B. Resnik, *The ethics of science: An introduction*, London, U.K., Routledge, 2019.
- [16] World Health Organization, *Laboratory biosafety manual*, 4th ed., Geneva, Switzerland, WHO, 2020.
- [17] World Trade Organization, *TRIPS agreement and public health*, Geneva, Switzerland, WTO, 2021.
- [18] World Intellectual Property Organization, *World intellectual property indicators 2023*, Geneva, Switzerland, WIPO, 2023.
- [19] Organisation for Economic Co-operation and Development, *The bioeconomy to 2030: Designing a policy agenda*, Paris, France, OECD Publishing, 2021.
- [20] Convention on Biological Diversity, *Cartagena protocol on biosafety: Implementation and compliance*, Montreal, Canada, CBD Secretariat, 2023.
- [21] Eur. Food Saf. Auth., "Guidance on risk assessment of genetically modified plants," *EFSA J.*, 2021.
- [22] R. Mackenzie, F. Burhenne-Guilmin, A. La Viña, J. Werksman, F. Ascencio, J. Kinderlerer, and R. Tapper, *An explanatory guide to the Cartagena protocol on biosafety*, Gland, Switzerland, IUCN, 2003.
- [23] N.R. Jordan, J. Mullen, A. Williams, and C. Whittaker, "Governance of gene editing in agriculture," *Nat. Plants*, vol. 7, pp. 136–143, 2021.
- [24] Ministry of Environment and Forests, Govt. of India, *Rules for the manufacture, use, import, export and storage of hazardous microorganisms/genetically engineered organisms or cells*, New Delhi, India, 1989.
- [25] V. Ahuja, *Biosafety regulations in India: Multi-tiered governance and its effectiveness*, Singapore, Springer, 2018.
- [26] Dept. of Biotechnology, *Guidelines for the safety assessment of genome-edited plants*, New Delhi, India, Ministry of Science and Technology, 2022.
- [27] S. Kumar, L. Arul, and M. Dasgupta, "Genome editing in India: Regulatory landscape and future perspectives," *Front. Plant Sci.*, vol. 14, Art. no. 112124, 2023.
- [28] Ministry of Environment, Forest and Climate Change, *GEAC decisions and biosafety oversight*, New Delhi, India, Govt. of India, 2023.
- [29] P. Bhatia and A. Chugh, "Regulatory landscape for biopharmaceuticals in India," *J. Generic Med.*, vol. 17, no. 3, pp. 123–132, 2021.
- [30] C. Klaassen and A. Casadevall, "Advances and challenges in synthetic biology: Implications for biosafety," *Trends Biotechnol.*, vol. 39, no. 5, pp. 455–467, 2021.
- [31] Natl. Acad. Sci., Eng., and Med., *Safeguarding the genome: Ethical and safety considerations in synthetic biology*, Washington, DC, USA, Natl. Acad. Press, 2017.
- [32] Organisation for Economic Co-operation and Development, *OECD guidance on responsible conduct in life sciences research*, Paris, France, OECD Publishing, 2019.
- [33] Centers for Disease Control and Prevention, *Biosafety in microbiological and biomedical laboratories*, 6th ed., Washington, DC, USA, U.S. Govt. Printing Office, 2020.
- [34] F. Lentzos, B. Rappert, and S. Roffey, "High-containment laboratories: Managing the risks of BSL-4 research," *Biosecurity Bioterrorism*, vol. 21, no. 1, pp. 12–26, 2023.
- [35] P. Rao, R. Sharma, and A. Singh, "High-containment laboratories and risk management in India," *Indian J. Biosafety*, vol. 12, no. 2, pp. 45–57, 2021.
- [36] D. Mueller, "Environmental risks of genetically engineered organisms," *J. Environ. Biotechnol.*, vol. 15, no. 3, pp. 101–115, 2019.
- [37] P. Macnaghten, "Responsible innovation in plant biotechnology," *Plants People Planet*, vol. 3, no. 6, pp. 564–572, 2021.
- [38] Eur. Food Saf. Auth., *Risk assessment of genetically modified plants: Guidance document*, Parma, Italy, EFSA, 2022.
- [39] Inter-Ministerial Committee on Biotechnology Policy, *Regulations and guidelines for recombinant DNA research and biocontainment*, New Delhi, India, Govt. of India, 2019.
- [40] R. Kumar, P. Singh, and A. Sharma, "Regulatory perspectives on genome-edited crops in India," *J. Plant Biotechnol.*, vol. 20, no. 2, pp. 101–115, 2023.
- [41] M.S. Andersson, A. Gheysen, R. Lemaire, J. Potrykus, R. Schiemann, J. Spok, and J. Sprink, "Post-release monitoring and stewardship of genetically modified crops," *Trends Plant Sci.*, vol. 26, no. 4, pp. 310–322, 2021.
- [42] J.A. DiMasi, H.G. Grabowski, and R.W. Hansen, "Innovation in the pharmaceutical industry," *J. Health Econ.*, vol. 47, pp. 20–33, 2016.
- [43] Govt. of India, *The patents act, 1970 (as amended)*, New Delhi, India, 1970.
- [44] Natl. Biodiversity Authority, *Guidelines on access and benefit sharing*, Chennai, India, Govt. of India, 2021.
- [45] World Trade Organization, *TRIPS and public health: Compulsory licensing*, Geneva, Switzerland, WTO, 2021.

- [46] Govt. of India, The protection of plant varieties and farmers' rights act, 2001, New Delhi, India, 2001.
- [47] F.M. Abbott and J.H. Reichman, "Facilitating access to vaccines and therapeutics during pandemics," *J. Int. Econ. Law*, vol. 25, no. 1, pp. 1–27, 2022.
- [48] World Trade Organization, TRIPS waiver for COVID-19 vaccines: Ministerial decision, Geneva, Switzerland, WTO, 2022.
- [49] Supreme Court of India, Novartis AG v. Union of India & Others, Civil Appeal Nos. 2706–2716, 2013.
- [50] Govt. of India, The patents act, 1970 (as amended in 2005), New Delhi, India, 2005.
- [51] Convention on Biological Diversity, Nagoya protocol on access to genetic resources and the fair and equitable sharing of benefits, United Nations, 2011.
- [52] A. Gupta, "Protecting traditional knowledge: The Indian experience with the Traditional Knowledge Digital Library," *J. Intellect. Prop. Rights*, vol. 24, pp. 1–10, 2019.
- [53] Convention on Biological Diversity, Digital sequence information on genetic resources, Montreal, Canada, CBD Secretariat, 2022.
- [54] S. Laird, R. Wynberg, M. Rourke, F. Humphries, M.R. Muller, and C. Lawson, "Access and benefit sharing in the digital age," *Nat. Plants*, vol. 6, pp. 26–27, 2020.

NANOPARTICLE BASED DRUG DELIVERY IN CANCER THERAPY AND ITS ROLE IN OVERCOMING DRUG RESISTANCE -A SYSTEMATIC REVIEW

Abstract

This chapter focuses on the use of nanoparticle-based drug delivery systems in cancer therapy. It begins by emphasizing why nanoparticles are considered superior to conventional cancer treatments. Traditional chemotherapy often suffers from poor drug stability, nonspecific distribution, and high systemic toxicity. In contrast, nanoparticles improve drug stability and biocompatibility, meaning they protect drugs from degradation and reduce harmful effects on healthy tissues. The chapter also highlights the Enhanced Permeability and Retention (EPR) effect, a phenomenon where nanoparticles preferentially accumulate in tumor tissues due to leaky blood vessels and poor lymphatic drainage. This property enables passive targeting, while surface modification of nanoparticles allows active targeting of cancer cells, improving treatment specificity and efficacy. The discussion of hybrid nanoparticles reflects recent advancements in nanomedicine. By combining different materials (such as polymers, lipids, or inorganic components), hybrid nanoparticles achieve multifunctionality, including controlled drug release, imaging, and simultaneous targeting of multiple pathways, leading to improved therapeutic outcomes. The review focuses on cancer drug resistance, a key challenge in oncology. It explains biological mechanisms such as overexpression of drug efflux transporters, which pump drugs out of cancer cells; defective apoptotic pathways, which prevent programmed cell death; and tumor hypoxia, which reduces drug sensitivity. Nanoparticles can bypass or modulate these mechanisms, thereby helping to reverse multidrug resistance (MDR). The inclusion of nanoparticle-based immunotherapy

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broadens the scope of the review, aligning it with modern immuno-oncology approaches that aim to stimulate the immune system to recognize and destroy cancer cells .

Keywords: Nanoparticle-based drug, drug stability, biocompatibility, enhanced retention permeability, overexpression of drug efflux, multidrug resistance.

I. INTRODUCTION

1. Need for New Cancer Treatments

Cancer still remains a major global health challenge despite advances in medical science. Although modern medicine has introduced multiple treatment strategies and personalized (individualized) therapies, stil limited therapeutic success is observed with many cancers. Cancer is currently the second leading cause of death worldwide, highlighting the urgent need for more effective and safer treatments. One of the main challenges in cancer therapy is drug resistance, which reduces treatment effectiveness over time [3].

2. Limitations of Conventional Chemotherapy

Chemotherapy is a widely used traditional cancer treatment; however, it lacks selectivity and therapeutic precision. A non-exhaustive list of drugs that combine anticancer and immunostimulatory effects includes imatinib mesylate, cyclophosphamide, anthracyclines and 5-fluorouracil [12]. Chemotherapeutic drugs indiscriminately target rapidly dividing cells, thereby affecting both cancerous and healthy tissues. This non-specific action results in severe side effects such as bone marrow suppression, alopecia (hair loss) and gastrointestinal toxicity [12]. In addition to systemic toxicity, the effectiveness of chemotherapy is further limited by physical barriers within the tumor microenvironment, insufficient drug penetration into deep tumor regions, and improper or abnormal tumor vascularization, which restrict uniform drug distribution. Although targeted therapies have improved treatment precision, they continue to produce adverse effects and frequently fail due to the development of drug resistance, thereby limiting their long-term clinical effectiveness [2].

3. Emergence of Nanotechnology in Cancer Therapy

To overcome these limitations, Nanoparticle (NP)-based drug delivery systems have emerged as a promising solution due to their ability to improve pharmacokinetics, tumor targeting, drug stability, and safety profiles. Nanoparticles are designed based on tumor pathophysiology, particularly tumor size, vascular permeability, and microenvironment [5].

4. Mechanism of Nanoparticle-Based Drug Delivery

Nanoparticles deliver drugs through two main mechanisms:(a)Passive targeting, mainly via the Enhanced Permeability and Retention (EPR) effect, which allows nanoparticles to accumulate in tumor tissues and (b)active targeting, where ligands on nanoparticles bind to specific receptors on tumor cells.

Once localized at the tumor site, nanoparticles release their payload, inducing tumor cell death. These payloads may include chemotherapeutic agents or nucleic acids, enabling both cytotoxic therapy and gene therapy. Nanoparticles also improve the delivery of poorly water-soluble drugs, enhancing their circulation time and bioavailability [1].

II. REDUCTION OF TOXICITY AND CLINICAL EVIDENCE

Clinical evidence shows that nanoparticle-based drug delivery reduces toxicity in cancer treatment. PEGylated liposomal doxorubicin lowers heart damage by limiting drug exposure to healthy tissues, while albumin-bound paclitaxel allows higher doses with fewer side effects. PEGylated liposomal doxorubicin reduces cardiotoxicity by encapsulating the drug within a PEG-coated carrier, which limits direct exposure of healthy heart tissue and prolongs circulation time [15]. Albumin-bound paclitaxel enhances drug solubility and tumor uptake, allowing higher tolerated doses while minimizing systemic and solvent-related side effects [16]. These examples demonstrate that nanotechnology reduces toxicity by limiting drug interaction with healthy tissues and improving therapeutic efficiency in cancer therapy [3].

Nanoparticles (NPs) used in medical treatment are carefully designed with specific size, shape, and surface properties because these factors directly affect how well drugs reach cancer cells. NPs with a size of 10–100 nm are ideal for cancer therapy since they can accumulate in tumors through the Enhanced Permeability and Retention (EPR) effect [14]. Very small particles can leak into normal tissues or be removed by the kidneys, while very large particles are quickly cleared by immune cells [14]. Further, modifying the surface of NPs—for example by coating them with polyethylene glycol (PEG)—helps them stay longer in the bloodstream by avoiding immune detection. This improves drug circulation time and increases drug accumulation in tumors. Overall, the combined properties of nanoparticles determine their effectiveness in cancer treatment.

Different types of nanoparticles (NPs) are currently available as drug delivery systems for cancer therapy. These can be categorized into organic, inorganic and hybrid NPs. The organic NPs include liposome-based NPs, polymer-based NPs and dendrimers. Among polymer-based NPs, polymeric NPs and polymeric micelles are common. Examples of inorganic NPs are gold NPs (Au NPs), carbon nanotubes, silica NPs, magnetic NPs, and quantum dots. Hybrid NPs combine the advantages of different NPs, including lipid-polymer hybrid NPs, organic-inorganic hybrid NPs, and cell membrane-coated NPs [4].

III. ORGANIC NPS

1. Liposomes Based NPs:

Organic nanoparticles, especially liposomes, have been widely used in cancer treatment. Liposomes are nano-sized carriers made of a lipid outer layer that can hold both hydrophilic and hydrophobic drugs. They can be modified to behave like living cells, which improves drug delivery to tumors. Liposomes are widely used to deliver anti-cancer drugs such as doxorubicin and paclitaxel, and are common in breast and prostate cancer therapy [1]. Compared to free drugs, liposomal drugs show better bioavailability, higher anti-tumor efficiency, and reduced side effects, such as lower heart toxicity with liposomal doxorubicin. Liposome-based systems can also carry multiple drugs together, improving treatment efficacy and helping overcome drug resistance [2].

2. Polymer Based NPs:

Polymer-based nanoparticles are drug carriers made from different polymers designed for efficient drug delivery. PLGA nanoparticles are widely used because they are biocompatible, biodegradable and can accumulate in tumors through the EPR effect.

- **Polymeric Micelles:** Polymeric micelles are formed by self-assembly of amphiphilic polymers and have a hydrophobic core to carry poorly soluble anticancer drugs, while the hydrophilic outer layer improves stability, reduces immune clearance, and prolongs circulation time. Overall, polymer-based nanoparticles enhance drug solubility, stability, and therapeutic effectiveness in cancer treatment.
- **Dendrimers:** Dendrimers are highly branched, three-dimensional polymers with many surface functional groups, which allow high drug loading and precise delivery [5].

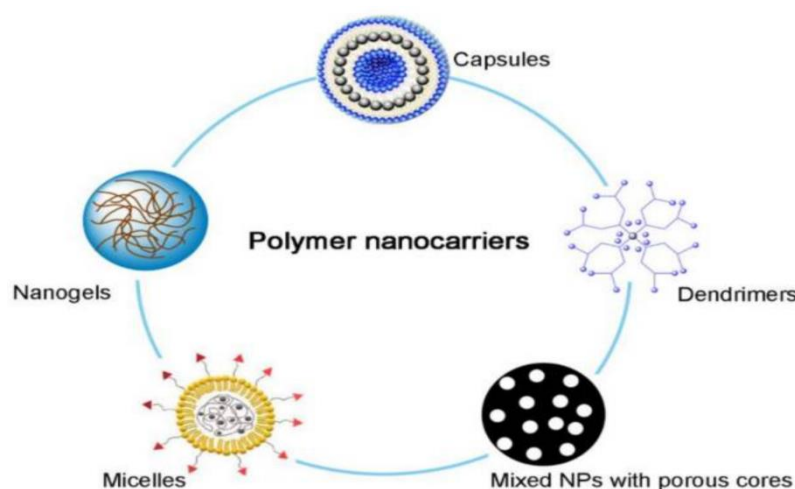


Figure 1: The different types of Polymer Nano carriers based drug delivery for cancer therapeutics DOI: [10.3390/ijms22179118](https://doi.org/10.3390/ijms22179118)

IV. INORGANIC NPS

1. Gold Nanoparticles

Inorganic nanoparticles used in cancer therapy include gold nanoparticles (AuNPs), carbon nanotubes, quantum dots, magnetic nanoparticles, and silica nanoparticles. Among these, AuNPs are the most widely studied because their gold core is chemically inert and non-toxic. Surface-modified AuNPs improve drug delivery by increasing drug accumulation in tumors and helping overcome drug resistance. In addition to drug delivery, AuNPs are also useful in multimodal cancer treatments, such as gene therapy, photothermal therapy, and immunotherapy, making them highly promising for cancer management [17, 18, 19].

2. Carbon Nanotubes (CNTs)

Carbon nanotubes are tube-shaped nanomaterials with unique physical and chemical properties, making them effective drug carriers for anticancer drugs like doxorubicin and paclitaxel. They can also generate heat under near-infrared radiation, which is useful for thermal ablation to destroy cancer cells [20].

3. Mesoporous Silica Nanoparticles (SNPs)

Mesoporous silica nanoparticles have a porous structure that allows them to store large amounts of anticancer drugs and release them in a controlled manner. They offer high stability, improved drug delivery efficiency, and better treatment outcomes. Additionally, porous silicon nanoparticles show promise in cancer immunotherapy by enhancing immune responses such as antigen presentation and interferon- γ secretion [2].

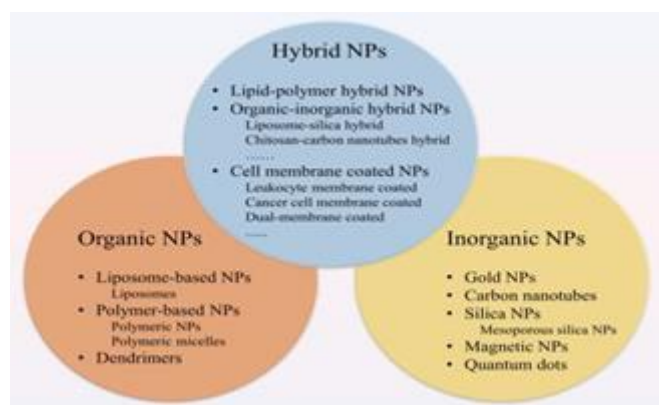


Figure 2: Different Types of Nanoparticles doi: 10.3389/fmolb.2020.00193

V. Hybrid NPs

Hybrid nanoparticles combine organic and inorganic materials to overcome the limitations of using a single type of nanoparticle. This combination improves drug delivery efficiency, enhances treatment effectiveness, and helps reduce drug resistance.

1. Lipid Polymer Hybrid Nps

Lipid–polymer hybrid nanoparticles have a polymer core and a lipid outer shell, making them highly suitable for cancer therapy. The lipid-polymer hybrid NPs consist of a biodegradable and biocompatible hydrophobic polymeric core made of poly D,L-lactic-co-glycolic acid (PLGA), a monolayer of phospholipids, and an outer corona layer made of polyethylene glycol (PEG) [13]. They merge the biocompatibility of lipids with the structural strength of polymers, allowing them to carry both hydrophilic and hydrophobic drugs. These hybrid nanoparticles are easily taken up by cancer cells, avoid rapid immune clearance, and have shown promising results in pancreatic, breast, and prostate cancer treatments [1].

2. Organic and Inorganic NPs

Hybrid nanoparticles made by combining organic and inorganic materials are widely used to improve cancer treatment [1]

- **Liposome-silica hybrid NPs:** Liposome–silica hybrid nanoparticles have a silica core with a lipid coating and can effectively deliver drugs to prostate, breast, and pancreatic cancer cells, including combination drugs like gemcitabine and paclitaxel. Advanced hybrid systems such as porous silicon nanoparticles with liposomes enable co-delivery of drugs and DNA, helping to kill drug-resistant cancer cells.

- **Carbon Nanotube Chitosan Hybrid NPs:** Carbon nanotube–chitosan hybrids improve anticancer activity while reducing toxicity to normal cells. Additionally, metal–polymer hybrid nanoparticles combine targeted drug delivery with heat-based therapy (hyperthermia), leading to more effective tumor destruction [4].

VI. Mechanism of Targeting

Targeting cancer cells is an important feature of nanoparticle drug carriers because it improves treatment effectiveness while reducing damage to normal cells. To design effective targeted nanoparticles, it is essential to understand tumor biology and how nanoparticles interact with cancer cells. Based on their mechanism, nanoparticle targeting strategies are mainly classified into two types: passive targeting and active targeting

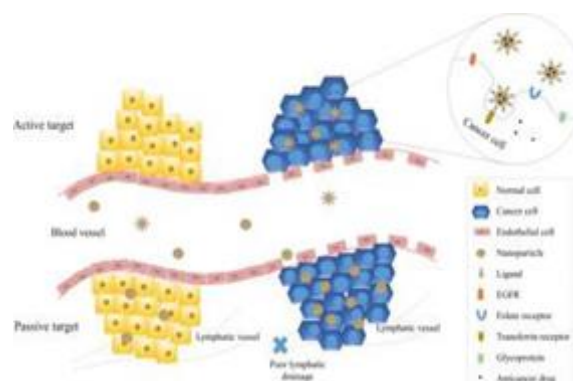


Figure 3: Active and Passive Targeting doi:10.3389/fmolb.2020.00193

Passive targeting takes advantage of the natural differences between tumor tissue and normal tissue. Tumors develop leaky blood vessels due to rapid and abnormal angiogenesis, allowing nanoparticles to escape from the bloodstream and accumulate in tumor tissue. Poor lymphatic drainage in tumors further helps retain these nanoparticles. This phenomenon is known as the Enhanced Permeability and Retention (EPR) effect [3].

Active targeting delivers drugs to cancer cells through specific ligand–receptor interactions. Nanoparticles are coated with ligands that bind to receptors overexpressed on cancer cells, allowing them to preferentially bind to cancer cells. This binding triggers receptor-mediated endocytosis, enabling nanoparticles to enter cancer cells and release drugs effectively. Active targeting is especially useful for delivering macromolecules such as proteins and siRNA. Common targeting ligands include antibodies, peptides, vitamins, and carbohydrates, which bind to receptors like the transferrin receptor, folate receptor, and EGFR [2].

VII. TARGETING THE CANCER CELLS

1. Transferrin is an iron-transport protein whose receptors are highly expressed on most cancer cells but present at low levels on normal cells. Therefore, transferrin-conjugated nanoparticles are used for active targeting, as they show higher cancer cell uptake, improved drug delivery, and help overcome drug resistance [1].
2. Folic acid (vitamin B9) is taken up by cells through folate receptors. While normal cells express few folate receptors, FR- α and FR- β is overexpressed in many cancers, making folate-conjugated nanoparticles effective for targeted cancer drug delivery [5].

3. Cancer cells express many glycoproteins and lectins on their surface. Targeting can be done either by attaching lectins to nanoparticles to bind cancer cell carbohydrates (direct lectin targeting) or by attaching carbohydrates to nanoparticles to bind lectins on cancer cells (reverse lectin targeting) [5].
4. Another important target is the epidermal growth factor receptor (EGFR), which is overexpressed in many cancers and plays a key role in tumor growth. Targeting EGFR, including HER-2 in breast and gastric cancers, allows nanoparticles to deliver drugs specifically to cancer cells. Using multiple targeting ligands on a single nanoparticle can further improve targeting accuracy and treatment effectiveness [2].

VIII.ROLE OF NANOPARTICLES IN CANCER IMMUNOTHERAPY

Immunotherapy in cancer treatment involves the use of the host immune capabilities in the recognition and destruction of tumor cells. It is clinically effective but remains associated with significant challenges, including inefficient delivery of immunomodulatory agents, systemic toxicity, inefficient antigen presentation rates and the suppressive microenvironment around the tumor (TME) [11]

Nanoparticles are beneficial in immunotherapy due to their ability to function as antigen carriers, adjuvants, cytokine delivery vehicles, or immune checkpoint inhibitors [11]. A promising approach to improve the efficacy of cancer immunotherapy is the use of nanoparticles (NPs) in drug delivery systems [8]. Owing to their Nano sized nature, NPs are readily internalized by antigen-presenting cells (APCs), particularly dendritic cells (DCs), through endocytosis, making them effective platforms for immune modulation [8]. This property has facilitated the development of Nano-vaccines capable of delivering tumor-associated antigens (TAAs) and immunostimulatory components directly to APCs [11]

Nano-vaccines have been shown to enhance antigen cross-presentation via major histocompatibility complex (MHC) class I pathways, leading to potent activation of cytotoxic CD8⁺ T cells [11]. In addition to acting as delivery carriers, certain nanoparticles exhibit inherent immunoadjuvant properties. Inorganic nanoparticles, such as mesoporous silica nanoparticles, have demonstrated immune-stimulatory effects including dendritic cell maturation, cytokine secretion and T-cell activation, comparable to polymeric nanoparticle systems [8].

Furthermore, nanoparticles have been engineered as artificial antigen-presenting cells through surface functionalization with MHC-peptide complexes and co-stimulatory molecules, enabling direct engagement and activation of T cells while overcoming limitations of natural APCs.

Nanoparticles also play a critical role in modulating the tumor immunosuppressive microenvironment. The TME is enriched with immunosuppressive cell populations such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), which collectively inhibit effective antitumor immune responses. Nanoparticle-based systems can selectively target these immunosuppressive cells, reprogramming pro-tumor M2 macrophages into antitumor M1 phenotypes and thereby restoring immune activity within the tumor. Additionally, nanoparticles enhance the therapeutic efficacy of immune checkpoint inhibitors by improving tumor accumulation and reducing off-target effects [11].

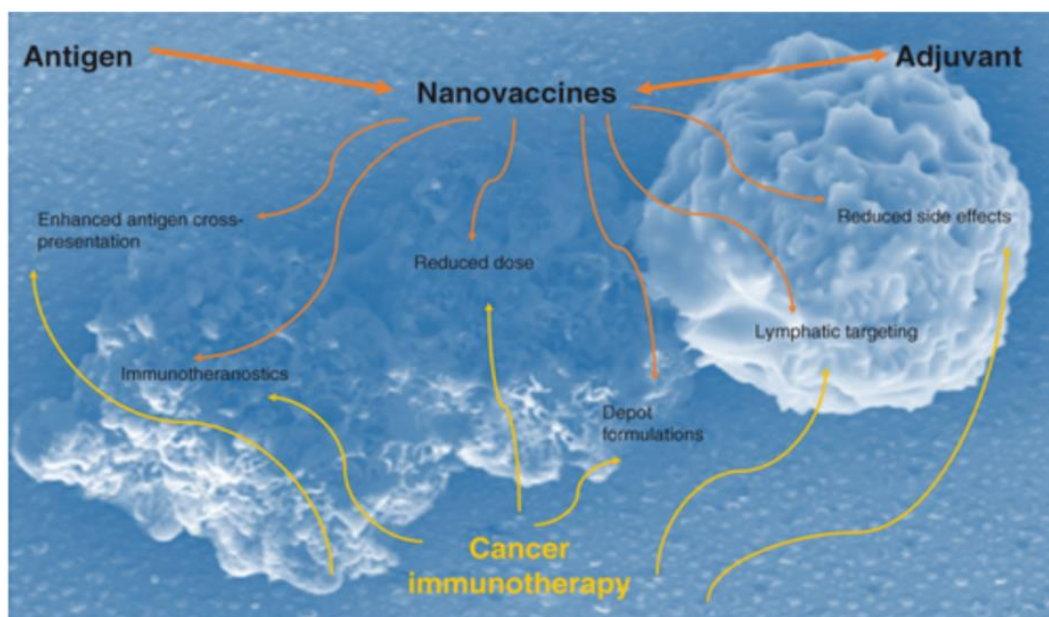


Figure 4: Role of Nanoparticles in Cancer Immunotherapy DOI: 10.1002/Wnan.1421

IX. MECHANISM OF NANOPARTICLES IN OVERCOMING DRUG RESISTANCE

Multidrug resistance (MDR) is one of the major obstacles to successful cancer chemotherapy. Resistance arises through several mechanisms, including overexpression of drug efflux transporters, evasion of apoptosis, hypoxic tumor conditions and altered intracellular drug distribution [6, 9]. Nanoparticle-based drug delivery systems have shown significant potential in overcoming these resistance mechanisms through both physical bypass strategies and biological modulation [7, 8]

1. Targeting Drug Efflux Transporters

A predominant mechanism of MDR is the overexpression of ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp), which actively expel chemotherapeutic agents from cancer cells, reducing intracellular drug concentration [6]. Nanoparticles can circumvent these efflux pumps by entering cells via endocytosis rather than passive diffusion, allowing drugs to be released intracellularly at perinuclear or subcellular sites away [7, 8]. Moreover, nanoparticles facilitate the co-delivery of chemotherapeutic agents along with efflux transporter inhibitors or gene-silencing molecules, enabling effective suppression of transporter expression or function. Certain polymeric nanoparticles also directly modulate MDR by inhibiting transporter activity or reducing ATP availability required for efflux pump function [6].

2. Evasion of Apoptosis

Defective apoptosis is a hallmark of drug-resistant cancer cells. Overexpression of anti-apoptotic proteins and dysregulation of apoptotic signaling pathways allow tumor cells to evade chemotherapy-induced cell death [6]. Nanoparticles provide efficient platforms for co-delivery of anticancer drugs and apoptosis-regulating agents, including gene therapy constructs and molecular inhibitors, to restore apoptotic pathways [7].

Nanoparticle-mediated delivery of tumor suppressor genes, particularly wild-type p53, was demonstrated to sustain anti-proliferative activity and restoration of apoptosis in resistant cancer cells [10]. Additionally, subcellular-targeted Nano carriers can induce mitochondrial dysfunction, leading to cytochrome-c release and caspase activation, thereby enhancing apoptosis while simultaneously reducing ATP-dependent drug efflux [7].

3. Targeting Hypoxia-Induced Drug Resistance

Tumor hypoxia significantly contributes to drug resistance by promoting genetic instability, slow cell proliferation and increased expression of drug efflux transporters [9]. Nanoparticles enable improved penetration and retention of therapeutic agents within hypoxic tumor regions, overcoming diffusion barriers associated with conventional chemotherapy [8, 9].

Furthermore, nanoparticle-based systems support controlled and stimuli-responsive drug release in hypoxic or acidic tumor microenvironments, ensuring higher localized drug concentrations and enhanced therapeutic efficacy within resistant tumor regions.

X. CONCLUSION

Nanoparticle-based drug delivery systems represent a significant advancement in cancer therapy by overcoming major limitations of conventional treatments. By improving drug stability, controlled release and tumor-specific accumulation, nanoparticles enhance therapeutic efficacy while reducing systemic toxicity through both passive (EPR effect) and active targeting strategies.

The availability of diverse nanoparticle platforms, including organic, inorganic and hybrid systems, allows for flexible drug design and multifunctional applications. Notably, hybrid nanoparticles enable the co-delivery of drugs, genes, and imaging agents, offering effective strategies for treating complex and drug-resistant cancers. Nanoparticles also play a critical role in overcoming multidrug resistance by bypassing efflux mechanisms, enhancing intracellular drug accumulation and restoring apoptotic pathways. Their expanding use in cancer immunotherapy further highlights their therapeutic relevance.

However, we still do not have a complete understanding of their potential toxicity. Their accumulation in different organs may lead to long-term toxicity [1]. Toxicity depends on the properties of the NP (e.g., size, shape, surface charge, chemical composition, and coatings) which can further complicate toxicity predictions. Similarly, while NPs have been shown to be capable of modulating immune responses to tumours, inflammation or hypersensitivity responses remain a distinct possibility. Further, the development of immune memory and its impact on efficacy needs to be addressed. Tumour heterogeneity, interaction of NPs with blood components and the possibility of their clearance by mononuclear phagocytes, thereby limiting efficacy are also aspects which require detailed investigation [21]. Moreover, challenges related to large-scale production and regulatory approval remain. In conclusion, while nanoparticle-based drug delivery systems hold strong potential to improve cancer treatment outcomes, continued research is essential for their successful clinical translation [1].

REFERENCES

- [1] Yao, Y., Zhou, Y., Liu, L., Xu, Y., Chen, Q., Wang, Y., Wu, S., Deng, Y., Zhang, J., & Shao, A. (2020) "Nanoparticle-based drug delivery in cancer therapy and its role in overcoming drug resistance. *frontiers in molecular biosciences*", 7, 193. DOI: 10.3389/fmolb.2020.00193.
- [2] Maeda, H., Wu, J., Sawa, T., Matsumura, Y., & Hori, K. (2000) "Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review". *Journal of Controlled Release*, 65(1–2), 271–284. DOI: 10.1016/S0168-3659(99)00248-5
- [3] Farokhzad, O. C., & Langer, R. (2009) "Impact of nanotechnology on drug delivery". *ACS Nano*, 3(1), 16–20. DOI: 10.1021/nn900002m
- [4] Shi, J., Kantoff, P. W., Wooster, R., & Farokhzad, O. C. (2017) "Cancer nanomedicine: Progress, challenges and opportunities". *Nature Reviews Cancer*, 17(1), 20–37. DOI: 10.1038/nrc.2016.108 PubMed
- [5] Xia, W., Tao, Z., Zhu, B., Zhang, W., Liu, C., Chen, S., & Song, M. (2021) "Targeted Delivery of Drugs and Genes Using Polymer Nano carriers for Cancer Therapy", 22(17):9118. DOI: 10.3390/ijms22179118
- [6] Litman, T., Druley, T. E., Stein, W. D., & Bates, S. E. (2001) "From MDR to MXR :New understanding of multidrug resistance systems". *Cellular and Molecular Life Sciences*, 58(7), 931–59. DOI: 10.1007/PL00000912
- [7] Murakami, M., Cabral, H., Matsumoto, Y., Wu, S., Kano, M. R., Yamori, T., Nishiyama, N., & Kataoka, K. (2011) "Improving drug potency and efficacy by nanocarrier-mediated subcellular targeting". *Science Translational Medicine*, 3(64), 64ra2. DOI: 10.1126/scitranslmed.3001385.
- Wang, A. Z., Langer, R., & Farokhzad, O. C. (2012) "Nanoparticle delivery of cancer drugs". *Annual Review of Medicine*, 63, 185–198.
- [8] DOI:10.1146/annurev-med-040210-162544
Jain, R. K. (2012) "Delivery of molecular and cellular medicine to solid tumors". *Advanced Drug Delivery Reviews*, 64, 353–365.
- [9] DOI: 10.1016/j.addr.2012.09.011
Prabha, S., & Labhasetwar, V. (2004) "Nanoparticle-mediated wild-type p53 gene delivery". *Molecular Pharmaceutics*, 1(3), 211–9.
- [10] DOI: 10.1021/mp049970+
- [11] Fontana, F., Liu, D., Hirvonen, J., & Santos, H. A. (2016) "Delivery of therapeutics with nanoparticles: new perspectives in cancer immunotherapy". *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2017 Jan; 9(1). DOI: 10.1002/wnan.1421
- [12] Laurence Zitvogel, Lionel Apetoh, François Ghiringhelli, Guido Kroemer "Immunological aspects of cancer chemotherapy" *Nat Rev Immunology* . 2008 Jan; 8(1):59-73. Doi: 10.1038/nri2216.
- [13] Hu C. M., Kaushal S., Tran Cao H. S., Aryal S., Sartor M., Esener S., et al. (2010). "Half-antibody functionalized lipid-polymer hybrid nanoparticles for targeted drug delivery to carcinoembryonic antigen presenting pancreatic cancer cells". *Mol. Pharm.* 7 914–920. 10.1021/mp900316a DOI: 10.1021/mp900316a
- [14] Venturoli D., Rippe B. (2005). "Ficoll and dextran vs. globular proteins as probes for testing glomerular permselectivity: effects of molecular size, shape, charge, and deformability". *Am. J. Physiol. Renal. Physiol.* 288 F605–F613. 10.1152/ajprenal.00171.2004 DOI: 10.1152/ajprenal.00171.2004
- [15] O'Brien M. E., Wigler N., Inbar M., Rosso R., Grischke E., Santoro A., et al. (2004). "Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer". *Ann. Oncol.* 15 440–449. 10.1093/annonc/mdh097 DOI: 10.1093/annonc/mdh097
- [16] Cortes J., Saura C. (2010). "Nanoparticle albumin-bound (nab (TM))-paclitaxel: improving efficacy and tolerability by targeted drug delivery in metastatic breast cancer". *EJC Suppl.* 8 1–10. 10.1016/s1359-6349(10)70002-1 [https://doi.org/10.1016/S1359-6349\(10\)70002-1](https://doi.org/10.1016/S1359-6349(10)70002-1)
- [17] Han G., Ghosh P., Rotello V. M. (2007). "Functionalized gold nanoparticles for drug delivery". *Nanomedicine* 2 113–123. 10.2217/17435889.2.1.113 <https://doi.org/10.2217/17435889.2.1.113>
- [18] Jiang Y., Huo S., Hardie J., Liang X. J., Rotello V. M. (2016). "Progress and perspective of inorganic nanoparticle-based siRNA delivery systems". *Expert. Opin. Drug Deliv.* 13 547–559. 10.1517/17425247.2016.1134486 <https://doi.org/10.1517/17425247.2016.1134486>
- [19] Riley R. S., Day E. S. (2017). "Gold nanoparticle-mediated photothermal therapy: applications and opportunities for multimodal cancer treatment". *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 9:e1449. 10.1002/wnan.1449 <https://doi.org/10.1002/wnan.1449>
- [20] Luo E., Song G., Li Y., Shi P., Hu J., Lin Y. (2013). "The toxicity and pharmacokinetics of carbon nanotubes as an effective drug carrier". *Curr. Drug Metab.* 14 879–890. Doi: 10.2174/138920021131400110
- [21] Niloufar Rashidi, Majid Davidson, Vasso Apostolopoulos, Kulmira Nurgali "Nanoparticles in cancer diagnosis and treatment: Progress, challenges, and opportunities" *Journal of Drug Delivery Science and Technology* 95 (2024) 105599 <https://doi.org/10.1016/j.jddst.2024.105599>

PLANT DISEASES, HOST IMMUNITY AND THE EMERGING CONCERN OF ANTIMICROBIAL RESISTANCE IN *BACILLUS*-BASED BIOPESTICIDES

Abstract

Plant diseases pose a serious threat to global food security by significantly reducing crop yield and quality. Infectious plant diseases caused by fungi, bacteria, viruses, nematodes, and other pathogens account for major agricultural losses worldwide. Plants rely on complex immune systems, including host and non-host resistance mechanisms, hypersensitive response, systemic acquired resistance and multi-layered signaling pathways, to defend against these pathogens. While chemical pesticides have traditionally been used for disease control, their environmental and ecological impacts have driven the adoption of biopesticides, particularly those based on *Bacillus* species. However, recent studies have revealed that commercially available *Bacillus*-based biopesticides may harbor antimicrobial resistance (AMR) genes. Phenotypic and genomic analysis demonstrates resistance to multiple clinically important antibiotics and reveals discrepancies between resistance genotypes and phenotypes. These findings highlight the need for rigorous evaluation and regulation of biopesticides to prevent unintended contributions to the global antimicrobial resistance crisis. Against this backdrop, this chapter reviews plant disease biology, host immunity, disease management strategies and antimicrobial resistance to critically examine the benefits and risks associated with modern biopesticide use.

Keywords: Plant diseases; Food security; Host resistance; Non-host resistance; Hypersensitive response; Systemic acquired resistance; Plant immunity; Biopesticides; *Bacillus* species; Antimicrobial resistance (AMR); Antibiotic resistance genes; Disease management; Sustainable agriculture; Plant pathogens; Biological control agents.

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I. INTRODUCTION

Sustaining agricultural productivity in the face of increasing biotic and abiotic stresses remains one of the most pressing challenges of the twenty-first century[45]. Plant diseases, driven by a wide range of fungal, bacterial, viral and nematode pathogens [1], continue to compromise crop yield, quality and stability across agro-ecosystems worldwide [16]. The impact of these diseases is amplified by climate change[19], intensification of agriculture and global trade, all of which facilitate pathogen persistence, dispersal and evolution [49]. As a result, plant health has become inseparably linked to food security, environmental sustainability and economic resilience [50].

Plants are not passive victims of disease but possess highly sophisticated immune systems that detect and respond to invading pathogens through multiple layers of defense [24]. However, under conditions of high pathogen pressure or complex co-infections involving multiple microbial species, these defenses may be overwhelmed, leading to severe disease outbreaks [2, 46]. Chemical pesticides have been the primary means of controlling plant diseases, but their widespread use has raised concerns regarding environmental degradation, non-target toxicity and disruption of beneficial soil microbiota . These limitations have accelerated the adoption of biological control agents, particularly biopesticides . They are generally perceived as environmentally safe alternatives to synthetic chemicals [43, 51]. Despite their benefits, the use of live microbial formulations introduces new and largely unexplored biosafety considerations potentially acting as reservoirs of antimicrobial resistance genes [44]

II. PLANT DISEASES

Plants form the foundation of life on Earth, acting as the main source of energy in ecosystems and being essential for the survival and progress of human society [1]. A plant becomes prone to disease when environmental conditions interfere with its normal structure, development or physiological processes. Plant diseases are generally classified into two groups: infectious (biotic) and non-infectious (abiotic). Non-infectious diseases arise from unfavorable growth conditions and do not spread from one plant to another. In contrast, infectious diseases result from living, multiplying agents such as bacteria, fungi, viruses and parasitic organisms[2]. Common symptoms include wilting from turgor loss and spotting (necrosis) from tissue death. Pathogens can cause rot, which destroys cell membranes or mummification, which shrinks and darkens plant organs. Pathological overgrowth, known as hypertrophy and hyperplasia, results in excessive tissue proliferation. Deformations and color changes, such as chlorosis and mosaic patterns, indicate nutrient or chloroplast dysfunction. These infectious agents spread through air, water, soil and vectors like insects or humans. Pathogens are highly resilient, often remaining infectious in natural reservoirs for several months or years [2].

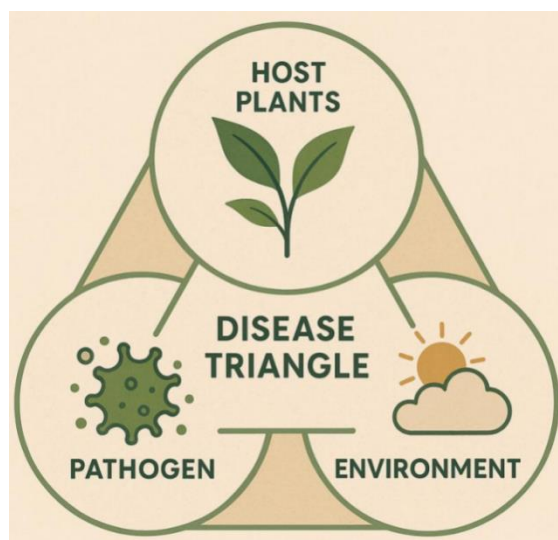


Figure 1: Disease Triangle: Interaction Between Host, Pathogen and Environment [1]

Table 1: Common Plant Pathogens and the Diseases Caused [1]

S.No	Pathogen	Disease
	Fungus	
1	<i>Phytophthora infestans</i>	Late blight in potatoes
2	<i>Puccinia graminis</i>	Stem rust in wheat
3	<i>Fusarium oxysporum</i>	Panama disease or Fusarium wilt in bananas
4	<i>Phytophthora ramorum</i>	Sudden oak death
5	Blue mold (<i>Penicillium spp.</i>) and Grey mold (<i>Botrytis cinerea</i>)	Postharvest diseases on fruits
6	<i>Hemileia vastatrix</i>	Coffee leaf rust
	Bacteria	
7	<i>Xanthomonas oryzae</i>	bacterial blight in rice
8	<i>Erwinia</i> and <i>Pectobacterium</i> species	Bacterial soft rots
9	<i>Pectobacterium amylovorum</i>	Fire blight of pear and apple trees
	Virus	
10	<i>Tobacco mosaic virus</i>	Tobacco mosaic disease
11	<i>Tomato yellow leaf curl virus</i>	Tomato yellow leaf curl disease
	Nematodes	
12	<i>Meloidogyne spp</i>	Root-knot in tomatoes, potatoes, and soybeans
	Phytoplasmas	Aster yellows and Coconut lethal yellowing
13	<i>Potato spindle tuber viroid (PSTVd)</i>	Stunted growth and reduced yields in potato

Table 2: Major Epidemic Plant Diseases, their Pathogens, Diagnostic Symptoms and Management Applications [1]

Plant disease (common name)	Scientific Name	Symptoms	Effective Management Methods & Agents
Wheat blast	<i>Magnaporthe oryzae Triticum</i>	Bleached, shriveled spikelets; lesions on leaves, nodes, and glumes; often leads to premature plant death.	Cultural: Burning infected crop residues, "wheat holidays" (banning cultivation for a period), crop rotation. Chemical: Fungicide sprays (e.g., strobilurins, triazoles) as protectants. Regulatory: Strict quarantine measures to prevent seed-borne spread.
Banana Fusarium wilt	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i>	Yellowing and wilting of leaves, starting from older leaves; splitting of pseudostems; internal vascular discoloration; plant death.	Genetic: Development and use of resistant/tolerant cultivars (ongoing). Cultural: Strict biosecurity measures (preventing movement of contaminated soil, plants, and equipment), exclusion, soil solarization, clean planting material. No effective chemical control for established infections.
Maize lethal necrosis	Maize chlorotic mottle virus	Stunting, severe mosaic or mottling on leaves, leaf reddening, premature drying of leaves, malformed ears, and significant yield loss.	Genetic: Use of resistant/tolerant maize varieties. Cultural: Vector control (e.g., for thrips, beetles), timely planting, rogueing infected plants, crop rotation, use of clean seed. Regulatory: Seed certification programs.
Coffee leaf rust	<i>Hemileia vastatrix</i>	Orange-yellow powdery lesions on the underside of leaves; premature defoliation; reduced berry set and vigor.	Genetic: Cultivation of resistant coffee varieties. Chemical: Fungicidal sprays (e.g., triazoles, strobilurins) applied preventatively or early in infection. Cultural: Pruning for improved air circulation, balanced fertilization, shade management.
Tomato brown rugose fruit virus	Tobamovirus (Tomato brown rugose fruit virus)	Mosaic patterns on leaves, rough or wrinkled fruit, brown necrotic spots on fruit, calyx necrosis, reduced yield and quality.	Cultural: Strict sanitation (tools, hands, clothing), rapid removal and destruction of infected plants, using certified virus-free seeds/transplants. Genetic: Breeding for resistance (emerging). Regulatory: Quarantine measures and strict import controls.
Potato late blight	<i>Phytophthora infestans</i>	Irregular, water-soaked lesions on leaves that turn brown/black; white fungal growth on undersides of leaves; potato tuber rot.	Genetic: Use of resistant potato varieties. Chemical: Fungicide sprays (e.g., protectants like chlorothalonil, systemic fungicides like propamocarb, dimethomorph), often in a scheduled program. Cultural: Crop rotation, proper plant spacing, hilling, destruction of cull piles, avoidance of overhead irrigation.
Ash dieback	<i>Hymenoscyphus fraxineus</i>	Lesions on stems and branches, often leading to necrosis; wilting of leaves; dieback of shoots; crown thinning; eventual tree death.	Genetic: Identification and breeding from naturally resistant ash trees. Cultural: Sanitation (removing infected trees), replanting with alternative tree species or resistant ash. Biological: Research into antagonistic fungi.

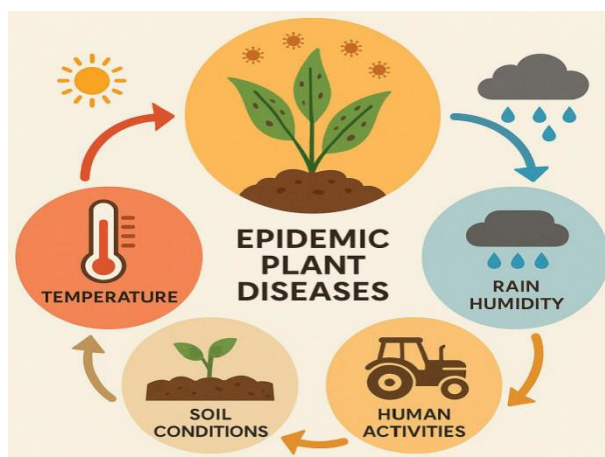


Figure2: Key Environmental Drivers of Epidemic Plant Diseases [1]

III.CO-INFECTIONS

Unlike mammalian disease models that emphasize multispecies synergistic interactions, plant pathology has traditionally focused more on monospecies or single-strain infections [15]. Colonization by more than one pathogen concurrently is known as co-infection and is widely observed in nature [46]. Co-infection can significantly modify disease dynamics throughout an extensive host–pathogen metapopulation [14].

A research study by Fang *et al.* in 2021 indicates that co-infection of alfalfa by *Fusarium oxysporum* f. sp. *medicaginis* (Fom) and *Rhizoctonia solani* (Rs) causes more severe disease than single infections and fundamentally alters disease-resistance responses across alfalfa varieties, revealing that resistance to individual pathogens does not translate into resistance under co-infection [3].

Zhang *et al.* in 2022 found out that a new, severe stem rot disease of *Aglaonema modestum* in South China is caused by the co-infection of two *Fusarium* species, a novel species *Fusarium aglaonematis* and known *Fusarium elaeidis* and that co-infection significantly increases disease severity compared to single infections [4].

More commonly, different pathogens with imperceptible relationships have been purified from the same disease plant tissue, such as the recent studies identifying *Dickeya zeae* and *Stenotrophomonas maltophilia* as the soft rot pathogens on *Clivia minita* [5], *Xanthomonas perforans* and *Pantoea ananatis* as the causal agents on water spinach (*Ipomoea aquatic*) [6], *Enterobacter asburiae* and *Pa. Ananatis* causing rice bacterial blight in China [7].

Understanding the interactions among different pathogens is equally crucial. Disease complexes often arise from the combined activity of multiple pathogens, including interactions between fungi, fungi and bacteria, fungi and nematodes or even closely related species. [4].

Both *Botryosphaeriaceae* and *Ilyonectria spp.* contributed to the decline of young grafted grapevines [9], the combination of *Didymella bryoniae* and pathogenic bacteria resulted in disease on Styrian oil pumpkin [10], co-infection of *Fusarium oxysporum* (*F. oxysporum*) f. sp. *lycopersici* and nematode (*Meloidogyne species*) on tomato caused serious damage in

Ethiopia [11], co-occurrence of two strains of the cassava mosaic virus led to severe symptoms on leaves of *Nicotiana benthamiana* [12]. Because multiple pathogens can interact through cooperation, competition or coexistence [4, 13], co-infections exhibit more intricate mechanisms than infections caused by a single pathogen. Therefore, it is essential to elucidate the pathogenic processes underlying co-infections [4].

IV. THREAT TO FOOD SECURITY

By 2050, the world's population will reach 9.7 billion and feeding them sustainably will be a difficult task [18]. Plant diseases have far-reaching consequences that extend beyond the immediate damage to crops, affecting economies, ecosystems and societies on a global scale [16]. However, agricultural productivity worldwide has dropped sharply due to plant diseases, of which fungal pathogens are responsible for nearly 70–80%. [19]. Fungal pathogens pose a serious risk to global food security, as many of the chemical fungicides used to control them show limited effectiveness. [20]. Economically, plant diseases are a major threat to agricultural productivity and food security, causing significant annual yield loss of more than 30% globally and financial hardships for farmers [16].

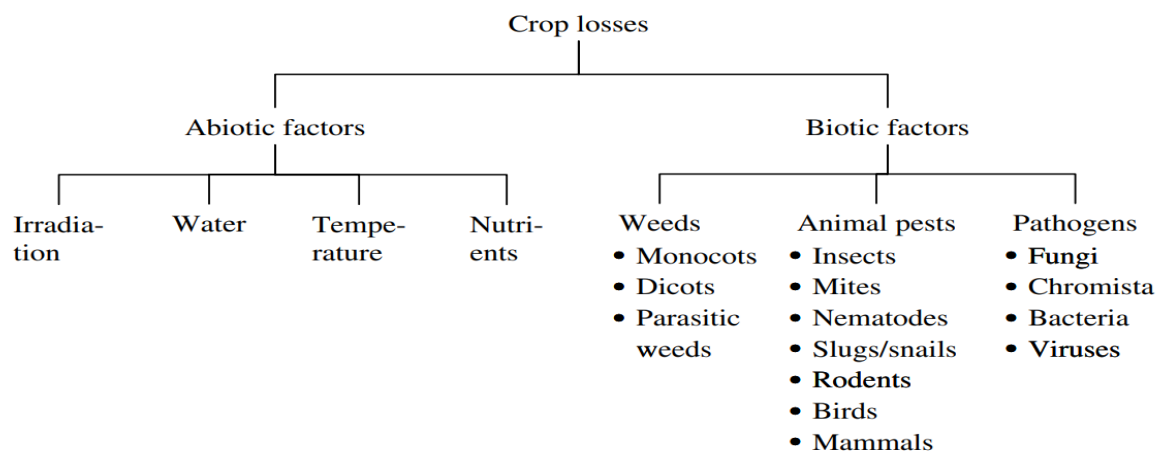


Figure 3: Abiotic and Biotic Elements Producing Crop Losses [17]

Table 3: Evaluated loss potential due to pathogens (fungi, bacteria), animal pests (nematodes, arthropods, birds, rodents, slugs and snails), weeds and viruses and actual losses due to pest classes in six important crops globally in 2001–03 [17].

Crop losses [%] ¹ due to											
Crop	Attainable production [M t]	Weeds		Animal pests		Pathogens		Viruses		Total	
		Potential	Actual	Potential	Actual	Potential	Actual	Potential	Actual	Potential	Actual
Wheat	785.0	23.0 (18–29)	7.7 (3–13)	8.7 (7–10)	7.9 (5–10)	15.6 (12–20)	10.2 (5–14)	2.5 (2–3)	2.4 (2–4)	49.8 (44–54)	28.2 (14–40)
Rice	933.1	37.1 (34–47)	10.2 (6–16)	24.7 (13–26)	15.1 (7–18)	13.5 (10–15)	10.8 (7–16)	1.7 (1–2)	1.4 (1–3)	77.0 (64–80)	37.4 (22–51)
Maize	890.8	40.3 (37–44)	10.5 (5–19)	15.9 (12–19)	9.6 (6–19)	9.4 (8–13)	8.5 (4–14)	2.9 (2–6)	2.7 (2–6)	68.5 (58–75)	31.2 (18–58)
Potatoes	517.7	30.2 (29–33)	8.3 (4–14)	15.3 (14–20)	10.9 (7–13)	21.2 (20–23)	14.5 (7–24)	8.1 (7–10)	6.6 (5–9)	74.9 (73–80)	40.3 (24–59)
Soybeans	244.8	37.0 (35–40)	7.5 (5–16)	10.7 (4–16)	8.8 (3–16)	11.0 (7–16)	8.9 (3–16)	1.4 (0–2)	1.2 (0–2)	60.0 (49–69)	26.3 (11–49)
Cotton	78.5 ²	35.9 (35–39)	8.6 (3–13)	36.8 (35–41)	12.3 (5–22)	8.5 (7–10)	7.2 (5–13)	0.8 (0–2)	0.7 (0–2)	82.0 (76–85)	28.8 (12–48)

¹ Figures in parentheses indicate variation among 19 regions.

² Seedcotton.

19 regions were specified according to the intensity of crop production and the production conditions: East Asia, Southeast Europe, North Africa, West Africa, East Africa, North America, Northern part of South America, Southern part of South America; Near East, South Asia, Central America, Southeast Asia, Asian states of the CIS; Northwest Europe, Southern Africa; Southern Europe, Northeast Europe, European part of the CIS; Oceania.[17]

More than 30% of annual crop production is lost to plant diseases, causing economic losses worth hundreds of billions of dollars, globally [16]. Food crops suffer significant yield losses worldwide due to microbial diseases related to 137 pathogens and pests, with mean losses of 30.3% for rice, 22.6% for maize, 21.5% for wheat, 21.4% for soybeans and 17.2% for potatoes in food security hotspots [22]. The damage to wheat, rice and maize by the fungi accounts for \$60 billion per year worldwide [21]. As the global population continues to expand while agricultural productivity declines, increasing strain is being placed on food availability, with plant diseases emerging as a significant threat to global food security. [16] The news published by Food and Agriculture Organization (FAO) on 3 April 2019, Rome says that according to FAO estimation, pests are responsible for the loss of approximately 20–40% of worldwide crop yields each year. Plant diseases alone result in economic losses of about USD 220 billion annually, while invasive insect species account for an additional USD 70 billion in damages [47].

V. PLANT IMMUNITY

Plant pathogens employ a range of mechanisms to infect their hosts, such as weakening plant immune responses and releasing toxins and cell-wall-degrading enzymes that facilitate colonization and nutrient acquisition [24]. Plant pathogens follow diverse infection strategies, with pathogenic bacteria commonly colonizing the apoplastic spaces after gaining entry via stomata, hydathodes or wounds [25].

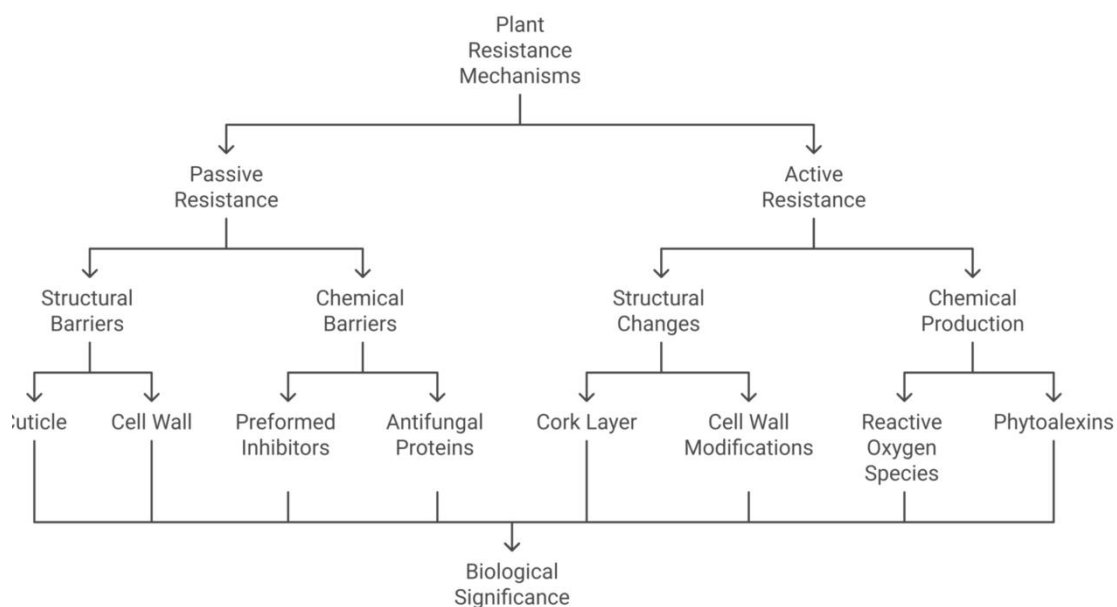


Figure 4: Plant Resistance Mechanisms

VI. HYPERSENSITIVE RESPONSE (HR)

One of the key defense strategies plants use against invading pathogens is the hypersensitive response (HR), which involves swift and localized cell death at the infection site. This targeted cell death helps protect the plant by restricting the pathogen's access to nutrients and preventing its further spread. [29]

VII. HOST AND NON HOST RESISTANCE

Based on the type of interaction between plants and pathogens, plant disease resistance is broadly classified into two categories: host resistance and nonhost resistance [26]. Host resistance and Nonhost resistance (NHR) are generally distinguished by whether a pathogen is specialized to infect a given species (the host) or is unable to establish infection in species to which it is not adapted (the nonhost) [26]. Single R genes commonly control host resistance, making it less durable, whereas nonhost resistance is a complex, multi-gene phenomenon that provides more sustained protection [26]. Nonhost resistance is again divided into type I and type II nonhost resistance. Type I nonhost resistance does not produce any visible symptoms whereas type II nonhost resistance results in a rapid hypersensitive response with cell death [27].

VIII. SYSTEMIC ACQUIRED RESISTANCE (SAR)

When plants are infected by necrotizing pathogens, they often develop increased resistance to subsequent pathogen attacks, even in tissues that were not initially infected. This broad, long-lasting defense response is known as systemic acquired resistance (SAR). Because systemic acquired resistance relies on recalling prior exposure, it exemplifies the concept that plants possess a memory-like mechanism. [30].

DIR1 (Defective in Induced Resistance 1) is a lipid transfer protein that plays a key role in systemic acquired resistance. It is transported via the plant vascular network and is thought to act as a carrier for lipid-derived signaling molecules, such as azelaic acid and glycerol-3-phosphate, thereby preparing remote, uninfected tissues to mount a faster and stronger defense upon later pathogen challenge[41]. .

IX. ZIGZAG MODEL OF PLANT IMMUNE RESPONSE IN NHR

In 2006, Jones and Dangl introduced the “zigzag” model to describe the coevolutionary dynamics between plants and pathogens, outlining two major layers of plant immunity. The first layer, known as pattern-triggered immunity (PTI), is activated by the detection of conserved microbial signatures such as PAMPs or MAMPs. The second layer, effector-triggered immunity (ETI), is initiated when plants recognize pathogen effectors that function to counteract or suppress PTI.[25]. During the first phase, plants perceive microbe- or pathogen-associated molecular patterns (MAMPs/PAMPs) through pattern-recognition receptors (PRRs), initiating PAMP-triggered immunity (PTI). In the second phase, adapted pathogens introduce effector molecules that suppress PTI or facilitate nutrient acquisition and spread, leading to effector-triggered susceptibility (ETS). The third phase occurs when a specific effector is detected by a nucleotide-binding leucine-rich repeat (NB-LRR) protein, thereby activating effector-triggered immunity (ETI), a heightened defense response that frequently exceeds the threshold required to induce hypersensitive cell death (HR). In the fourth phase, pathogen populations evolve through the loss of recognized effectors and the acquisition of novel ones, often via horizontal gene transfer, enabling them to evade or

suppress ETI. In response, plants undergo selection for new NB-LRR variants capable of recognizing these newly evolved effectors, once again restoring ETI [25, 28]

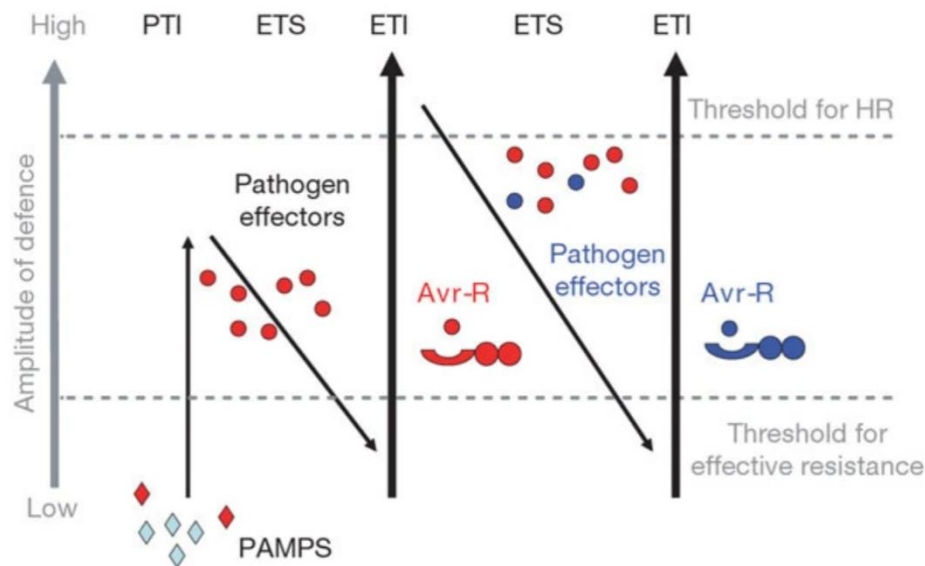


Figure 5: The Zigzag Framework Explains The Quantitative Dynamics Of Immune Responses In Plants. (red diamonds-PAMPS, red balls-effectors, blue balls-new effectors) [25].

X. GENES INVOLVED IN PLANT IMMUNITY

1. Genes Involved in Host Resistance

There are significant similarities between host and nonhost resistance mechanism [26]. Plant disease resistance (R) genes function by recognizing invading pathogens and activating defense responses to combat the infection[32]. Plant defense signaling is activated when host resistance (R) genes identify specific pathogen-derived effector molecules. The resistance mediated by R proteins is typically strain-specific, providing protection only against pathogens that produce the corresponding avirulence (Avr) effector recognized by the host. This type of defense response is frequently linked to the hypersensitive response (HR), characterized by the rapid programmed death of infected cells and occasionally nearby cells to restrict pathogen spread [32]

Host resistance involves single R genes like RPS4 , RPM1 in *Arabidopsis thaliana* [52], Yr5, Yr18, Yr26 [36] in wheat recognizing specific pathogen effectors (Avr protein) [52, 36] Pathogen detection within the cell is mainly controlled by immune receptors belonging to the nucleotide-binding and leucine-rich repeat (NLR) family [31].

2. Genes Involved in Non host resistance

Pre-existing physical defenses along with pre-existing chemical defenses such as continuously produced antimicrobial secondary metabolites, enable some plants to resist infection by non-adapted pathogens [8]. The leaf cuticle plays a role in providing non-host resistance against poplar leaf rust [38]. Many plants contain antifungal saponins that act as pre-existing chemical defenses against infection by phytopathogenic fungi [39]. In addition to these constitutive defenses, plants rely on two well-studied immune responses: pathogen-

associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) [8, 28].

Non host resistance genes involve multiple genes like NHO, PAD, and PEN and so on [37]. Salicylic acid (SA) is a central regulator of SAR, and its biosynthesis is activated by the transcription factors SARD1 and CBP60g, which are themselves stimulated by N-hydroxyphenylpyruvic acid (NHP) signalling. The immune regulators EDS1 and PAD4 function upstream, promoting NHP accumulation and SA signalling, thereby reinforcing systemic defence gene activation during SAR. Additionally, lipid-derived signalling molecules cooperate with NHP and SA pathways to transmit long-distance immune signals, strengthening systemic resistance throughout the plant [40, 42, 48].

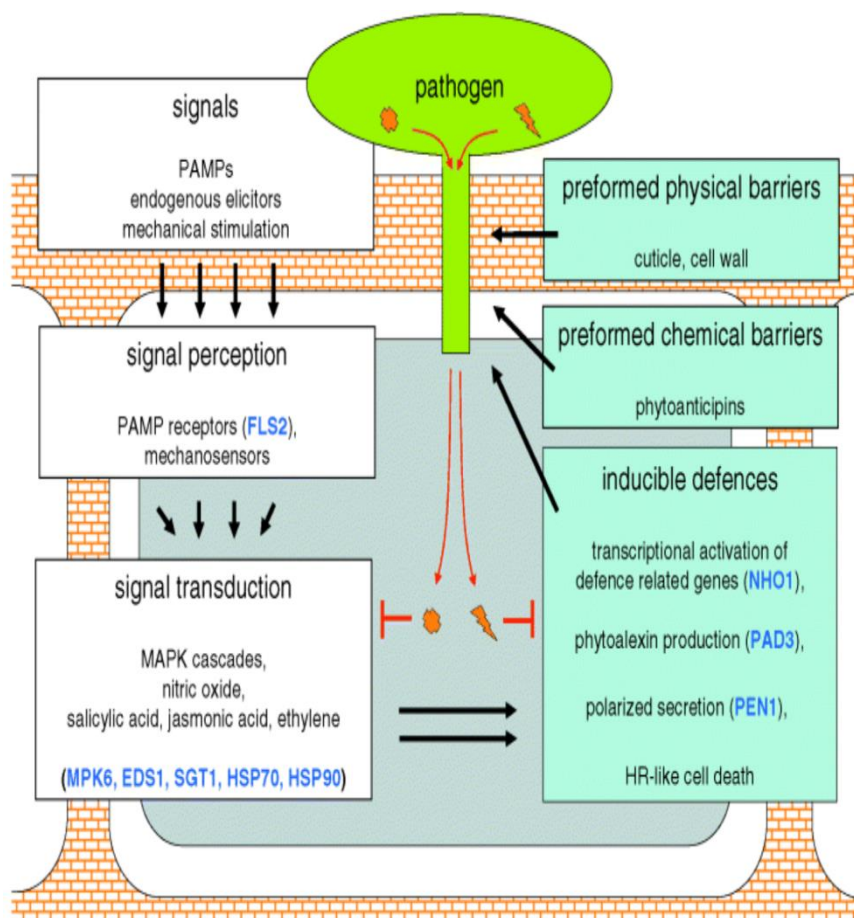


Figure 6: Non Host Resistance Through PAMP [37]

Abbreviations: MPK6, mitogen-activated protein kinase 6; SGT1, suppressor of G2 allele of Skp1; NHO1, non-host resistance 1; HSP, heat shock protein; PEN, penetration; FLS2, flagellin sensing 2; PAD3, phytoalexin deficient 3 [37].

Table 4: R Loci Conferring Resistance To Host-Selective And Host Non-Selective Toxins Produced By Fungal And Bacterial Pathogens [34]

Toxin type	Plant R Locus	Plant Species	Resistance mechanism	Pathogen	Infection type/ Organ attacked	Toxin name	Toxin type	Toxin function	Race specific	Reference
Host-selective										
1	<i>Hm1</i>	Maize	Detoxifying enzyme HC-toxin reductase	<i>Cochliobolus carbonum</i> (race 1)	Fungal necrotroph/ leaf	HC	Cyclic tetrapeptide	Inhibition of histone deacetylase enzyme activity which is thought to interfere with the transcriptional activation of plant defence genes	Yes	Johal and Briggs (1992)
2	<i>Asc-1</i>	Tomato	TM helix-LAG1 motif facilitates a sphingolipid-dependent transport mechanism	<i>Alternaria alternata</i> f.sp. <i>lycopersici</i>	Fungal necrotroph/ leaf	AAL	Sphinganine analogue mycotoxins – (SAMs) – aminopenitol esters	Triggers programmed cell death which likely involves ceramide signalling and cell cycle disruption	No	Brandwagt <i>et al.</i> (2000)
3	Minus <i>T-urf13</i> gene in mitochondrial genome	Maize	Lack of pore formation in mitochondrial membrane	<i>Cochliobolus heterostrophus</i> (race T)	Fungal necrotroph/ leaf	T-toxin	Polyketide – linear polyketols	Direct binding to the tetrameric URF13 protein in the mitochondrial membrane, causes pore formation and leakage of mitochondrial contents	Yes	^a
4	vb/pc-2 (not cloned)	Oats	Not known	<i>Cochliobolus victoriae</i>	Fungal necrotroph/ leaf	Victorin	Cyclized chlorinated peptide	Binds to the P protein of the glycine decarboxylase complex in mitochondria	Yes	^a
5	ToxA insensitivity locus located to chromosome 5BL (not cloned)	Wheat	Lack of entry (internalization) from the extracellular space into the plant mesophyll cells of the resistant genotype	<i>Pyrenophora tritici-repentis</i>	Fungal necrotroph/ leaf	Ptr Tox A and Ptr Tox B	13.2 and 6.6 kDa proteins	Induction of host cell death in the absence of the pathogen. Binding to the chloroplast protein ToxABP1	Yes	(^a) and Manning <i>et al.</i> (2007)
Host nonselective										
6	<i>Fhb1</i> (Chr3B)	Wheat	Masking of toxins by the formation of conjugated toxin-glycosides	<i>Gibberella zeae</i> and <i>Fusarium culmorum</i>	Fungal, nonbiotroph/ floral, stem base and roots	Deoxynivalenol	B-type sesquiterpenoid trichothecenes	Binds to the peptidyl transferase protein in the ribosome and inhibits protein translation	No	Lemmens <i>et al.</i> (2005)
7	None	Tobacco	Toxin efflux or toxin reduction (yeast)	<i>Cercospora nicotianae</i>	Fungus	Cercosporin	Superoxide generating perylenequinone photosensitizer	Singlet oxygen generation leading to peroxidation of membranes	No	Daub and Ehrenshaft (2000)
8	Tabtoxin resistance gene (ttr) from <i>Pst</i> overexpressed in plants	Beans, soybean	Acetyltransferase activity	<i>Pseudomonas syringae</i> pv. <i>tabaci</i>	Bacteria	Tabtoxin	Dipeptide	Hydrolysed by plant cells to release tabtoxinine, the active toxin which inactivates glutamine synthetase and leads to ammonia accumulation	No	Anzai <i>et al.</i> (1989)

XI. PLANT DISEASE CONTROL

Traditional farming methods have long been applied to control plant diseases, such as maintaining field hygiene, regulatory measures, use of resistant cultivars, crop rotation systems, soil solarization, biofumigation, soil conditioning, anaerobic soil disinfection, steam sterilization of soil, soilless cultivation and maintaining soil nutrient balance. In addition, chemical pesticides, which are designed to act on specific targets include nematicides,insecticides, nematicides,bactericides, herbicides and fungicides [43]. The excessive application of chemical pesticides can disturb soil microbial diversity by modifying soil properties. They interfere with important soil biochemical processes, including nitrogen fixation, ammonification and nitrification, by influencing the activity of certain microorganisms and enzymes. Additionally, these chemicals slow down the mineralization of

soil organic matter, which plays a crucial role in maintaining soil fertility and productivity [43].

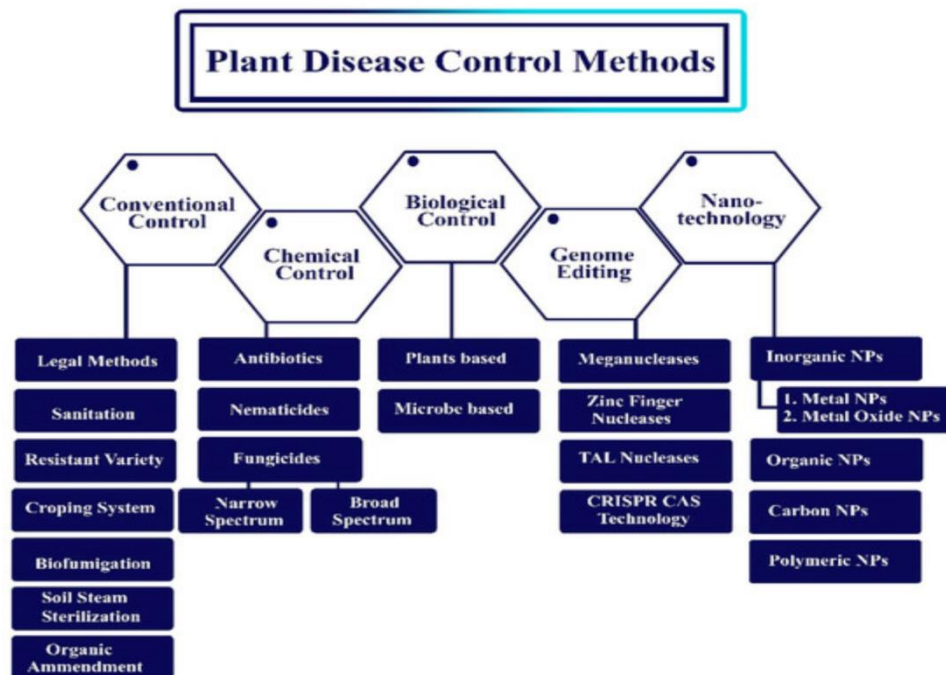


Figure 7: Plant Disease Control Methods [43]

1. Biopesticides:

Biopesticides are pest control agents obtained from natural sources such as microorganisms, plants or compounds produced from biological organisms [43].

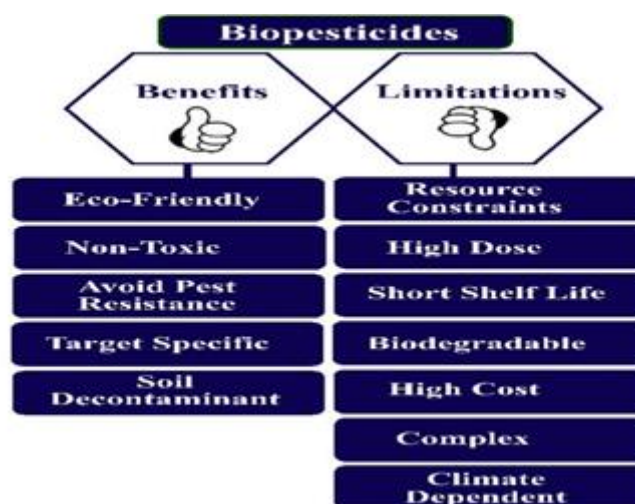


Figure 8: Benefits and Limitations of Biopesticides [43]

These bio pesticides contain live bacteria [43] but very little attention has been given to whether these bacteria carry antimicrobial resistance (AMR) genes [44], which could be harmful to human, animal and environmental health [35]. Bio pesticides made from *Bacillus* bacteria are widely used in agriculture to control pests and plant diseases [23]. Antimicrobial resistance is the ability of microorganisms such as bacteria, fungi, viruses and parasites to survive and multiply despite the presence of medications intended to eliminate them. The spread of AMR is a global health problem [35]

2. Analysis of four commercially available *Bacillus*-based biopesticides

Kaze *et al.* in 2021 analysed four *Bacillus*-related biopesticide strains available in the market: *Bacillus thuringiensis* subsp. *kurstaki* strain SA12 (two products from different manufacturers), *B. amyloliquefaciens* D747 and *B. subtilis* QST 713, designated *Bt-kurstaki*-1, *Bt-kurstaki*-2, *B. amyloliquefaciens* and *B. subtilis*, respectively using a combination of bioinformatics and antimicrobial susceptibility testing [44].

XII. ANTIMICROBIAL SUSCEPTIBILITY TESTING

Products were cultured in Luria–Bertani (LB) medium at 22 °C for 8 h, standardized to 0.5 McFarland turbidity ($\sim 1.5 \times 10^8$ cells), and spread onto Mueller–Hinton agar plates. Antimicrobial susceptibility was assessed using the Kirby–Bauer disc diffusion method with Oxoid antibiotic discs, including clindamycin, doxycycline, linezolid, sulfamethoxazole/trimethoprim and vancomycin, incubated at 24 °C for 8 h. Control plates without bacteria were included to exclude contamination [44].

Minimum inhibitory concentrations (MICs) were assessed according to Clinical and Laboratory Standards Institute guidelines using Liofilchem MIC test strips under aerobic conditions. Tested antimicrobials included cephalazolin, clindamycin, ceftazidime, quinupristin/dalfopristin, ertapenem, imipenem, erythromycin and tetracycline, with MIC breakpoints interpreted according to EUCAST (The European Committee on Antimicrobial Susceptibility Testing) standards [44].

XIII. GENOMIC ANALYSIS

For genomic analysis, single colonies were isolated by streak plating to avoid mixed-strain contamination. DNA was extracted directly from products and from LB cultures using a modified Qiagen DNeasy protocol incorporating a 90 °C incubation step to disrupt Gram-positive cell walls. DNA quantity and quality were assessed using Qubit fluorometry and Eppendorf Biospec measurements. Sequencing libraries were prepared with the Illumina Nextera Flex kit and sequenced on an Illumina MiSeq platform. Reads were quality-checked with FastQC, trimmed using Trimmomatic, assembled with SPAdes (St. Petersburg genome assembler) and assembly quality assessed using QUAST (Quality Assessment Tool for Genome Assemblies). Genomes were annotated using RAST (Rapid Annotation using Subsystem Technology) and screened against CARD (Comprehensive Antibiotic Resistance Database), with AMR gene predictions manually curated and verified using UniProt. Identified AMR genes were quantified using RStudio [44].

All tested biopesticide products showed resistance to at least one clinically important antibiotic. Low-level resistance was observed to clindamycin, erythromycin and ertapenem across products. Imipenem resistance varied, with *Bt-kurstaki*-1 fully resistant, *Bt-kurstaki*-2 partially resistant, and *B. amyloliquefaciens* mostly susceptible. *B. amyloliquefaciens* and *B.*

subtilis were resistant to quinpristin/dalfopristin and tetracycline. *Bt-kurstaki*-1 and *Bt-kurstaki*-2 showed complete resistance to the cephalosporins ceftazidime and cefazolin. Disc diffusion tests confirmed resistance to clindamycin and sulfamethoxazole/trimethoprim in some *Bt-kurstaki* samples [44].

All products remained susceptible to doxycycline, linezolid and vancomycin. Resistance phenotypes were observed across five antibiotic classes: cephalosporins, lincosamides, streptogramins, sulfonamides and tetracyclines. Genomic analysis using CARD and genome annotation identified many resistance genes, but less than half were phenotypically expressed. Differences between the two genomic methods highlight inconsistencies between resistance genes and observed antibiotic resistance. Across the five drug classes corresponding to the resistance phenotypes, CARD detected twelve genes, while genome annotation revealed seven. Among the total antimicrobial resistance genes identified, fifty-two were detected by CARD and forty-seven by genome annotation, yet fewer than half translated into observable resistance phenotypes, accounting for 44% and 47%, respectively [44].

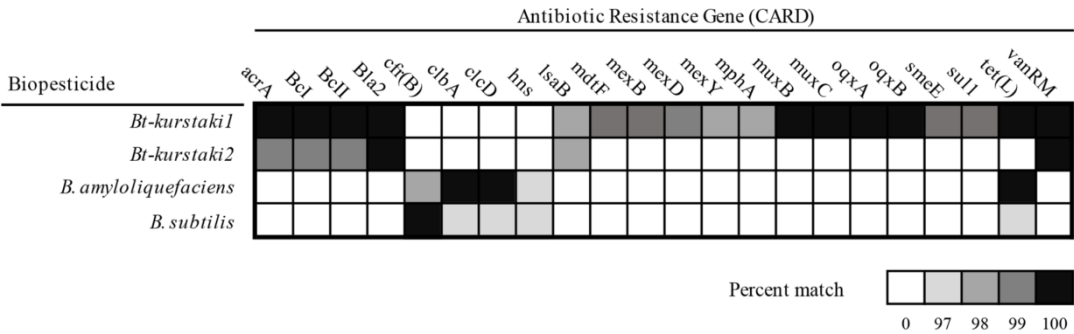


Figure 9: CARD-identified Antibiotic Resistance Genes for Each Commercial Biopesticide Product Studied . Square Shade Indicates Percent Match [44]

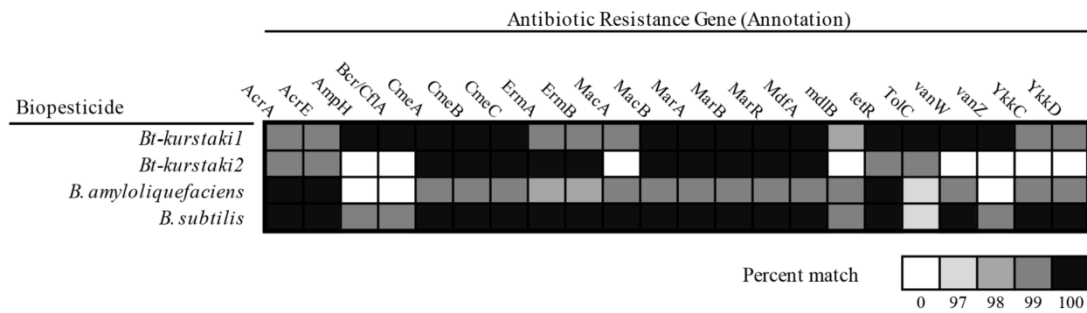


Figure 10: Annotation-identified Antibiotic Resistance Genes for each commercial biopesticide studied . Square shade indicates percent match [44]

The study by Kaze *et al.* in 2021 represents the first work to examine antimicrobial resistance in commonly distributed bacterial strains employed as commercial biopesticides. This study indicates that biopesticides may act as important sources and carriers of antimicrobial resistance within the wider environmental resistome, an area that remains largely unexplored [44].

XIV. CONCLUSION

Plant diseases continue to be a major constraint on global agricultural productivity and food security, driven by diverse pathogens, complex co-infections and changing environmental conditions [46, 49, 50, 51]. Plants possess robust immune systems that provide multilayered defense against pathogens, yet these defenses are often insufficient under high disease pressure [2, 24]. Biopesticides, particularly those based on *Bacillus* species, represent an important alternative to chemical pesticides and play a growing role in sustainable disease management [51].

However, evidence of antimicrobial resistance in *Bacillus*-based biopesticide products raises important safety concerns. Phenotypic resistance to multiple antibiotic classes and the presence of numerous resistance genes suggest that these products may contribute to the environmental spread of AMR. The observed mismatch between resistance genotypes and phenotypes further underscores the complexity of resistance expression and the limitations of current detection methods [44]. Therefore, comprehensive risk assessment, standardized screening for antimicrobial resistance and improved regulatory oversight are essential to ensure the safe and sustainable use of biopesticides. Addressing these challenges is critical to protecting crop health, environmental integrity and public health simultaneously.

REFERENCES

- [1] Abdelhak Rhouma Plant Diseases: Types, Causes and Impacts Egypt. J. Agric. Sci., 76.(3) 21-46. 2025
- [2] 10.21608/EJARC.2025.441655 DOI:10.21608/ejarc.2025.441655
- [3] P A Nazarov , D N Baleev , M I Ivanova , L M Sokolova , M V Karakozova Infectious Plant Diseases: Etiology, Current Status, Problems and Prospects in Plant Protection Acta Naturae . 2020 Jul-Sep;12(3):46–59. doi: 10.32607/actanaturae.11026
- [4] Xiangling Fang, Caixia zhang, Zi Wang, Tingyu Duan, Binhua Yu, Xitao Jia, Jiayin Pang, Lisong Ma, Yanrong Wang, Zhibiao Nan Co-infection by Soil Borne Fungal Pathogens Alters Disease Responses Among Diverse Alfalfa Varieties Frontiers in Microbiology July 2021 | Volume 12 | Article 664385 doi: 10.3389/fmicb.2021.664385
- [5] Zhang Y, Chen C, Mai Z, Lin J, Nie L, Maharachchikumbura SSN,
- [6] You C, Xiang M, Hyde KD and Manawasinghe IS (2022) Co-infection of *Fusarium aglaonematis* sp. nov. and *Fusarium elaeidis* Causing Stem Rot in *Aglaonema modestum* in China. Front. Microbiol. 13:930790. doi: 10.3389/fmicb.2022.930790
- [7] Ming Hu, Chuhao Li, Yang Xue, Anqun Hu, Shanshan Chen, Yufan Chen, Guangtao Lu, Xiaofan Zhou and Jianuan Zhou Isolation, Characterization, and Genomic Investigation of a Phytopathogenic Strain of *Stenotrophomonas maltophilia* Phytopathology 2021. 111: 2088 - 2099 <https://doi.org/10.1094/PHYTO-11-20-0501-R>
- [8] Ming Hu, Chuhao Li, Xiaofan Zhou, Yang Xue, Si Wang, Anqun Hu, Shanshan Chen, Xiuwen Mo and Jianuan Zhou Microbial Diversity Analysis and Genome Sequencing Identify *Xanthomonas perforans* as the Pathogen of Bacterial Leaf Canker of Water Spinach (*Ipomoea aquatic*) Front. Microbiol. 12:752760. doi: 10.3389/fmicb.2021.752760
- [9] Yang Xue, Ming Hu, Shanshan Chen, Anqun Hu, Shimao Li, Haiya Han, Guangtao Lu, Lisha Zeng and Jianuan Zhou *Enterobacter asburiae* and *Pantoea ananatis* Causing Rice Bacterial Blight in China 2021 105: 2078-2088 <https://doi.org/10.1094/PDIS-10-20-2292-RE>
- [10] Wu Y, Sexton W, Yang B, Xiao S. Genetic approaches to dissect plant nonhost resistance mechanisms. Mol Plant Pathol. 2023 Mar;24(3):272-283. doi: 10.1111/mpp.13290. Epub 2023 Jan 8. PMID: 36617319; PMCID: PMC9923397.
- [11] M. A. Whitelaw-Weckert , L. Rahman , L. M. Appleby , A. Hall , A. C. Clark ,H. Waite and W. J. Hardie Co-infection by *Botryosphaeriaceae* and *Ilyonectria* spp.fungi during propagation causes decline of young grafted grapevines 2013 British Society for Plant Pathology (2013) 62, 1226–1237 Doi: 10.1111/ppa.12059
- [12] Martin Grube, Michael Fürnkranz, Sabine Zitzenbacher, Herbert Huss Emerging multi-pathogen disease caused by *Didymella bryoniae* and pathogenic bacteria on Styrian oil pumpkin November 2011European Journal of Plant Pathology 131(3):539-548 DOI:10.1007/s10658-011-9829-8

- [13] Yitayih Kassie Status of Root-Knot Nematode (Meloidogyne Species) and Fusarium Wilt (Fusarium oxysporum) Disease Complex on Tomato (Solanum lycopersicum L.) in the Central Rift Valley, Ethiopia January 2019 Agricultural Sciences 10(08):1090-1103 DOI:10.4236/as.2019.108082
- [14] V N Fondong 1, J S Pita, M E Rey, A de Kochko, R N Beachy, C M Fauquet Evidence of synergism between African cassava mosaic virus and a new double-recombinant geminivirus infecting cassava in cameroon J Gen Virol . 2000 Jan;81(Pt 1):287-97. doi: 10.1099/0022-1317-81-1-287
- [15] Abdullah AS, Moffat CS, Lopez-Ruiz FJ, Gibberd MR, Hamblin J and Zerihun A (2017) Host–Multi-Pathogen Warfare: Pathogen Interactions in Co-infected Plants. Front. Plant Sci. 8:1806. doi: 10.3389/fpls.2017.01806
- [16] Hanna Susi, Benoit Barrès, Pedro F. Vale & Anna-Liisa Laine Co-infection alters population dynamics of infectious disease Nature Communications volume 6, Article number: 5975 (2015) <https://doi.org/10.1038/ncomms6975>
- [17] Jay Ram Lamichhane, Vittorio Venturi Synergisms between microbial pathogens in plant disease complexes: a growing trend Front Plant Sci . 2015 May 27;6:385. doi: 10.3389/fpls.2015.00385
- [18] Gai, Y. and Wang H. (2024). Plant Disease: A Growing Threat to Global Food Security. Agronomy, 14(8), 1615. <https://doi.org/10.3390/agronomy14081615>
- [19] E.-C. OERKE Crop losses to pests Journal of Agricultural Science (2006), 144, 31–43. f 2005 Cambridge University Press 31 doi:10.1017/S0021859605005708
- [20] Ahmed Hamad, Aya Tayel Food 2050 concept: Trends that shape the future of food Journal of Future Foods, 2025, ISSN 2772-5669, <https://doi.org/10.1016/j.jfutfo.2025.03.003>.
- [21] Zhou, J.; Zhang, X.; Qu, Z.;Zhang, C.;Wang, F.; Gao, T.; Yao, Y.;Liang, J. Progress in Research on Prevention and Control of Crop Fungal Diseases in the Context of Climate Change. Agriculture 2024, 14,1108. <https://doi.org/10.3390/agriculture14071108>
- [22] Delgado-Baquerizo, M., Guerra, C.A., Cano-Díaz, C. et al. The proportion of soil-borne pathogens increases with warming at the global scale. Nat. Clim. Chang. 10, 550–554 (2020). <https://doi.org/10.1038/s41558-020-0759-3>
- [23] Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A. The global burden of pathogens and pests on major food crops. Nature ecology & evolution. 2019 Mar;3(3):430-9.
- [24] Ali S., Tyagi A., Bae H. Plant microbiome: An ocean of possibilities for improving disease resistance in plants. Microorganisms. 2023; 11:392. doi: 10.3390/microorganisms11020392
- [25] Madan Lal Verma 1 , Ashutosh Kumar , Anjani Devi Chintagunta , Prashant Jeevan Kumar Samudrala , Marc Bardin and Eric Lichtfouse Microbial Production of Biopesticides for Sustainable Agriculture Sustainability 2024,16, 7496. <https://doi.org/10.3390/su16177496> <https://www.mdpi.com/journal/sustainability>
- [26] Sajad Ali , Anshika Tyagi , Zahoor Ahmad Mir Plant Immunity: At the Crossroads of Pathogen Perception and Defense Response Plants (Basel). 2024 May 22; 13(11):1434. doi: 10.3390/plants13111434
- [27] Jonathan D. G. Jones & Jeffery L. Dangl The plant immune system Nature volume 444, pages323–329 (2006) doi: 10.1038/nature05286
- [28] Upinder S. Gill, Seonghee Lee and Kirankumar S. Mysore Host Versus Nonhost Resistance: Distinct Wars with Similar Arsenals Volume 105 Issue 5 Pages: 580 -712 2015 American Phytopathological Society <https://doi.org/10.1094/PHYTO-11-14-0298-RVW>
- [29] Kirankumar S Mysore ksmysore@noble.org · Choong-Min Ryu Nonhost resistance: how much do we know? Trends in Plant Science Volume 9, Issue 2P97-104February 2004 <https://doi.org/10.1016/j.tplants.2003.12.005>
- [30] Peter N. Dodds & John P. Rathjen Plant immunity: towards an integrated view of plant–pathogen interactions Nature Reviews Genetics volume 11, pages539–548 (2010) <https://doi.org/10.1038/nrg2812>
- [31] Jonathan Cohn , Guido Sessa a , Gregory B. Martin Innate immunity in plants Current Opinion in Immunology Volume 13, Issue 1, 1 February 2001, Pages 55-62 [https://doi.org/10.1016/S0952-7915\(00\)00182-5](https://doi.org/10.1016/S0952-7915(00)00182-5)
- [32] Uwe Conrath Systemic Acquired Resistance Plant Signal Behav. 2006 Jul-Aug; 1(4):179–184. doi: 10.4161/psb.1.4.3221
- [33] Mauricio P Contreras , Daniel Lüdke , Hsuan Pai AmirAli Toghani , Sophien Kamoun NLR receptors in plant immunity: making sense of the alphabet soup EMBO Rep. 2023 Aug 21;24(10):e57495. doi: 10.15252/embr.202357495
- [34] Mayank Anand Gururani, Jelli Venkatesh, Chandrama Prakash Upadhyaya, Akula Nookaraju, Shashank Kumar Pandey, Se Won Park Plant disease resistance genes: Current status and future directions Physiological and Molecular Plant Pathology Volume 78, April 2012, Pages 51-65
- [35] Boyes DC, Nam J, Dangl JL. The Arabidopsis thaliana RPM1 disease resistance gene product is a peripheral plasma membrane protein that is degraded coincident with the hypersensitive response. Proc

- Natl Acad Sci U S A. 1998 Dec 22; 95(26):15849-54. Doi: 10.1073/pnas.95.26.15849. PMID: 9861059; PMCID: PMC28133.
- [36] Kim E Hammond-Kosack, Kostya Kanyuka Resistance Genes (R Genes) in Plants *ENCYCLOPEDIA OF LIFE SCIENCES & 2007*, John Wiley & Sons, Ltd. www.els.net doi: 10.1002/9780470015902.a0020119
- [37] Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, Alqumber MAA. Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare (Basel)*. 2023 Jul 5;11(13):1946. doi: 10.3390/healthcare11131946. PMID: 37444780; PMCID: PMC10340576.
- [38] Zhang M, Saimi A, Liu Q, Ma Z, Chen J. The Detection of Yr Genes in Xinjiang Wheat Cultivars Using Different Molecular Markers. *Int J Mol Sci*. 2023 Aug 29;24(17):13372. doi: 10.3390/ijms241713372. PMID: 37686178; PMCID: PMC10487826.
- [39] Thorsten nürnberg er and Volkerlipka Non-host resistance in plants: new insights into an old phenomenon *Molecular plant pathology* (2005) 6 (3) , 335 – 345 doi : 10 . 1111 / j . 1364 - 3703 . 2005 . 00279.x
- [40] Yu, Z.D. , Shen, K.C. , Newcombe, G. , Fan, J.F. & Chen, Q.W. (2019) Leaf cuticle can contribute to nonhost resistance to poplar leaf rust. *Forests*, 10, 870, 2019
- [41] Bowyer, P. , Clarke, B.R. , Lunness, P. , Daniels, M.J. & Osbourn, A.E. (1995) Host - range of a plant - pathogenic fungus determined by a saponin detoxifying enzyme. *Science*, 267, 371 – 374.
- [42] Lin Wang, Kenichi Tsuda, William Truman, Masanao Sato, Le V Nguyen, Fumiaki Katagiri, Jane Glazebrook CBP60g and SARD1 play partially redundant critical roles in salicylic acid signaling *Plant J* . 2011 Sep;67(6):1029-41. doi: 10.1111/j.1365-313X.2011.04655.x. Epub 2011 Jul 6. PMID: 21615571.
- [43] Champigny MJ, Shearer H, Mohammad A, Haines K, Neumann M, Thilmony R, He SY, Fobert P, Dengler N, Cameron RK. Localization of DIR1 at the tissue, cellular and subcellular levels during Systemic Acquired Resistance in Arabidopsis using DIR1:GUS and DIR1:EGFP reporters. *BMC Plant Biol*. 2011 Sep 6;11:125. doi: 10.1186/1471-2229-11-125. PMID: 21896186; PMCID: PMC3180652.
- [44] Tian, H., Xu, L., Li, X., and Zhang, Y. (2025). Salicylic acid:The roles in plant immunity and crosstalk with other hormones.*J. Integr. Plant Biol*. 67: 773–785 <https://doi.org/10.1111/jipb.13820>
- [45] Akhtar, Hira, Muhammad Usman, Rana Binyamin, Akhtar Hameed, Sarmad Frogh Arshad, Hafiz Muhammad Usman Aslam, Imran Ahmad Khan, Manzar Abbas, Haitham E. M. Zaki, Gabrijel Ondrasek, and et al. 2024. "Traditional Strategies and Cutting-Edge Technologies Used for Plant Disease Management: A Comprehensive Overview" *Agronomy* 14, no. 9: 2175. <https://doi.org/10.3390/agronomy14092175>
- [46] Mo kaze, Lauren Brooks and Mark Sistrom Antimicrobial resistance in Bacillus- based bio pesticide product, *Microbiology (Reading)* 2021 Aug;167(8). doi: 10.1099/mic.0.001074. PMID: 34351257.
- [47] Daniel P. Roberts and Autar K. Mattoo Sustainable Agriculture—Enhancing Environmental Benefits, Food Nutritional Quality and Building Crop Resilience to Abiotic and Biotic Stresses *Agriculture* 2018,8, 8; doi:10.3390/agriculture8010008 www.mdpi.com/journal/agriculture
- [48] Hadjer Bellah, Nicolas F. Seiler, Daniel Croll Divergent Outcomes of Direct Conspecific Pathogen Strain Interaction and Plant Co-Infection Suggest Consequences for Disease Dynamics *ASM Journals Microbiology Spectrum* Vol. 11, No. 2 <https://doi.org/10.1128/spectrum.04443-22>
- [<https://www.fao.org/newsroom/detail/New-standards-to-curb-the-global-spread-of-plant-pests-and-diseases/en>]
- [49] Nair A, Goyal I, Voß E, Mrozek P, Prajapati S, Thurow C, Tietze L, Tittmann K, Gatz C. N-hydroxy-pipecolic acid-induced transcription requires the salicylic acid signaling pathway at basal SA levels. *Plant Physiol*. 2021 Dec 4;187(4):2803-2819. doi: 10.1093/plphys/kiab433. PMID: 34890459; PMCID: PMC8644824.
- [50] Jean B. Ristaino et al., The persistent threat of emerging plant disease pandemics to global food security *PNAS* 2021 Vol. 118 No. 23 e2022239118 <https://doi.org/10.1073/pnas.2022239118>
- [51] S. Chakrabortya and A. C. Newton, Climate change, plant diseases and food security: an overview *Plant Pathology* (2011) 60, 2–14 Doi: 10.1111/j.1365-3059.2010.02411.x
- [52] Ayilara MS, Adeleke BS, Akinola SA, Fayose CA, Adeyemi UT, Gbadegesin LA, Omole RK, Johnson RM, Uthman QO, Babalola OO. Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides. *Front Microbiol*. 2023 Feb 16;14:1040901. doi: 10.3389/fmicb.2023.1040901. Erratum in: *Front Microbiol*. 2024 Jan 03;14:1258968. doi: 10.3389/fmicb.2023.1258968. PMID: 36876068; PMCID: PMC9978502.
- [53] Gassmann W, Hinsch ME, Staskawicz BJ. The Arabidopsis RPS4 bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes. *Plant J*. 1999 Nov;20(3):265-77. doi: 10.1046/j.1365-313x.1999.t01-1-00600.x. PMID: 10571887.

NATURAL PHARMACY IN THE MUSHROOM AND PHARMACEUTICAL INDUSTRY

Abstract

Mushrooms, from time immemorial, have been used for various purposes in human culture. It has a very strong anthropological significance in human civilisations. It is exploited basically for edible and pharmaceutical purposes. The key reason why they stand out as excellent candidates of therapeutic exploitation can be very strongly attributed to their diverse and versatile secondary metabolite production. Mushrooms have a significant reservoir of bioactive secondary metabolites with diverse structural complexity and medicinal potential. These organisms produce a vast array of compounds, primarily categorized as polysaccharides, terpenoids, phenolics, and proteins, which are synthesized through dedicated biosynthetic gene clusters (BGCs). Key chemical classes include polyketides (PKS), synthesized from primary metabolites like acyl-CoAs, and non-ribosomal peptides (NRPS), derived from proteinogenic and non-proteinogenic amino acids.

Extensive research characterizes mushroom metabolites as potent anticancer agents that function by modulating key oncogenic signaling pathways in human body. Furthermore, these fungi act as effective prebiotics, shaping gut microbiota composition to enhance the production of short-chain fatty acids (SCFAs), which assist in maintaining immune homeostasis and host metabolic regulation. Studies on wild and cultivated species like *Agaricus bisporus*, *Ganoderma lucidum*, and *Pleurotus spp.* emphasize their roles in managing chronic diseases, oxidative stress, and nutrient deficiencies

Despite their long history in traditional medicine, the integration of mushrooms into modern therapy requires expanded clinical trials and standardized production methods.

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While thousands of species are known, only a fraction have been explored, leaving many metabolites unidentified and representing an untapped resource for future drug discovery. Advancements in genomics, proteomics, and metabolomics are essential for identifying "orphan" biosynthetic pathways and validating their efficacy in treating chronic and life-threatening diseases

Keywords: Mushroom, Secondary metabolites, PKS, NRPS, Anti-cancer

I. INTRODUCTION

Mushrooms are macro fungi that belong to the Division Basidiomycota and Ascomycota; they can be hypogeous and epigeous in nature. They are also known as wild edible fungi, because we are able to cultivate around 20-25 types of mushrooms found in the wild, the rest are collected from the jungles all over the world. Since ancient times, they have been used as a valuable food source and as traditional medicines around the world [1] Egyptians were the pioneers in this field; according to them, mushrooms were considered a gift from the Storm God Set and sent to earth via lightning. During ancient times, only Pharaohs were supposed to consume that; common people were not allowed to touch them. Temple pillars, ornaments can be seen in the shape of *Amanita* and *Psilocybe* and various other bracket-fungi[2], [3].

Ancient world fungus agarikon (*Fomitopsis officinalis*), a cure for all, was recommended by John Geard in his Herbal 1597, used to cure asthma, poor digestion, worms and the coughing of blood from the lungs [4], [5]. The warriors were instructed to eat mushrooms before the wars and battle, that help to improve their strength and endurance. Physician Dioscorides mentions in his herbal *De Materia Medica* that mushrooms grow on decaying, damp earth, are poisonous, least indigestible and have no nutritional value as food. But in China and Japan, mushrooms were considered to be life-extending and even the leaders of immortality. China and Japan are the leading countries in the field of traditional medicine concerning mushrooms. [6].

The mythical "Sage King" Shennong is considered the Father of traditional Chinese medicine (TCM). He classifies mushrooms as aid of the life forces, for different parts of bodies. Europe, America, China, and Japan have different views regarding the medicinal value of mushrooms. In the Rigveda, mushrooms have been mentioned that in ancient India, Aryans used to drink "Somras" was believed to be obtained from the diluted decoction of *Amanita muscaria*. [7]

In the present article, various mushrooms associated with the cure of various diseases and their chemical constituents will be discussed. These days, mushrooms are creating a boom in the Pharmaceutical Industry, enabling in establishing startups and entrepreneurship.

II. GLOBAL SCENARIO OF MUSHROOM PRODUCTION

Mushroom farming is gaining widespread traction all over the world, with the advancement in the technology of cultivation. The global mushroom market is expanding horizontally and

vertically due to its edible and medicinal properties. They help in achieving Sustainable Development Goals (SDGs) and ensure food security[8].

France was the leader in the cultivation of mushrooms. It was documented to be grown in the Caves of Paris. Then it was introduced in England, where gardeners found it easy to grow. Now, in present-day China is the leading producer country in the field of mushroom cultivation, producing 70 per cent of the World's mushroom production. China is generating revenue by cultivating and selling of mushrooms [9]. USA is the second largest mushroom producing country after China, followed by European Union countries, i.e. France, Germany, the Netherlands, Italy, Poland, Spain, Hungary and others [10].

Latin America is producing about 55 tonnes of *Agaricus* mushroom per day, followed by Argentina, Colombia, Brazil, Chile, Peru etc [11]. United States of America is the largest consumer of mushrooms in the world these days. US trade flows for canned mushrooms are dominated by imports, especially from China, India and Indonesia. Canada is the main market source for US exports of fresh mushrooms; it also exports to Japan and France [10].

Africa constitutes about 25% of the total mushroom biodiversity worldwide, but contributes only 0.4% of total mushroom sales and new mushroom products on the global market. Still, mushrooms are well known in most indigenous African recipes [12]. In many African countries, at the start of the rainy season, people search rural people for wild mushrooms.

The cultivation of mushrooms for food and medicine is increasingly popular across the world, including Africa. China, India and Indonesia are the three most important global mushroom-exporting countries in Asia. All three countries became the major suppliers of canned mushrooms import volume in 2007-2008. India produces about 250 thousand tons of edible mushrooms annually. Indian mushroom growers are using the latest technology in the production of mushrooms. Agro-Dutch estimated production capacity of 50,000-60,000 metric tons of fresh mushrooms for canning annually [10].

III.MEDICINAL MUSHROOMS

In ancient Chinese manuscripts, many records are available regarding the use of mushrooms in traditional medicines [13]. If we go through the history of mushrooms, medicinal mushroom species were scarce and were collected from the forests, where they grew on dead or living trees and forest litter. Mushrooms can be used in powder and liquid form, extract, decoction and concentrates for medicinal purposes. Nowadays, there is a rapid surge in this industry, due to the simultaneous agro-technological advancements. The key reason is the secondary metabolite production by the various species of mushrooms. Most of the medicinal mushrooms are subjected to large-scale artificial cultivation [14]. Mushrooms produce various types of bioactive molecules, polysaccharides, lectins, phenolic compounds, sterols, fats, tocopherols, glycosides, carotenoids, enzymes and folates [15], [16].

1. *Agaricus* Species

Agaricus commonly known as button mushroom. 80% *Agaricus* is grown all over the world. *Agaricus* species have been used to treat heart, stomach ulcers, tumors, digestive problems, diabetes and other medical problems

[17]. *Agaricus* sp. has been consumed to boost the immune system and to cope with physical and emotional stress. *Agaricus campestris* is known for its insulin releasing and glucose level-lowering activities [18]. Lectins isolated from the *Agaricus* is anti-diabetic in nature.

2. *Ganoderma lucidum*

Ganoderma is commonly known as lingchi and is the main constituent of Chinese medicine. *Ganoderma* have a typical structure i.e. kidney shape, hard and woody in texture. It is of various colour, commonly reddish brown in colour. It has a bitter taste due to the presence of various fatty acids, Terpenoids, Sterols, and 'Ganodermin'. It can be used in the form of extract, powder, capsule and coffee [19] *Ganoderma lucidum* contains many bioactive compounds i.e., triterpenoids, polysaccharides and peptidoglycans, which contribute to medicinal properties [20]. They have antioxidant properties, anti-inflammatory and anticancer effects. It exhibits immuno-boosting, immuno-modulatory effects ; significantly boosting the activity of the T-lymphocytes [21].

3. *Podaxis pistillaris*

Podaxis pistillaris belongs to family Agaricaceae. It is known for its medicinal and nutritional value. Generally it is collected by the people of rural area [22]. It has antimicrobial properties, used for skin diseases and inflammation [23].

In India has been reported from Haryana that people considered it good for health. They used it externally for the treatment of skin and wounds, worked as a healing agent. Fresh and dried fruiting bodies after mashing mixed with mustard oil can be applied on wounds. As this mushroom is a rich source of calcium can be used for bone cracks and for pregnant women [24]. In people of *Sindh*, they consume this mushroom by frying and it is recommended for diabetic and heart patients by traditional herbal doctors [15]

From Australia reports, instances are available that *Podaxis* is used by old man to darken their white hair and it's use as an anti-fly repellent [25]. *Podaxis pistillaris* has antibacterial activity against human pathogenic bacteria *Pseudomonas aeruginosa* and *Proteus mirabilis*[24].

4. *Phellorina herculeana*

Phellorina herculeana (Pers.) Kreisel is a wild edible mushroom found in arid and semi-arid regions. It usually grows on sandy soil, under high temperature and low humidity and is highly acceptable to xeric conditions [26]. From India, first time it was reported by Ahmad (1939) in the Punjab region, and from different parts of India [27], [28]. Commonly it is known as Marusthali Khumbi, relished by the local people since centuries and people of Thar people Desert of India, known for its deliciousness and medicinal value.

The villagers collected the wild mushroom for its their consumption, medicinal purposes and business. For very short period it is available in market during rainy season, cost of this mushroom is about ₹ 400-500 per kilogram. *Phellorina inquinans* is highly nutritive, contains high amount of proteins, minerals and essential amino-acids reported the presence of: 23.30% crude protein, 9.38% carbohydrate, 2.43% reducing sugars, 6.68% non-reducing sugars, 1.77% lipid, 88.65% moisture, 13.07% crude fibres, 24.60% ash on a dry weight basis [29]. *Phellorina* sps are antidiabetic, antimicrobial, repair of cracked bones and in cure of heart diseases[26].

5. *Pleurotus* Species

Pleurotus mushrooms are commonly known as Oyster mushrooms. Various species of mushrooms can be grown whole of the year as they can grow at various temperatures i.e., *P. florida*, *P. sajor cajo*, *P. djmaor*, *P. citrenopileatus* etc. The secondary metabolites of the *Pleurotus* genus, which can be isolated from both the fruiting bodies and the mycelia, are responsible for the mushroom's diverse health-promoting and therapeutic effects [30], [31]. These bioactive substances are generally classified into high-molecular weight compounds, such as polysaccharides (notably β -glucans like pleuran), peptides, and proteins, and low-molecular weight compounds, including terpenoids, fatty acid esters, and polyphenols[32]. Specific low-molecular weight metabolites include lovastatin, which helps regulate cholesterol, and phenolic acids such as ferulic and p-coumaric acid [33], [34], [35]. Furthermore, advanced chemical analyses such as Gas Chromatography-Mass Spectrometry (GC-MS) have identified unique compounds like squalene, piperidin-4-carboxylic acid, and various alkaloids, while qualitative tests confirm the presence of tannins, flavonoids, and steroids in different species [36]. Collectively, these metabolites exhibit a wide range of biological activities, including immunostimulatory, anti-neoplastic, antidiabetic, anti-inflammatory, and anti-oxidative properties[37].

IV. SECONDARY METABOLITES IN MACROFUNGAL SPECIES

Secondary metabolites are low-molecular-weight compounds produced by fungi, primarily filamentous Ascomycota and Basidiomycota, that are not essential for basic growth but play critical roles in environmental interaction, defense, and communication[38], [39]. These compounds are often biosynthesized via specialized pathways such as polyketide, terpenoid, and non-ribosomal peptide routes and are frequently encoded by contiguous biosynthetic gene clusters (BGCs) [39], [40].

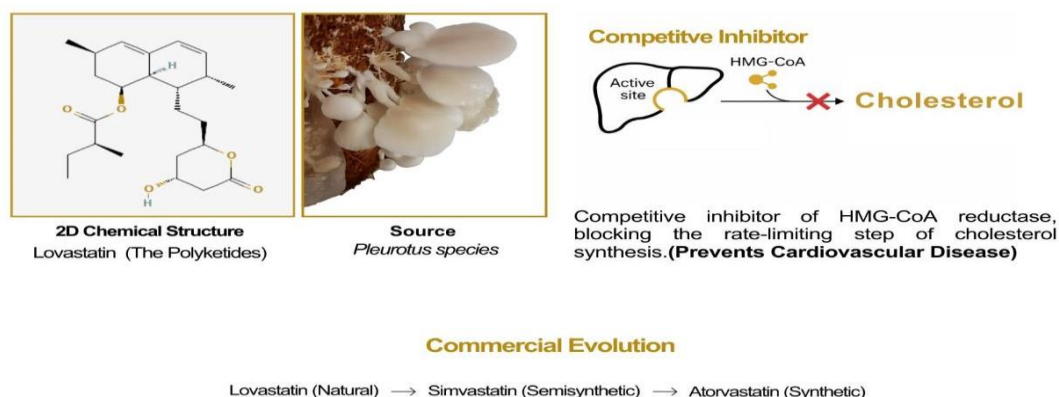


Figure 1: Infographic exhibiting the 2D- skeletal (bond-line) chemical structure, shown using wedge–dash stereochemical notation. (sourced and derived from <https://pubchem.ncbi.nlm.nih.gov/compound/Lovastatin> , The figure outlines its origin source, class, bioactivity and commercial evolution from its natural form. Diagram based upon [39]

One such secondary metabolite is Lovastatin, it's chemically classified as a polyketide. Its carbon backbone is synthesized from primary metabolite pools, specifically acyl-CoAs such as acetyl-CoA and malonyl-Co. The production is catalyzed by two highly reducing type I

polyketide synthases (HR-PKSs). As highlighted previously, it acts as vigorous cholesterol lowering agent. It functions by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is the rate-limiting step in the cholesterol biosynthetic pathway (as shown in figure 1). It's mentioned that it is found predominantly in the fruiting bodies of multiple *Pleurotus* species. it's role is cogent in preventing cardiovascular disease.[39], [41], [42].

The following table 1. summarizes the key secondary metabolites produced by macro-fungi, categorized by their chemical class and biological activity.

Table 1: Secondary Metabolites Obtained From Various Mushrooms

Chemical Class	Biological Activity Category	Metabolite Name	Producing Mushroom Genus/Species	Major Bioactivity and Description
Terpenoids	Anticancer	Ganoderic Acids (T, D, DM)	<i>Ganoderma lucidum/lingzhi</i>	Induce apoptosis in metastatic lung and cervical cancer cells via mitochondrial dysfunction. Inhibit transcription factors; Activator
				Protein-1 (AP-1) and Nuclear Factor kappa B (NF-κB) to suppress breast cancer invasion.
		Lucidenic Acids	<i>Ganoderma lucidum</i>	Exhibit significant anti-invasive effects against hepatoma cells.
	Neuroprotective	Radianspenes	<i>Coprinellus radians</i>	Guanacastane-type diterpenoids showing potent inhibitory activity against breast cancer cell lines.
		Erinacines (A, C)	<i>Hericium erinaceus</i>	Pass through the blood-brain barrier to stimulate the synthesis of Nerve Growth Factor (NGF).• Protect against Parkinson's disease and ischemic stroke by

				reducing neuronal apoptosis.
		Sarcodonins	<i>Sarcodon scabrosus</i>	Cyathane diterpenoids that promote significant neurite outgrowth in neuronal cells.
	Antimicrobial	Pleuromutilin	<i>Clitopilus passeckerianus</i>	Diterpene antibiotic that targets the peptidyl transferase center of bacterial ribosomes.
		Astraodoric Acids	<i>Astraeus odoratus</i>	Lanostane triterpenes showing moderate activity against <i>Mycobacterium tuberculosis</i> .
	Anti-inflammatory	Ganoderic Acid DM	<i>Ganoderma lucidum</i>	Arrests osteoclastogenesis by suppressing the expression of c-Fos and NFATc1 transcription factors in bone marrow cells.
Phenolics	Antioxidant	Inoscavins	<i>Inonotus xeranticus</i>	Styrylpyrone polyphenols that act as potent free radical scavengers.
		Phelligradins	<i>Phellinus igniarius</i>	Oxygenated styrylpyrones exhibiting significant antioxidant and lipid peroxidation inhibition.
	Anti-inflammatory	Hispolon	<i>Phellinus linteus</i>	Suppresses ROS-mediated NF- κ B pathways to reduce inflammation in

				macrophages.
	Anticancer	Hispidin	<i>Phellinus linteus</i>	Induces apoptosis in gastric and epidermoid cancer cells and inhibits bladder cancer growth.
Polyketides	Antimicrobial	Strobilurins	<i>Strobilurus tenacellus</i>	Potent antifungal agents that interfere with mitochondrial respiratory electron transport.
		Bikaverin	<i>Fusarium spp.</i>	Antibacterial compound induced during interspecies confrontations to limit microbial competition.
	Antifungal	Griseofulvin	<i>Penicillium griseofulvum</i>	Inhibits mitosis by binding to tubulin, primarily used to treat infections of skin, hair, and nails.
	Hypolipidemic	Lovastatin (Monacolin K)	<i>Pleurotus ostreatus</i>	Inhibits HMG-CoA reductase, preventing the conversion of mevalonate into cholesterol.
	Cytotoxic	Oosporein	<i>Beauveria bassiana</i>	Weaponized polyketide used to poison insect cadavers and limit microbial competition for nutrients.
Alkaloids	Neuroactive	Psilocybin	<i>Psilocybe spp.</i>	Active pharmacological principle used in traditional ceremonies and under investigation for psychiatric medicine.

		Ergotamine	<i>Claviceps purpurea</i>	Neurotransmitter mimic used as a vasoconstrictor to treat migraine headaches.
	Antimalarial	Cordypyridones	<i>Cordyceps nipponica</i>	N-hydroxy-2-pyridones exhibiting potent in vitro activity against malarial parasites.
	Hypocholesterolemic	Eritadenine	<i>Lentinula edodes</i>	Adenosine analogue that reduces blood cholesterol by modifying hepatic phospholipid metabolism.
Peptides	Immunomodulatory	Cyclosporin A	<i>Tolypocladium inflatum</i>	Binds to cyclophilin A to inhibit calcineurin, used to prevent organ transplant rejection.
	Cytotoxic	Amatoxins (α -amanitin)	<i>Amanita phalloides</i>	Potent inhibitors of RNA polymerase II, leading to severe hepatic and renal cell death.
		Phallotoxins (Phalloidin)	<i>Amanita spp.</i>	Bind specifically to F-actin to stabilize the cytoskeleton, used as a tool in cell biology.
		Ustiloxins	<i>Villosiclava virens</i>	Toxic cyclic peptides that act as antimitotic agents by inhibiting microtubule assembly.

Polysaccharides	Immunomodulatory	Lentinan	<i>Lentinula edodes</i>	β -1,3-D-glucan that activates Natural Killer Cells (NK) cells and macrophages to enhance antitumor response.
		PSK (Krestin)	<i>Coriolus versicolor</i>	Protein-bound glycan that improves helper T-cells i.e. Th1/Th2 balance and increases overall survival in gastric cancer patients.
		Pleuran	<i>Pleurotus ostreatus</i>	β -glucan used to prevent recurrent respiratory tract infections by stimulating humoral and cellular immunity.
	Antiviral	PSP (Polysaccharide Peptide)	<i>Coriolus versicolor</i>	Triggers Toll-like receptors (TLR4)-dependent immune responses to downregulate viral replication in HIV models.
Small Molecules	Antitumor	Cordycepin	<i>Cordyceps militaris</i>	Toxic adenosine analogue that inhibits polyadenylation and acts as an RNA chain terminator.
	Antioxidant	Kojic Acid	<i>Aspergillus oryzae</i>	Inhibits tyrosinase to suppress melanogenesis; used in cosmetic products for skin lightening.

	Neurotrophic	Theanine	<i>Xerocomus badius</i>	Amino acid analogue that improves focus and relaxation by modulating neurotransmitters.
Source: [17], [38], [39], [40], [43], [44], [45], [46], [47], [48]				

Based on recent breakthroughs in genomic sequencing, studies have indicated that mushrooms possess between 30 and 70 secondary metabolite gene clusters per species, most of which are "cryptic" or unexpressed under standard laboratory conditions [40], [47]. The use of epigenetic manipulation (e.g., using HDAC inhibitors) and heterologous expression systems in *Aspergillus nidulans* or *Saccharomyces cerevisiae* is rapidly expanding the catalog of known mushroom metabolites, offering vast potential for future drug discovery in oncology and infectious disease management.

V. PHARMACEUTICAL AND THERAPEUTIC BIOACTIVITY OF MUSHROOMS

The pharmaceutical potential of medicinal mushrooms is heavily influenced by the environmental and developmental context of their secondary metabolites. Metabolite diversity and potency often differ between wild and cultivated specimens due to stress-induced bioactive molecule production. For instance, wild-grown *Ganoderma lucidum* typically exhibits a higher abundance of triterpenoids produced in response to environmental stressors, whereas cultivated versions often show elevated levels of total phenolic content (TPC) and water-soluble polysaccharides. Geographic, microclimatic, and edaphic factors directly impact the chemical composition and antioxidant capacity of wild species like *Armillaria mellea*, which has significant implications for bioavailability and drug discovery [49], [50]. While wild mushrooms offer a "treasure trove" of unexplored compounds, cultivation provides the standardized protocols and extraction techniques necessary for systematic quality evaluation and consistent pharmacological application[51].

Mushroom-derived metabolites serve as potent redox modulators and prebiotics through complex pharmacological pathways. Key antioxidant compounds include phenolics (such as gallic and caffeic acids), flavonoids, ergothioneine, and heteropoly saccharides These function via Reactive Oxygen Species (ROS) scavenging, inhibiting lipid oxidation, and metal chelation, with ergothioneine specifically protecting mitochondrial components from oxidative damage[52], [53], [54], [55].. Extracts from species like *Hericium coralloides* have been shown to upregulate endogenous antioxidant enzyme systems, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) [56]. As prebiotics, fungal polysaccharides like chitin, xylans, and α - β -glucans shape gut microbiota composition by selectively enhancing the growth of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*.This process increases the production of short-chain fatty acids (SCFAs) (acetate, propionate, and butyrate), which influence host metabolic pathways, regulate immune homeostasis, and suppress systemic inflammation [54], [57].

The immunomodulatory efficacy of mushroom β -glucans is intrinsically linked to their β -1,3/ β -1,6 structural architecture. These polysaccharides are recognized by innate immune receptors, primarily Dectin-1 (isoforms a and b), complement receptor 3 (CR3), and TLR2/4. Binding triggers intracellular cascades, such as the Spleen Tyrosine Kinase (Syk)-mediated pathway and the activation of NF- κ B, resulting in the secretion of pro-inflammatory

cytokines such as Tumor Necrosis Factor Alpha, Interlukin-6 (TNF- α , IL-6) and adaptive immune priming [58], [59], [60]. Exposure to β -glucans induces trained immunity, a functional reprogramming of myeloid cells and their bone-marrow progenitors involving metabolic shifts (increased glycolysis and oxidative phosphorylation) and epigenetic priming. This allows the innate system to exhibit a memory-like enhanced response to subsequent stimuli [51]. These actions support their use as immunostimulants or immunoadjuvants in combination therapies, such as with immune checkpoint blockades, i.e. Programmed cell death-1 / Programmed death-ligand 1 blockade (e.g., PD-1/PD-L1 inhibitors), to reverse resistance and enhance tumor clearance [57].

Based on studies made earlier, the pharmacological efficacy of medicinal mushrooms in oncology is attributed to their ability to modulate specific intracellular signalling cascades and the tumor microenvironment [53], [61], [62], [63], [64], [65], [66]. These secondary metabolites, including polysaccharides (β -glucans), triterpenoids, and polyphenols, target various hallmarks of cancer such as immune evasion, proliferative signalling, and multidrug resistance (MDR). Secondary metabolites mediate multi-targeted anticancer mechanisms by modulating key oncogenic signalling pathways and the tumor microenvironment. Compounds like triterpenoids from *G. lucidum* and polysaccharides from *Grifola frondosa* induce apoptosis via caspase-3, -8, and -9 activation and trigger cell-cycle arrest (G0/G1 or S phase)[67]. They also inhibit angiogenesis and metastasis by downregulating VEGF and suppressing matrix metalloproteinases (MMP2/9), as seen with hispolon from *Phellinus linteus* [68], [69]. Key targeted pathways include PI3K/AKT (inhibited by *Pleurotus spp.* to sensitise cells to chemotherapy), Wnt/ β -catenin (targeted by *Inonotus obliquus* to suppress tumor initiation), and MAPK/NF- κ B (blocked to prevent inflammatory-driven oncogenesis). To overcome multidrug resistance (MDR), specific metabolites like zhankuic acids can inhibit P-glycoprotein (Pgp) efflux pumps, increasing the intracellular accumulation of agents like paclitaxel. Pharmaceutical translation focuses on standardised extracts (e.g., Lentinan from *L. edodes*, PSK from *C. versicolor*) and strategies to enhance oral bioavailability, such as simulated digestion processing and micronutrient fortification (e.g., Vitamin D2 or Selenium enrichment), to preserve bioactive integrity for systemic immune education [51], [57].

The table 2. summarizes the anticancer mechanisms and molecular targets of various medicinal mushrooms

Table 2: Anticancer Mechanisms and Molecular Targets of Medicinal Mushrooms

Mushroom Species	Primary Molecular Targets	Mechanism of Action
<i>Antrodia camphorata</i>	Lgr5 /Wnt/β-catenin, JAK–STAT, PI3K/AKT/mTOR, MAPK, NF-κB	Inhibits colon and pancreatic carcinoma by downregulating proliferative signalling and inducing G0/G1 phase arrest and apoptosis.
<i>Ganoderma lucidum</i>	PD-1, NF-κB,PI3K/AKT, MAPK, p27kip,Cyclin D1/CDK4, Caspase 3/9	Suppresses tumour growth by inhibiting immune checkpoints, blocking Pgp expression via NF- κ B, and inducing apoptosis/autophagy.

<i>Phellinus linteus</i>	Wnt/β-catenin, ERK1/2, PI3K/AKT, FAK, NF-κB, MAPK, MMP2/9, uPa	Inhibits motility, invasion, and angiogenesis by downregulating matrix metalloproteinases and Wnt signalling genes like TCF/LEF.
<i>Inonotus obliquus</i>	CTLA-4/CD80, β-catenin	Blocks inhibitory immune checkpoint interactions to increase T-cell activity and suppresses nuclear β -catenin in colorectal cancer.
<i>Grifola frondosa</i>	NOTCH1, NF-κB, IκB-α, FLIP, Caspase 3/8	Induces apoptosis and S phase arrest through the Notch1–NF- κ B pathway and modulation of pro-inflammatory cytokines.
<i>Cordyceps militaris</i>	NF-κB, eIF2α, TORC1, Polyadenylation	Sensitises cells to TNF- α -induced apoptosis and terminates DNA/RNA elongation.
<i>Lentinula edodes</i>	PD-L1, MAPK	Suppresses chemo-induced PD-L1 expression to recover cell chemosensitivity and induces cytotoxicity in breast cancer cells.
<i>Trametes versicolor</i>	NF-κB, MAPK, PSK/PSP	Induces B-cell activation and enhances the immune status of immunocompromised patients by stimulating NK cells and T-cells.
<i>Pleurotus spp.</i>	PI3K/AKT, BAD (p-Ser 136)	Suppresses PI3K/AKT signalling to sensitise liver and ovarian cancer cells to chemotherapy agents like cisplatin.
<i>MDR</i> <i>Reve</i> <i>rsal Species</i> (e.g., <i>C. cibarius</i>, <i>R. emetica</i>)	P-glycoprotein (Pgp)	Blocks Pgp-mediated drug efflux to increase the intracellular accumulation and efficacy of Paclitaxel (TAX) and Doxorubicin (DOX).
Source: [50], [57], [61], [62], [64], [65], [66], [67]		

Mushroom metabolites act synergistically with conventional chemotherapy by modulating oncogenic signalling and efflux mechanisms (see table 2). They act as efficacious biological response modifiers. Various pharmacological strategies increasingly utilize these secondary metabolites as immunoadjuvants or in synergistic combinations with conventional chemotherapy. As noted earlier, β -glucans can restore PD-1/PD-L1 therapy responsiveness. While specific compounds like zhankuic acids from *Taiwano fungus camphoratus* serve as cytotoxicity enhancers by regulating Pgp in multi-drug resistant cancer cells. These multi-targeted mechanisms provide a robust framework for integrating fungal secondary metabolites into standardized therapeutic formulations[70], [71].

VI. CONCLUSION

In conclusion, medicinal and edible mushrooms represent a uniquely complex gold mine of

secondary metabolite primarily polysaccharides, terpenoids, and phenolics, with profound pharmaceutical and nutritional potential. These bio-factories produce diverse phytochemicals whose potency and diversity are intrinsically linked to species, environmental stressors, and geographic origins.

The pharmacological efficacy of these fungi is rooted in their multi-targeted mechanisms of action, most notably their ability to modulate the innate immune system through "Trained Immunity". Fungal β -glucans, recognised by innate receptors such as Dectin-1, trigger intracellular signalling cascades and functional reprogramming characterised by metabolic shifts and epigenetic priming. This immunomodulatory activity is further complemented by their role as prebiotics, in which fungal polysaccharides modulate the gut microbiota composition to enhance the production of short-chain fatty acids (SCFAs), thereby maintaining host metabolic regulation and immune homeostasis.

In the realm of oncology, mushroom-derived metabolites act as potent agents that can induce apoptosis, arrest the cell cycle, and inhibit key oncogenic signalling pathways such as PI3K/Akt, MAPK, and Wnt/ β -catenin. Crucially, specific compounds like triterpenoids and zhangic acids offer a strategic solution to multidrug resistance (MDR) by inhibiting P-glycoprotein (Pgp) efflux pumps, which enhances the intracellular accumulation and potency of conventional chemotherapeutic drugs. Beyond anticancer applications, mushroom extracts demonstrate significant antioxidant and anti-inflammatory properties, offering a natural and safe alternative to synthetic treatments for chronic diseases, including diabetes, cardiovascular disorders, and neurodegeneration.

While wild and cultivated mushrooms provide a robust foundation for drug discovery, the transition to standardised therapeutic formulations requires consistent molecular profiling and rigorous extraction protocols to ensure bioavailability and safety. Future pharmacological advancement depends on large-scale clinical trials and the development of synergistic drug-metabolite combinations to fully translate the vast biological potential of mushrooms into effective preventative and therapeutic regimens.

REFERENCES

- [1] R. Gupta and A. Singh, *Text Book of Mushroom Cultivation*. Astral Publishing House, 2024. [Online]. Available: <https://www.astralint.com>
- [2] S. R. Berlant, "The entheomycological origin of Egyptian crowns and the esoteric underpinnings of Egyptian religion," *Journal of Ethnopharmacology*, vol. 102, no. 2, pp. 275–288, 2005.
- [3] A. J. Kadmon and J. M. A. was born February, "THE SACRED MUSHROOM AND THE CROSS", Accessed: Feb. 03, 2026. [Online]. Available: https://www.academia.edu/download/87832680/John_Marco_Allegro_The_Sacred_Mushroom_and_the_Cross.pdf
- [4] P. E. Stamets, "Antipox properties of *Fomitopsis officinalis* (Vill.: Fr.) bond. et singer (agarikon) from the Pacific Northwest of North America," *International Journal of Medicinal Mushrooms*, vol. 7, no. 3, 2005, Accessed: Feb. 03, 2026. [Online]. Available: <https://www.dl.begellhouse.com/journals/708ae68d64b17c52,0d0f121956dd501b,0a3878105512f782.html>
- [5] B. Muszyńska *et al.*, "*Fomitopsis officinalis* : a Species of Arboreal Mushroom with Promising Biological and Medicinal Properties," *Chemistry & Biodiversity*, vol. 17, no. 6, p. e2000213, Jun. 2020, doi: 10.1002/cbdv.202000213.
- [6] M. Zázik and M. Daňová, "The Food of the Gods in the Empire of Man: Mushrooms and their use in everyday life and medicine in classical antiquity.," *History of Sciences & Technology / Dějiny Věd a Techniky*, vol. 55, no. 1, p. 78, Jan. 2022, doi: 10.70391/7e6.1-2.d.
- [7] S. Lawrence, *The Magic of Mushrooms: Fungi in Folklore, Superstition and Traditional Medicine*. Royal Botanic Garden Kew, 2022. [Online]. Available: <https://www.welbeckpublishinggroup>
- [8] V. V. Pandey, A. Kumari, M. Kumar, J. Saxena, C. Kainthola, and A. Pandey, "Mushroom

- cultivation: Substantial key to food security.,” *Journal of Applied & Natural Science*, vol. 10, no. 4, 2018, Accessed: Feb. 05, 2026. [Online]. Available: <https://core.ac.uk/download/pdf/188662739.pdf>
- [9] B. L. Muhammad and B. Suleiman, “Global Development of Mushroom Biotechnology,” *International Journal of Emerging Trends in Science and Technology*, vol. 2, no. 6, pp. 2660–2669, 2015.
- [10] B. L. Dhar, “Changing Global Scenario in Mushroom Industry,” in *Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8)*, 2014, p. 602.
- [11] M. Martinez-Carrera, “Mushroom Biotechnology in Tropical America,” *International Journal of Mushroom Sciences*, vol. 3, pp. 9–20, 2000.
- [12] P. M. Mpeketula, “Indigenous Mushroom Species Cultivation, Processing and Utilization for Food Security and Conservation,” in *National Research Council of Malawi Conference Proceedings*, 2008, pp. 95–105.
- [13] D. Bensky and A. Gamble, *Chinese Materia Medica*, 2nd ed. Seattle: Eastland Press, 1993.
- [14] P. Stamets, *Growing Gourmet and Medicinal Mushrooms*, 3rd ed. Olympia, WA: Ten Speed Press, 2000.
- [15] N. S. Atri and Mridu, “Podaxis pistillaris- A common wild edible mushroom from Haryana (India) and its Sociobiology,” vol. 44, Jul. 2015.
- [16] S. P. Wasser and A. L. Weis, “Medicinal Properties of Substances Occurring in Higher Basidiomycetes Mushrooms: Current Perspectives,” *International Journal of Medicinal Mushrooms*, vol. 1, pp. 31–62, 1999, doi: 10.1615/IntJMedMushrooms.v1.i1.30.
- [17] A. Bhambri, M. Srivastava, V. G. Mahale, S. Mahale, and S. K. Karn, “Mushrooms as Potential Sources of Active Metabolites and Medicines,” *Front. Microbiol.*, vol. 13, p. 837266, Apr. 2022, doi: 10.3389/fmicb.2022.837266.
- [18] S. M. Prasanna, S. Khanam, and K. K. Hullatti, “Effect of *Agaricus bisporus* and *Pleurotus ostreatus* on alloxan-induced hyperglycemic animals,” *Pharmacognosy Magazine*, vol. 4, no. 13, pp. 57–59, 2008.
- [19] B. S. Sanodiya, G. S. Thakur, R. K. Baghel, G. B. K. S. Prasad, and P. S. Bisen, “Ganoderma lucidum: a potent pharmacological macrofungus,” *Curr Pharm Biotechnol*, vol. 10, no. 8, pp. 717–742, Dec. 2009, doi: 10.2174/138920109789978757.
- [20] S. Sudheer, I. Alzorqi, S. Manickam, and A. Ali, “Bioactive Compounds of the Wonder Medicinal Mushroom ‘Ganoderma lucidum,’” pp. 1863–1893, 2019, doi: 10.1007/978-3-319-78030-6_45.
- [21] X. Zhu and Z. Lin, “Effects of Ganoderma lucidum polysaccharides on proliferation and cytotoxicity of cytokine-induced killer cells,” *Acta Pharmacol Sin*, vol. 26, no. 9, pp. 1130–1137, Sep. 2005, doi: 10.1111/j.1745-7254.2005.00171.x.
- [22] D. Arora, *Mushrooms Demystified: A Comprehensive Guide to the Fleshy Fungi*. Berkeley: Ten Speed Press, 1986.
- [23] X. L. Mao, *The Macrofungi in China*. Zhengzhou: Henan Publishing House of Science & Technology, 2000.
- [24] C. Panwar and D. K. Purohit, “Antimicrobial activity of Podaxis pistillaris and Phellorinia inquinans against Pseudomonas aeruginosa and Proteus mirabilis,” *Mushroom Research*, vol. 11, no. 1, 2002, Accessed: Feb. 03, 2026. [Online]. Available: <https://epubs.icar.org.in/index.php/MR/article/view/53400>
- [25] jurisdiction:Commonwealth of A. corporateName:Australian N. B. Gardens,; “Aboriginal use of fungi.” Accessed: Feb. 03, 2026. [Online]. Available: <https://www.anbg.gov.au/fungi/aboriginal.html>
- [26] P. Gehlot, R. Pathak, and S. K. Singh, “Phellorinia: An Under Exploited Mushroom,” *Indian Journal of Tropical Biodiversity*, vol. 24, no. 1, pp. 1–7, 2016.
- [27] S. Ahmad, “Higher Fungi of the Punjab Plains—II: Gasteromycetes,” *Journal of Botanical Society*, vol. 8, pp. 47–58, 1939.
- [28] R. D. Singh, “Mushroom Cultivation in Rajasthan,” in *Advances in Horticulture*, vol. 13, K. L. Chadha and S. R. Sharma, Eds., New Delhi: Malhotra Publishing House, 1995, pp. 489–496.
- [29] A. Doshi and B. Bohra, “Studies on Basidiospore Germination of Gasteromycetes Fungus *Phellorinia inquinans*,” *Micologia Italiana*, vol. 28, pp. 33–37, 2000.
- [30] I. Golak-Siwulska, A. Kałużewicz, T. Spizewski, M. Siwulski, and K. Sobieralski, “Bioactive compounds and medicinal properties of Oyster mushrooms (*Pleurotus* sp.),” *Folia Horticulturae*, vol. 30, no. 2, pp. 191–201, Dec. 2018, doi: 10.2478/fhort-2018-0012.
- [31] A. S. Sekan, O. S. Myronycheva, O. Karlsson, A. P. Gryganskyi, and Y. B. Blume, “Green potential of *Pleurotus* spp. in biotechnology,” *PeerJ*, vol. 7, p. e6664, Mar. 2019, doi: 10.7717/peerj.6664.
- [32] M. Jesenak, J. Majtan, Z. Rennerova, J. Kyselovic, P. Banovcin, and M. Hrubisko, “Immunomodulatory effect of pleuran (β-glucan from *Pleurotus ostreatus*) in children with recurrent respiratory tract infections,” *International immunopharmacology*, vol. 15, no. 2, pp. 395–399, 2013.
- [33] M. Gąsecka, M. Mleczek, M. Siwulski, and P. Niedzielski, “Phenolic composition and antioxidant

- properties of *Pleurotus ostreatus* and *Pleurotus eryngii* enriched with selenium and zinc,” *Eur Food Res Technol*, vol. 242, no. 5, pp. 723–732, May 2016, doi: 10.1007/s00217-015-2580-1.
- [34] N. Gunde-Cimerman and A. Cimerman, “Pleurotus fruiting bodies contain the inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase—lovastatin,” *Experimental mycology*, vol. 19, no. 1, pp. 1–6, 1995.
- [35] A. Matkovits, M. Fodor, and Z. Jókai, “Analysis of Polyphenol Patterns of *Pleurotus ostreatus* Cultivars by UHPLC-ESI-MS/MS; Application of FT-NIR and Chemometric Methods, Classification Options,” *Chemosensors*, vol. 12, no. 2, p. 19, 2024.
- [36] S. Madaan, S. I. A. Jabar, and B. P. Panda, “Fatty acids of *Pleurotus florida* mushroom: Potential molecules for blood glucose control,” *Food Bioscience*, vol. 46, p. 101558, 2022.
- [37] S. Govindaraj and S. Renganathan, “Molecular Characterization and Phytochemical Studies of *Pleurotus* sp to Unveil Species Diversity and Pharmacological Activities,” *J PURE APPL MICROBIO*, vol. 11, no. 2, pp. 891–901, Jun. 2017, doi: 10.22207/JPAM.11.2.28.
- [38] T. Anke, “Secondary metabolites from mushrooms,” *J Antibiot*, vol. 73, no. 10, pp. 655–656, Oct. 2020, doi: 10.1038/s41429-020-0358-6.
- [39] G. F. Bills and J. B. Gloer, “Biologically Active Secondary Metabolites from the Fungi,” *Microbiol Spectr*, vol. 4, no. 6, p. 4.6.01, Dec. 2016, doi: 10.1128/microbiolspec.FUNK-0009-2016.
- [40] N. P. Keller, “Fungal secondary metabolism: regulation, function and drug discovery,” *Nat Rev Microbiol*, vol. 17, no. 3, pp. 167–180, Mar. 2019, doi: 10.1038/s41579-018-0121-1.
- [41] M. S. Talkad, R. K. Das, P. Bhattacharjee, S. Ghosh, and U. P. Shivajirao, “Establishment of Enzyme Inhibitory Activities of Lovastatin, Isolated From *Pleurotus ostreatus*,” *Int J Appl Sci Biotechnol*, vol. 3, no. 3, pp. 408–416, Sep. 2015, doi: 10.3126/ijasbt.v3i3.12932.
- [42] J. A. Tobert, “Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors,” *Nat Rev Drug Discov*, vol. 2, no. 7, pp. 517–526, Jul. 2003, doi: 10.1038/nrd1112.
- [43] V. K. Chaturvedi, S. Agarwal, K. K. Gupta, P. W. Ramteke, and M. P. Singh, “Medicinal mushroom: boon for therapeutic applications,” *3 Biotech*, vol. 8, no. 8, p. 334, Aug. 2018, doi: 10.1007/s13205-018-1358-0.
- [44] D. D. De Silva *et al.*, “Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry,” *Fungal Diversity*, vol. 62, no. 1, pp. 1–40, Sep. 2013, doi: 10.1007/s13225-013-0265-2.
- [45] M. L. Gargano *et al.*, “Medicinal mushrooms: Valuable biological resources of high exploitation potential,” *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, vol. 151, no. 3, pp. 548–565, May 2017, doi: 10.1080/11263504.2017.1301590.
- [46] N. P. Keller, G. Turner, and J. W. Bennett, “Fungal secondary metabolism — from biochemistry to genomics,” *Nat Rev Microbiol*, vol. 3, no. 12, pp. 937–947, Dec. 2005, doi: 10.1038/nrmicro1286.
- [47] J. Macheleidt *et al.*, “Regulation and Role of Fungal Secondary Metabolites,” *Annual Review of Genetics*, vol. 50, no. Volume 50, 2016, pp. 371–392, Nov. 2016, doi: 10.1146/annurev-genet-120215-035203.
- [48] X. Sun, Y. Shi, D. Shi, Y. Tu, and L. Liu, “Biological Activities of Secondary Metabolites from the Edible-Medicinal Macrofungi,” *JoF*, vol. 10, no. 2, p. 144, Feb. 2024, doi: 10.3390/jof10020144.
- [49] M. Al Qutaibi and S. R. Kagne, “Exploring the Phytochemical Compositions, Antioxidant Activity, and Nutritional Potentials of Edible and Medicinal Mushrooms,” *International Journal of Microbiology*, vol. 2024, no. 1, p. 6660423, Jan. 2024, doi: 10.1155/2024/6660423.
- [50] M. Vlassopoulou *et al.*, “Immunomodulating Activity of *Pleurotus eryngii* Mushrooms Following Their In Vitro Fermentation by Human Fecal Microbiota,” *JoF*, vol. 8, no. 4, p. 329, Mar. 2022, doi: 10.3390/jof8040329.
- [51] S. Case *et al.*, “β-glucans from *Agaricus bisporus* mushroom products drive Trained Immunity,” *Front. Nutr.*, vol. 11, p. 1346706, Feb. 2024, doi: 10.3389/fnut.2024.1346706.
- [52] M. Fogarasi *et al.*, “Bioactive secondary metabolites in mushrooms: A focus on polyphenols, their health benefits and applications,” *Food Bioscience*, vol. 62, p. 105166, 2024.
- [53] N. Li *et al.*, “Separation and purification of the antioxidant compounds, caffeic acid phenethyl ester and caffeic acid from mushrooms by molecularly imprinted polymer,” *Food chemistry*, vol. 139, no. 1–4, pp. 1161–1167, 2013.
- [54] K. S. Teja *et al.*, “Mushrooms: A Potential Option in the Management of Deficiency and Diseases in Humans,” *J. Pure Appl. Microbiol.*, vol. 17, no. 2, pp. 749–760, Jun. 2023, doi: 10.22207/JPAM.17.2.55.
- [55] J.-J. Zhong and J.-H. Xiao, “Secondary Metabolites from Higher Fungi: Discovery, Bioactivity, and Bioproduction,” in *Biotechnology in China I*, J.-J. Zhong, F.-W. Bai, and W. Zhang, Eds., Berlin, Heidelberg: Springer Berlin Heidelberg, 2009, pp. 79–150. doi: 10.1007/10_2008_26.
- [56] L. M. Williams, B. S. Berthon, I. L. Stoodley, E. J. Williams, and L. G. Wood, “Medicinal Mushroom Extracts from *Hericium coralloides* and *Trametes versicolor* Exert Differential

- Immunomodulatory Effects on Immune Cells from Older Adults In Vitro,” *Nutrients*, vol. 15, no. 9, p. 2227, May 2023, doi: 10.3390/nu15092227.
- [57] H.-J. Park, “Current Uses of Mushrooms in Cancer Treatment and Their Anticancer Mechanisms,” *International Journal of Molecular Sciences*, vol. 23, no. 18, p. 10502, Jan. 2022, doi: 10.3390/ijms231810502.
- [58] G. Camilli, G. Tabouret, and J. Quintin, “The Complexity of Fungal β -Glucan in Health and Disease: Effects on the Mononuclear Phagocyte System,” *Front. Immunol.*, vol. 9, Apr. 2018, doi: 10.3389/fimmu.2018.00673.
- [59] E. De Marco Castro, P. C. Calder, and H. M. Roche, “Back Cover: β -1,3/1,6-Glucans and Immunity: State of the Art and Future Directions,” *Molecular Nutrition & Food Research*, vol. 65, no. 1, p. 2170003, 2021, doi: 10.1002/mnfr.202170003.
- [60] J. Vetter, “The Mushroom Glucans: Molecules of High Biological and Medicinal Importance,” *Foods*, vol. 12, no. 5, p. 1009, Jan. 2023, doi: 10.3390/foods12051009.
- [61] T. P. Joseph *et al.*, “A Preclinical Evaluation of the Antitumor Activities of Edible and Medicinal Mushrooms: A Molecular Insight,” *Integr Cancer Ther*, vol. 17, no. 2, pp. 200–209, Jun. 2018, doi: 10.1177/1534735417736861.
- [62] H.-C. Lin *et al.*, “Antroquinonol, a Ubiquinone Derivative from the Mushroom *Antrodia camphorata*, Inhibits Colon Cancer Stem Cell-like Properties: Insights into the Molecular Mechanism and Inhibitory Targets,” *J. Agric. Food Chem.*, vol. 65, no. 1, pp. 51–59, Jan. 2017, doi: 10.1021/acs.jafc.6b04101.
- [63] P. Mattila *et al.*, “Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms,” *J Agric Food Chem*, vol. 49, no. 5, pp. 2343–2348, May 2001, doi: 10.1021/jf001525d.
- [64] P. Nowakowski, R. Markiewicz-Żukowska, J. Bielecka, K. Mielcarek, M. Grabia, and K. Socha, “Treasures from the forest: Evaluation of mushroom extracts as anti-cancer agents,” *Biomedicine & Pharmacotherapy*, vol. 143, p. 112106, Nov. 2021, doi: 10.1016/j.biopha.2021.112106.
- [65] D. K. Park, Y. H. Lim, and H.-J. Park, “*Antrodia camphorata* Grown on Germinated Brown Rice Inhibits HT-29 Human Colon Carcinoma Proliferation Through Inducing G0/G1 Phase Arrest and Apoptosis by Targeting the β -Catenin Signaling,” *Journal of Medicinal Food*, vol. 16, no. 8, pp. 681–691, Aug. 2013, doi: 10.1089/jmf.2012.2605.
- [66] H.-J. Park, J.-B. Park, S.-J. Lee, and M. Song, “*Phellinus linteus* Grown on Germinated Brown Rice Increases Cetuximab Sensitivity of KRAS-Mutated Colon Cancer,” *International Journal of Molecular Sciences*, vol. 18, no. 8, p. 1746, Aug. 2017, doi: 10.3390/ijms18081746.
- [67] M. Spano *et al.*, “Dehydrated mycelia (*Cordyceps militaris*, *Grifola frondosa*, *Hericium erinaceus* and *Laricifomes officinalis*) as Novel Foods: A comprehensive NMR study,” *LWT*, vol. 199, p. 116123, May 2024, doi: 10.1016/j.lwt.2024.116123.
- [68] B. Boh, M. Berovic, J. Zhang, and L. Zhi-Bin, “*Ganoderma lucidum* and its pharmaceutically active compounds,” *Biotechnol Annu Rev*, vol. 13, pp. 265–301, 2007, doi: 10.1016/S1387-2656(07)13010-6.
- [69] G.-S. Wu *et al.*, “Anti-cancer properties of triterpenoids isolated from *Ganoderma lucidum* – a review,” *Expert Opinion on Investigational Drugs*, vol. 22, no. 8, pp. 981–992, Aug. 2013, doi: 10.1517/13543784.2013.805202.
- [70] V. Ramesh *et al.*, “Novel Bioactive Wild Medicinal Mushroom−*Xylaria* sp. R006 (Ascomycetes) against Multidrug Resistant Human Bacterial Pathogens and Human Cancer Cell Lines,” *IJM*, vol. 17, no. 10, 2015, doi: 10.1615/IntJMedMushrooms.v17.i10.100.
- [71] Y.-N. Teng, Y.-H. Wang, T.-S. Wu, H.-Y. Hung, and C.-C. Hung, “Zhankuic Acids A, B and C from *Taiwanofungus camphoratus* Act as Cytotoxicity Enhancers by Regulating P-Glycoprotein in Multi-Drug Resistant Cancer Cells,” *Biomolecules*, vol. 9, no. 12, p. 759, Nov. 2019, doi: 10.3390/biom9120759.

APOPTOSIS OF MITOCHONDRIAL MEMBRANE BY DEPOLARIZATION AND REACTIVE OXYGEN SPECIES ACCUMULATION OF COLORECTAL CANCER CELLS USING ETHANOLIC EXTRACT OF ALLIUM PRZEWALSKIANUM

Abstract

Allium przewalskianum is used for medicinal purposes but there are few reports on the anticancer effects of this plant. The present study was aimed to investigate the antitumor potential of the extract of A. przewalskianum and mechanism of action against cancer cell lines. Crude extract of A. przewalskianum was checked for anti-proliferative activity against lung, colon, and glioma cell lines followed by fractionation of the ethanolic extract. The effect on the cell morphology, cell cycle, cytoskeleton and nuclei were analysed by different techniques like flow cytometry, and confocal microscopy. Alteration in the expression of caspase 8 and 9, loss in the mitochondrial membrane potential and apoptotic percentage was also studied. Results showed the potential of A. przewalskianum as an important source of chemotherapeutics for cancer therapy. The chloroform fraction induced apoptosis and arrested the cells in the S phase of cell cycle after 24 h of treatment. The increased ROS levels, caspase 8, caspase 9 and lowered mitochondrial membrane potential might lead to the release of pro apoptotic factors from the mitochondria, leading to apoptosis. The data obtained assure the potential chloroform fraction of ethanolic extract of A. przewalskianum as a promising candidate to combat colorectal cancer.

Keywords: Allium przewalskianum; cell cycle arrest; apoptosis; chemotherapeutics, mitochondrial membrane potential

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I. INTRODUCTION

With the increase in the epidemiology of cancer, it has become a major concern in the world to seek out options for its treatment. As per WHO, 7 million deaths occur annually due to Cancer throughout the world and 19 million new cancer cases were reported in 2020 [1]. Due to the side effects of different synthetic drugs available in the market, there's a need to find solutions from nature [2]. Studies have been reported that anticancer drugs damage the hair follicles as well as affect the blood-forming cells, nephrotoxicity, myelosuppression, etc [3]. Natural products are gaining attention for anticancer drugs due to their biocompatibility and lesser toxicity to normal cell [2]. The findings of anticancer components like taxols, camptothecins, etc. open up the scope of the plant sources to be used in the treatment of cancer [4].

Ladakh flora is one of the least unexplored, although being used for multiple health problems locally viz. *Allium przewalskianum*, a member of the Amaryllidaceae family is used against dysentery and stomach ache [5]. The anticancer properties of this monocotyledon still need to be explored. *A. przewalskianum* is grown in dry slopes, plains, rock crevices at altitudes of 2000--4800 m above mean sea level and has great potential to be explored for its anticancer properties. *Allium przewalskianum* has bulb (swollen leaf base) formation in clusters, which are narrowly ovoid-cylindric, 0.5-1 cm in diameter; sometimes covered with common red to light brown tunic. Leaves are tubular, shorter to slightly longer than scape, 0.5--1.5 mm wide, semi-terete to terete, quad to penta angular. The scape is 10--40 cm long, covered with leaf sheaths only at base. Spathe is 1-valved, persistent. Umbel is hemispheric to globose; flowers are densely crowded with red to dark purplish flowers. Flowers and fruits formation is during June to September. *A. przewalskianum*, a wild onion species growing, has long been commonly used as an important vegetable and/or condiment by Tibetans, Indians, and Nepalese in the highlands of the Himalayas and adjacent regions and referred as Jimbu in Nepalali.

Studies have been reported for the medicinal properties of *Allium*, and it has been recognized as 'Russian penicillin' and 'natural antibiotic'. The usage of garlic components as a hypocholesterolemic, hypolipidemic and antihypertensive activity agent has been reported [6]. Members of the genus have sulfur-containing amino acids and it has been reported that the sulfur-sulfur bond is responsible for their anticancer properties [7]. Studies have demonstrated that the organosulfur compounds show anticancer properties either by causing the release of tumor necrosis factor, interleukins, or by activation of nuclear factor-kappa β due to oxidative stress [8,9]. Studies have reported the role of reactive oxygen species in induction of mitogen-activated protein kinase pathway which leads to apoptosis [10]. Another report has studied extensively on the mechanism of cell cycle arrest with Diallyl trisulfide (DATS) using human prostate cancer cells, PC-3 and DU145. The cell cycle arrest by DATS at G2/M phase in prostate cancer cells was associated with reactive oxygen species (ROS)-dependent hyperphosphorylation and destruction of the cell division cycle 25C (Cdc25C) phosphatase [11]. The main aim of our study was to explore the potential of *A. przewalskianum* for its use as a chemotherapeutic followed by further fractionation, with an orientation to purify the bioactive compounds for its anticancer potential.

1. Chemicals and Reagents

Phosphate buffer saline (PBS), foetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), McCoy 5A, trypsin-EDTA, trypan blue, trichloroacetic acid, and penicillin–streptomycin was obtained from HiMedia. Ethanol, chloroform, and dimethyl sulfoxide (DMSO) were purchased from Merck. Propidium iodide (PI), tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), RNase, and Dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma Chemical. All chemicals were of the highest grade commercially available and used without any modification or further purification.

2. Cell lines and Culture

The cell lines, lung cancer (A549) and glioma (C6) were cultured in DMEM whereas colon cancer (HCT116) cultured in McCoy's 5A with 10% Fetal bovine serum and 1% antibiotic antimycotic solution (penicillin, amphotericin B, and streptomycin) at 37°C and humidified atmosphere of 5% CO₂.

3. Plant Material

Allium przewalskianum was collected from the Nubra valley of Ladakh. The collected specimens were identified and deposited in Herbarium, Punjabi University, Patiala (India) under an accession number 62892. The plant name was authenticated by Prof. Manish Kapoor, Department of Botany, Punjabi University and was checked for its accepted names in accordance with the International Plant Names Index (IPNI) with <http://www.theplantlist.org>,

4. Preparation of Plant Extract

The bulb/ swollen leaf base of *A. przewalskianum* was collected from the Nubra valley of Ladakh region, washed, dried, disintegrated, and stored in dark. Solvent-based extracts were prepared using the Soxhlet extraction method, followed by drying in a rotary evaporator with little modifications. 16 g of the ground plant powder was put in the solvent extractor and extraction was done with 200 mL of ethanol until the colourless solvent was obtained in the Soxhlet extractor. Further chloroform fractionation of ethanolic extract was done using separating funnel, and dried using rotary evaporator. [11].

5. Cell Viability Assay

As per a modified method by Moglianetti et al., cell viability by the extracts was estimated by performing MTT assay on different cancer cell lines. 6×10^3 cells/well were seeded in 96 well plate and incubated for 24 h. Cells were then treated with different concentrations (0.062, 0.125, 0.25, 0.5 and 1mg/mL) of the ethanolic extracts for 24 and 48 h. A control group was also put in each set of cell-based experiments, which was not given treatment. On completion of the treatment periods, 20µL of MTT (5mg/mL, HiMedia) and 180µL media were added to each well and incubated for another 3-4 h at 37°C. After completion of the incubation, the supernatant was aspirated and 100µL of DMSO were added to each well. The plate was then

6. Morphological Immunofluorescence

Three cancer cell lines [lung (A549), colon (HCT116), and glioma (C6)] were treated with 0.5 mg/mL of chloroform fraction of ethanolic extract of *A. przewalskianum* for 24 h. The cells were then stained with fluorescent dyes, Hoechst 33342 (50 µL) and Phalloidin (10 µg/mL) for 10 min, and the images were recorded by Zeiss LSM880 confocal microscope (Carl Zeiss, Thornwood, New York) to check the effect on the nucleus and cytoskeleton [13].

7. ROS Generation Assay

5x10³ cells/well were seeded in a 96 well plate for 24 h and then treated with different concentration (0.062, 0.125, 0.25, 0.5, and 1mg/mL) of the chloroform fraction of the ethanolic extract of *A. przewalskianum* for 24 h in a humidified atmosphere of 5% CO₂. To determine the levels of ROS, DCFH-DA was used to calculate the oxidative stress. DCFH-DA (10 µg/mL) was then added to the wells and the plate was kept at 37°C for 40 min. Using a microplate reader, the plate was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm using a microplate reader [14].

8. Anti-Migration Assay

Cells were cultured in 6-well plates at 1 × 10⁵ cells/well. After 24 h, the cells were then starved serum free medium overnight. A 200 µL pipette tip was used to create straight wounds and then treated with 0.25, 0.5 and 1 mg/mL concentration of chloroform fraction. No treatment was given to the control group. The wound was then imaged at different time intervals (0 h, 12 h and 24 h) [15].

9. Mitochondrial Membrane Potential (MMP) Assay

MMP assay was performed using 3,3'-dihexyloxacarbocyanine iodide (DiOC6). This dye is taken by the mitochondria of live cells when used at lower concentrations (100 nM). 5x10⁴ HCT116 cells were seeded in 35 mm petri plate and then treated with 0.5 mg/mL. After 24 h of treatment, 100 µL of the dye was added and incubated for 20 min. Imaging was done immediately after time of incubation under Zeiss LSM880 confocal microscope [16].

10. Cell Cycle Analysis

Cell cycle analysis was done according to the protocol by Kumar et al., (2017) with few modifications. Seeded 7x10⁴ cancer cells in a 35 mm petri plate and incubated for 24 h. The cells were treated with chloroform fraction of *A. przewalskianum* with different concentrations (0.25, 0.5 and 1 mg/mL) for 24 h. Cells were then trypsinized followed by centrifugation at 2000 rpm for 5 min at 37° C. Then the pellet was collected, washed with ice-cold PBS, and fixed by 70% ethanol at -20°C for 30 min. Cells were then washed with PBS and stained 50 µL of 50 µg/mL PI on the addition of 100 µg/mL RNase for 30 min. Cells were analysed using a flow cytometer (Fluorescence-activated cell sorting (FACS) system, BD Biosciences, UK). Data was collected from 10,000 events per sample. The concentrations chosen for treatment were. 0.25, 0.5 and 1 mg/mL [17].

The detection of apoptosis and necrosis was performed using fluorescent dyes, Annexin V and PI (Invitrogen). 3×10^5 cells/mL cells were seeded for 24 h and then treated with chloroform fraction. After 24 h of the treatment, Annexin V and PI staining were performed as per the manufacturer's protocol and analysis was done using flow cytometry. 10,000 events per sample were analysed and data was generated as dot plots [18].

12. Caspase 8 and 9 Activity Assays

The caspase assay was performed using thermo fisher caspase kits. After seeding 4×10^3 cells in 96 well plate. The steps were followed as instructed in the kit and absorbance was measured at 450 nm within 30 minutes of adding stop solution [19].

13. Statistical Analysis

The experiments were performed in triplicate. The data are expressed as mean $\pm$ standard deviation. Statistical analysis of percentage counts was carried out with GraphPad using t-test At 5% level of significance ($p \leq 0.05$).

III. RESULTS AND DISCUSSION

1. Anti-Proliferative Effects of A. Przewalskianum

Ethanol extract was prepared and then MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was performed to evaluate the percentage of cell cytotoxicity caused by ethanolic extract of A. przewalskianum. The viability of lung (A549), colon (HCT116), and glioma (C6) cancer cell lines were checked on treatment with different concentrations of ethanolic extract of A. przewalskianum (Fig. 1). Although a significant difference was not observed in the cell viability at 24 h and 48 h of treatment but a dose dependent pattern was observed. The ethanolic extract of A. przewalskianum at 0.5 mg/mL concentration was observed to be LD50 (50% cell death) in C6 cells, whereas in case of A549 it observed to be LD45 (Fig. 1) and in HCT116 it was LD55 at the same concentration (Fig. 1B). The A549 cell line was observed to be relatively less sensitive to growth inhibition compared to C6 and HCT116 (Fig. 1). On being treated with the same concentration of the extract, a 10% cell viability difference was observed in A549 and HCT116. Studies have shown that higher anti-proliferative effects was obtained in organic extracts than the aqueous extract of garlic [20], therefore , ethanolic extract was chosen over aqueous extract in the present study.

Further fractionation of the ethanolic extract to chloroform fraction was done. As seen in Fig. 1, the p value for 24 h and 48 h is non-significant so in the further experiments, the cells were only treated for 24 h. The cell viability decreased to 30% at 0.5 mg/mL of the chloroform fraction of ethanolic extract for HCT116 as shown in Fig. 2A At 0.5 mg/mL, the cell viability was only 38% and 49% in case of C6 and A549 respectively. The chloroform fraction showed the most promising anticancer activity against HCT116 at 0.5 mg/mL concentration; therefore, chosen for further investigations. It is expected that the decrease in cell viability through chloroform fraction was due to an increase in the concentration of the bioactive component with anticancer potential [4].

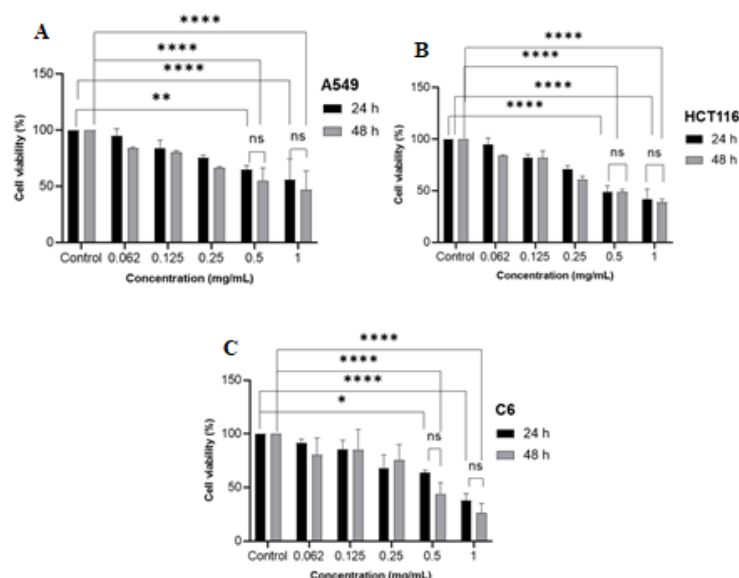


Figure 1: Cell viability determination by MTT of *A. przewalskianum* ethanolic extract on different cancer cell. A lung (A549) ; B colon (HCT116) and C glioma (C6). The cells were treated with different concentrations (0.062, 0.125, 0.25, 0.5 and 1 mg/mL) for 24 h and 48 h. Data are expressed as the mean + standard deviation. * $p < 0.05$, **** $p < 0.0001$, ns – non significant

2. Morphological Changes and Cytoskeleton Analysis

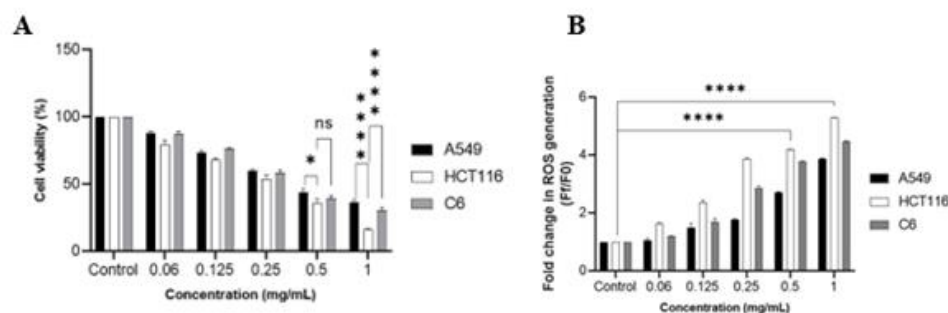
The effect on the actin filament and nucleus was observed under confocal microscopy, after treatment with the chloroform fraction followed by staining with fluorescent dyes. Hoechst 33342 staining was done to check the effects on the nucleus, as it binds with the DNA whereas to observe the changes in cell morphology and cytoskeleton, phalloidin staining was performed. The treated cells show a completely different morphology compared to the control indicating the effects on the actin filaments of cells. Fig. 2C. shows the changes in morphology, nuclear condensation, and effects on the cytoskeleton on the cancer cell lines after the treatment. On staining with Hoechst 33342 and Phalloidin, small and short cytoplasmic processes is detected in the cancer cells by the chloroform fraction. Phalloidin is found intact to the nucleus in untreated cells, whereas nuclear shrinkage is observed in the case of treated cells. Significant changes were observed in the cellular shape. Very distinct and noticeable differences in the morphology of A549 and HCT116 (Fig. 2C) were observed after treatment with the chloroform fraction for 24 h [21]. The nuclear condensation and cytoskeleton damage are some of the changes observed in the cells undergoing apoptosis, as observed under confocal microscope (Fig. 2C).

3. Determination of ROS Generation

Several studies have described the antioxidant properties of medicinal plants, which are rich in phenolic compounds [22]. ROS levels play a key role in anticancer therapy as high ROS levels leads to excessive cell damage, ultimately leading to cell death. So as to investigate the factors causing the death of cancer cells, the determination of ROS was done. The dye, dichlorofluorescein diacetate (DCFDA) gets oxidized to fluorescent DCFH-DA by ROS in the cells. Higher is the cellular ROS levels, higher is the reduction of DCF-DA to DCFH-DA

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[23]. The intracellular level of ROS generated was determined to study the mechanism underlying cell death. As numerous studies suggested that the maximum amount of ROS is generated after 24 h of treatment [24], (Fig. 1), shows to be non-significant at 5% level of significance for cell viability 48 h, so the cells were checked for ROS generation after 24 h of treatment at different concentrations. In the present study, it was observed that oxidative stress increased in a dose-dependent manner and maximum cell death was observed at the highest concentration. Fig. 4 summarizes the results obtained after treatment of cancer cell lines with the chloroform fraction of ethanolic extract of *A. przewalskianum*. In Fig. 2B, HCT116 resulted in higher oxidative stress than other cell lines. The ROS generation increased four times on treating with 1 mg/mL for 24 h as compared with the untreated cells (control). Such high ROS generation indicated that it is the synergistic effect of fractionation that leads to the significant therapeutic response, potentially involving DNA damage as a consequence of chemotherapy [25]. The precise mechanism has not yet been established, but the results indicate that it is probably due to ROS produced in the cellular environment that causes cancer cell death. It has been reported by some workers that, the increased ROS levels lead to lysosomal disruption, which cause autophagic cell death whereas it has also been reported that high ROS levels, prevents the fusion of lysosomes with autophagosomes, which eventually cause the release of hydrolytic enzymes due to lysozyme disruption leading to cell death [26].



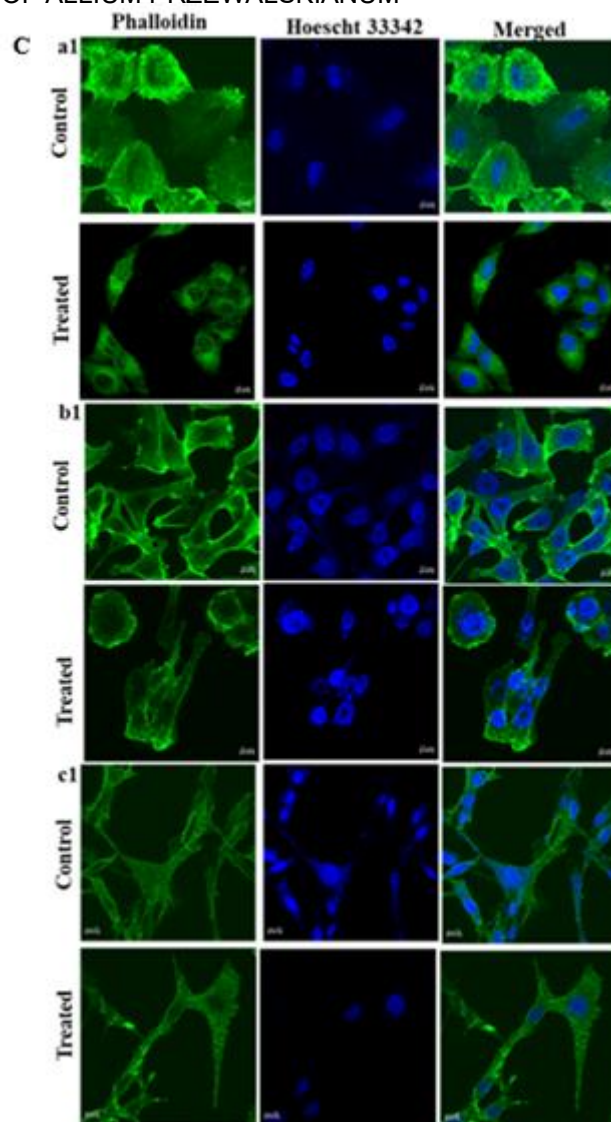


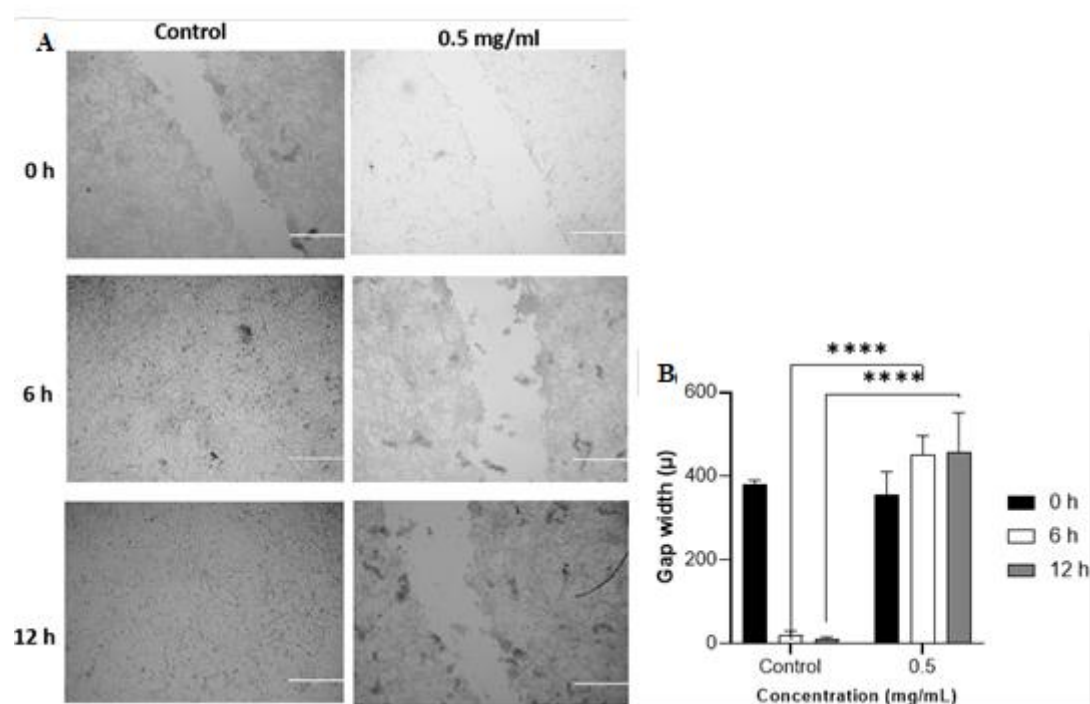
Figure 2 : A Effect of using chloroform fraction of ethanolic extract of *A. przewalskianum* on cancer cells. Cells viability of different cancer cell lines was determined, after treatment with 0.062, 0.125, 0.25, 0.5 and 1 mg/mL of chloroform fraction for 24 h; B Intracellular ROS determination. Ff represents the fluorescence intensity of test sample and F0 represents the fluorescence intensity of control (untreated sample). Data are expressed as the mean + standard deviation. **** $p < 0.0001$, C Morphological analysis of cancer cell lines treated with chloroform fraction (0.5mg/mL) of *A. przewalskianum*. The nucleus was stained blue with Hoescht-33342 (blue) and actin filaments are stained using Fluorescein Phalloidin (green). a1 A549, b1 HCT116 and c1 C6. (Scale bar = 10 μ m)

Cancer Cell Migration Analysis

Migration was performed to determine the effect on the migration of HCT116 and the results illustrated that the chloroform fraction effectively inhibited the migration of HCT116 as compared to the control. The higher is the concentration of treatment, the better is the anti-migratory effect observed on the treated cells as seen in Fig. 3A [21]. The gap width was calculated using ImageJ software and the results display that the gap width increased after treatment which indicate the decreased migratory activity of cells (Fig. 3B).

Mitochondrial Membrane Potential Loss Analysis

To further explore the pathway involved in the apoptosis of cancer cells, the mitochondrial membrane integrity was observed under confocal imaging microscope. Studies show that the loss in mitochondrial membrane potential leading to loss in membrane integrity, which releases ROS and triggers the initiation of apoptotic cascade [27]. These results encouraged us to check if the mitochondrial membrane was depolarised by chloroform fraction in HCT116 cells. Fig. 3C shows a significant decrease in the mitochondrial trans-membrane potential of treated cells compared to the control. The quantitative estimation of dye showed 20% decrease in the uptake by treated cells indicating loss in mitochondrial integrity (Fig. 3D). This data clearly demonstrates the interlink between increase ROS generation, loss of membrane potential, which leads to leakiness of mitochondria [28]. Under physiological conditions, ATP generation in mitochondria occurs due to the high magnitude in the outer and inner membrane of mitochondria. On generation of higher ROS and calcium accumulation, there is an increase in conductance in the permeability transition pore complex, which allows the entry of small molecules into the mitochondrial matrix, resulting in the immediate loss of mitochondrial membrane potential. The loss in membrane potential leads to the release of cytochrome-C and thereby leading to apoptosis [29]. The loss in the MMP leads to formation of mitochondrial permeability transition pore, which leads to the release of apoptogenic proteins which cause apoptosis.



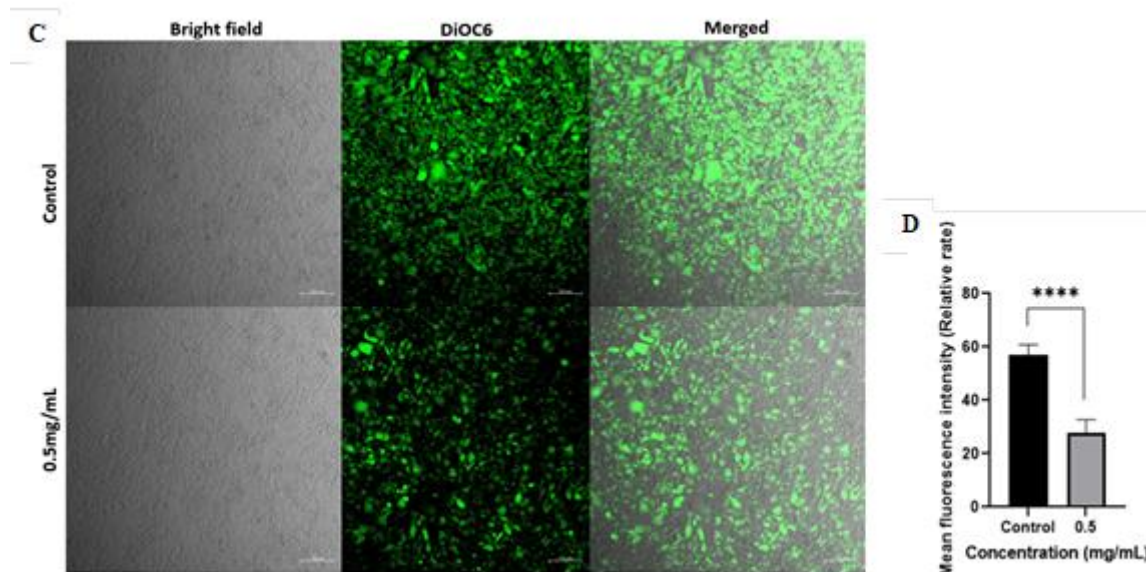


Figure 3: Anti migratory effect of chloroform fraction (0.25, 0.5 and 1mg/mL) on HCT116 cells at 0 h, 6 h and 12 h of time intervals. A Representative images of scratch in control and treated sample (0.5 mg/mL of chloroform fraction) at 0 h, 6 h and 12 h; B The gap width calculated using Image J, in each sample at 0 h, 6 h and 12 h. Data are expressed as the mean + standard deviation . ****p<0.0001; C Loss of mitochondrial membrane potential of HCT116 cells after treatment with 0.5 mg/mL of chloroform fraction of *A. przewalskianum* (Scale bar = 100 μ m); D Mean fluorescence intensity of DiOC6 for MMP analysis calculated using ImageJ

Cell cycle analysis

The chloroform fraction displayed highest cell death against colon cancer cell line, therefore, HCT116 was chosen to further investigate the effects of treatment. Cell cycle analysis was done to determine on which cell cycle stage the chloroform fraction affects the cancer cells using flow cytometry. The analysis showed that HCT116 cells were mainly arrested in the S phase of the cell cycle. The number of cells in the S phase of the cell cycle increased whereas a decrease in the G₀/ G₁ was observed. The cells in the S phase were 40% whereas it was only 15% in the untreated sample (Fig. 4A-B). At 0.5 mg/mL of the treatment of chloroform fraction of ethanolic extract of *A. przewalskianum*, the rise in the percentage of cells in the S phase was higher as compared to the increase in percentage of cells in G₂/M phase. The cells need protein kinases to move into different phases of the cell cycle and any interference to these kinases causes the cell to get arrested in a particular phase of the cell cycle [30]. An arrest in the S phase of the cell cycle could possibly be due to the effect of the selected chloroform fraction on kinases.

Analysis of apoptosis and necrosis

The percentage of live cells present, early apoptosis, late apoptosis and necrosis phase was observed (Fig 8). On comparing with the percentage of live HCT116 cells in control and treatment groups, the cell percentage decreased from 99.5% in control group to 4.8% in the treatment group (1mg/mL) (Fig. 4C-D). The cells in the late apoptosis increased to 90% at the highest concentration of treatment. A significant change (86%) was also observed at 0.5

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mg/mL concentrations of the chloroform fraction whereas only 12% increase of cells in late apoptosis was observed at 0.25 mg/mL. At 0.25 mg/mL, the highest number of cells were observed in early apoptosis (53.6%) (Fig. 4C-D). This also indicates the cells moving from an early apoptotic phase to late apoptotic phase with increased concentration of the chloroform fraction [31].

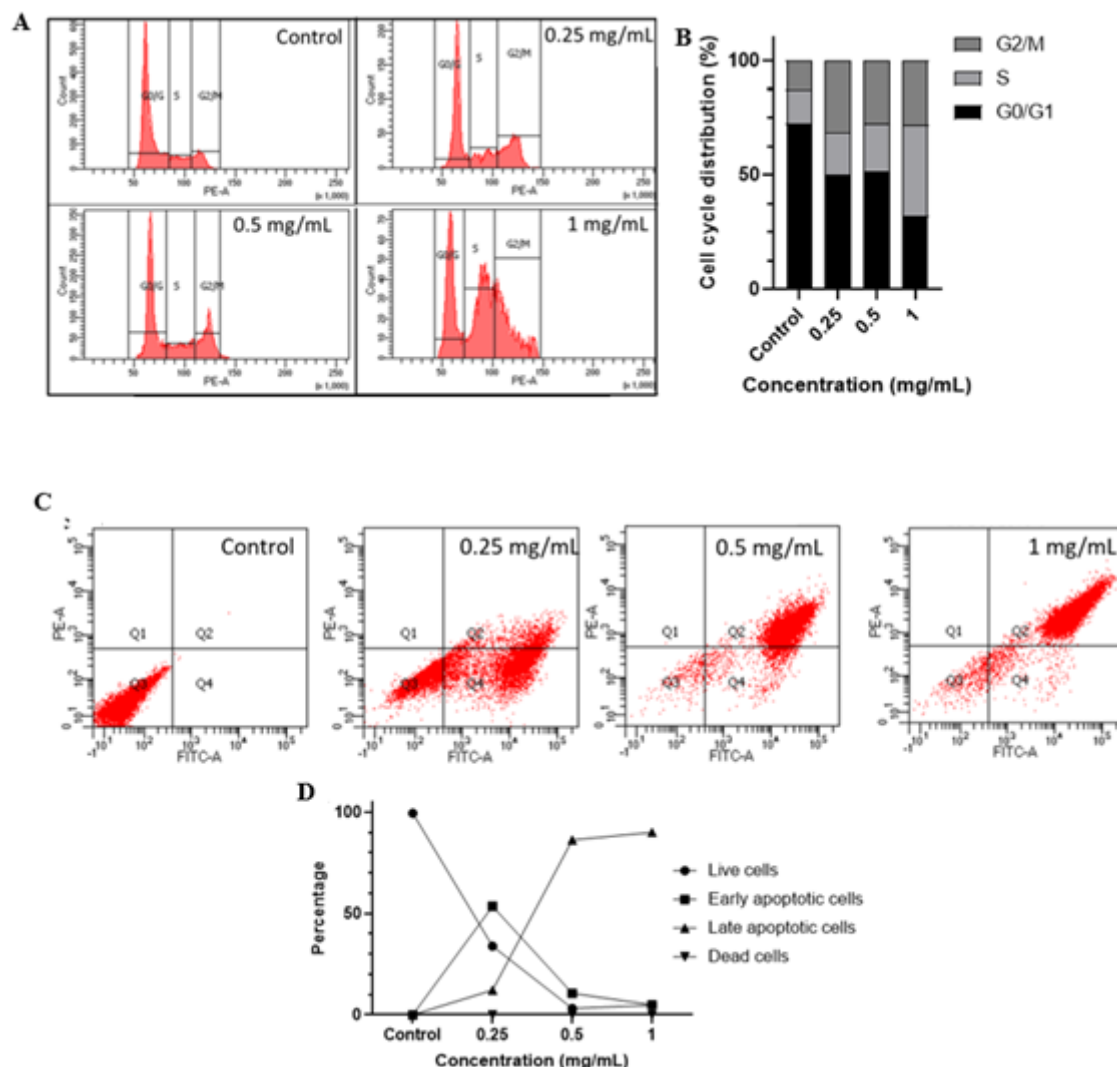


Figure 4 : A Cell cycle analysis of the chloroform fraction at different concentration for HCT116 cell line; B Bar graphs represent the total population of cells in different phases of cell cycle after appropriate treatments of HCT116 cells; C Induction of cell death by different concentrations (0.25, 0.5 and 1mg/mL) of chloroform fraction on HCT116 cell line; D Bar graphs represent the percentage of cells in each quadrant. Q1, Q2, Q3 and Q4 represent the quadrant for cells in Necrosis, Late apoptosis, Live and Early apoptosis phase

Caspase 8 And 9 Assay

The induction of apoptosis depends on the interaction of the cascade complexes. The expression of initiator caspases (8 and 9) was examined to determine the pathway involved in apoptosis after treatment with 0.5mg/mL of chloroform fraction. The treated cells show higher levels of both caspase 8 and 9 (Fig. 5)

The initiator caspases was involved in the initiation of caspase cascade. Caspase 8 is involved in the extrinsic pathway of apoptosis, whereas intrinsic pathway is initiated by caspase 9. The chloroform fraction increased the levels of both caspase 8 and caspase 9. Hence, the apoptotic effect in the HCT116 cells was induced by both, extrinsic and intrinsic pathways (Fig. 5). Both these pathways converge at the proteolytic activation of effector caspases, 3, 6 and 7. The activation of caspase 9 is led by the release of cytochrome-C from mitochondria [32]. The leaky membrane of mitochondria was also verified by our study on the mitochondrial membrane potential [12].

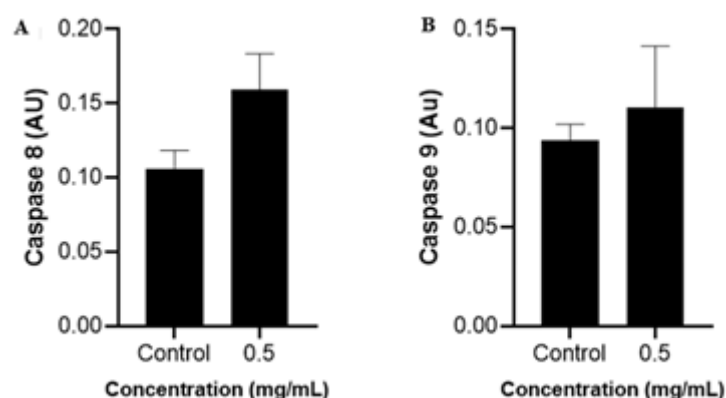


Figure 5 : Effect on caspase 8 and 9 levels in HCT116 cells treated with 0.5 mg/mL of chloroform fraction; A Caspase 8 levels in the control and treated group; B Levels of caspase 9 in the control and treated HCT116 cells. Data are expressed as the mean + standard deviation

Hence, our in vitro data implicates the anti-proliferative effects of chloroform fraction chloroform fraction of ethanolic extract of *A. przewalskianum* through the activation of both extrinsic and intrinsic pathway which further causes leaky channels in the membrane of mitochondria. The increased ROS levels also disrupts the mitochondrial membrane integrity. All of the above factors lead to the apoptosis of cancer cells on treatment with *A. przewalskianum*.

IV. CONCLUSION

Results demonstrate the first evidence for the involvement of *A. przewalskianum* in antiproliferative activity against cancer cell lines. This study shows the apoptotic effects and increased ROS accumulation in the cancer cell lines, indicating the possibility of chloroform fraction to be used for the treatment of cancer. The results also suggest the death of cancer cells by disruption of cytoskeleton, higher ROS generation and mitochondrial damage by causing loss in the integrity of membrane after treatment. The increased levels of both caspase 8 and 9 indicate the involvement of both extrinsic and intrinsic pathway in apoptosis of cells. Although both these pathways converge at the effector caspase, caspase 3 which leads to apoptosis. Therefore it could be concluded that the ethanolic plant extract chloroform fraction of *A. przewalskianum* contain bioactive compound is capable of apoptosis of malignant cells by apoptosis.

REFERENCES

- [1] Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA. Cancer J. Clin.* 2021, 71 (3), 209–249.
- [2] Calle, E. E.; Rodriguez, C.; Walker-Thurmond, K.; Thun, M. J. Overweight, Obesity, and Mortality from Cancer in a Prospectively Studied Cohort of US Adults. *N. Engl. J. Med.* 2003, 348 (17), 1625–1638.
- [3] van den Boogaard, W. M.; Komninos, D. S.; Vermeij, W. P. Chemotherapy Side-Effects: Not All DNA Damage Is Equal. *Cancers* 2022, 14 (3), 627.
- [4] Nicastro, H. L.; Ross, S. A.; Milner, J. A. Garlic and Onions: Their Cancer Prevention Properties. *Cancer Prev. Res. (Phila. Pa.)* 2015, 8 (3), 181–189.
- [5] Petrovska, B.; Cekovska, S. Extracts from the History and Medical Properties of Garlic. *Pharmacogn. Rev.* 2010, 4 (7), 106.
- [6] Melguizo-Rodríguez, L.; García-Recio, E.; Ruiz, C.; De Luna-Bertos, E.; Illescas-Montes, R.; Costela-Ruiz, V. J. Biological Properties and Therapeutic Applications of Garlic and Its Components. *Food Funct.* 2022, 13 (5), 2415–2426.
- [7] Agbana, Y. L.; Ni, Y.; Zhou, M.; Zhang, Q.; Kassegne, K.; Karou, S. D.; Kuang, Y.; Zhu, Y. Garlic-Derived Bioactive Compound S-Allylcysteine Inhibits Cancer Progression through Diverse Molecular Mechanisms. *Nutr. Res.* 2020, 73, 1–14.
- [8] Yu, F.-L.; Bender, W.; Fang, Q.; Ludeke, A.; Welch, B. Prevention of Chemical Carcinogen DNA Binding and Inhibition of Nuclear RNA Polymerase Activity by Organosulfur Compounds as the Possible Mechanisms for Their Anticancer Initiation and Proliferation Effects. *Cancer Detect. Prev.* 2003, 27 (5), 370–379.
- [9] Kwon, K.-B.; Yoo, S.-J.; Ryu, D.-G.; Yang, J.-Y.; Rho, H.-W.; Kim, J.-S.; Park, J.-W.; Kim, H.-R.; Park, B.-H. Induction of Apoptosis by Diallyl Disulfide through Activation of Caspase-3 in Human Leukemia HL-60 Cells. *Biochem. Pharmacol.* 2002, 63 (1), 41–47.
- [10] Powolny, A. A.; Singh, S. V. Multitargeted Prevention and Therapy of Cancer by Diallyl Trisulfide and Related Allium Vegetable-Derived Organosulfur Compounds. *Cancer Lett.* 2008, 269 (2), 305–314.
- [11] Baldosano, H. Y.; Castillo, M.; Elloran, C. D. H.; Bacani, F. T. Effect of Particle Size, Solvent and Extraction Time on Tannin Extract from *Spondias Purpurea* Bark through Soxhlet Extraction. In *Proceedings of the DLSU Research Congress*; 2015; Vol. 3, pp 4–9.
- [12] Moglianetti, M.; De Luca, E.; Pedone, D.; Marotta, R.; Catelani, T.; Sartori, B.; Amenitsch, H.; Retta, S. F.; Pompa, P. P. Platinum Nanozymes Recover Cellular ROS Homeostasis in an Oxidative Stress-Mediated Disease Model. *Nanoscale* 2016, 8 (6), 3739–3752.
- [13] Wu, D.; Wang, Z.; Lin, M.; Shang, Y.; Wang, F.; Zhou, J.; Wang, F.; Zhang, X.; Luo, X.; Huang, W. In Vitro and in Vivo Antitumor Activity of Cucurbitacin C, a Novel Natural Product from Cucumber. *Front. Pharmacol.* 2019, 10, 1287.
- [14] Sharafutdinov, I. S.; Ozhegov, G. D.; Sabirova, A. E.; Novikova, V. V.; Lisovskaya, S. A.; Khabibrakhmanova, A. M.; Kurbanalieva, A. R.; Bogachev, M. I.; Kayumov, A. R. Increasing Susceptibility of Drug-Resistant *Candida Albicans* to Fluconazole and Terbinafine by 2 (5 H)-Furanone Derivative. *Molecules* 2020, 25 (3), 642.
- [15] Lee, J.; Gupta, S.; Huang, J.-S.; Jayathilaka, L. P.; Lee, B.-S. Preparation and Purification of Garlic-Derived Organosulfur Compound Allicin by Green Methodologies. *Green Chromatogr. Tech. Sep. Purif. Org. Inorg. Anal.* 2014, 123–139.
- [16] Kumar, R. A.; Sridevi, K.; Kumar, N. V.; Nanduri, S.; Rajagopal, S. Anticancer and Immunostimulatory Compounds from *Andrographis Paniculata*. *J. Ethnopharmacol.* 2004, 92 (2–3), 291–295.
- [17] Barroso, G.; Taylor, S.; Morshedi, M.; Manzur, F.; Gaviño, F.; Oehninger, S. Mitochondrial Membrane Potential Integrity and Plasma Membrane Translocation of Phosphatidylserine as Early Apoptotic Markers: A Comparison of Two Different Sperm Subpopulations. *Fertil. Steril.* 2006, 85 (1), 149–154.
- [18] Idelchik, M. del P. S.; Begley, U.; Begley, T. J.; Melendez, J. A. Mitochondrial ROS Control of Cancer. In *Seminars in cancer biology*; Elsevier, 2017; Vol. 47, pp 57–66.
- [19] Sung, B.; Kang, Y. J.; Kim, D. H.; Hwang, S. Y.; Lee, Y.; Kim, M.; Yoon, J.-H.; Kim, C. M.; Chung, H. Y.; Kim, N. D. Corosolic Acid Induces Apoptotic Cell Death in HCT116 Human Colon Cancer Cells through a Caspase-Dependent Pathway. *Int. J. Mol. Med.* 2014, 33 (4), 943–949.
- [20] Bar, M.; Binduga, U. E.; Szychowski, K. A. Methods of Isolation of Active Substances from Garlic (*Allium Sativum* L.) and Its Impact on the Composition and Biological Properties of Garlic Extracts. *Antioxidants* 2022, 11 (7), 1345.
- [21] Abbruzzese, C.; Matteoni, S.; Persico, M.; Ascione, B.; Schenone, S.; Musumeci, F.; Amato, R.; Perrotti, N.; Matarrese, P.; Paggi, M. G. The Small Molecule SI113 Hinders Epithelial-to-Mesenchymal Transition

Chapter 12: APOPTOSIS OF MITOCHONDRIAL MEMBRANE BY DEPOLARIZATION AND REACTIVE OXYGEN SPECIES ACCUMULATION OF COLORECTAL CANCER CELLS USING ETHANOLIC EXTRACT OF ALLIUM PRZEWALSKIANUM

- and Subverts Cytoskeletal Organization in Human Cancer Cells. *J. Cell. Physiol.* 2019, 234 (12), 22529–22542.
- [22] Xu, D.-P.; Li, Y.; Meng, X.; Zhou, T.; Zhou, Y.; Zheng, J.; Zhang, J.-J.; Li, H.-B. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *Int. J. Mol. Sci.* 2017, 18 (1), 96.
- [23] Kim, H.; Xue, X. Detection of Total Reactive Oxygen Species in Adherent Cells by 2', 7'-Dichlorodihydrofluorescein Diacetate Staining. *JoVE J. Vis. Exp.* 2020, No. 160, e60682.
- [24] Lossi, L. The Concept of Intrinsic versus Extrinsic Apoptosis. *Biochem. J.* 2022, 479 (3), 357–384.
- [25] Agbana, Y. L.; Ni, Y.; Zhou, M.; Zhang, Q.; Kassegne, K.; Karou, S. D.; Kuang, Y.; Zhu, Y. Garlic-Derived Bioactive Compound S-Allylcysteine Inhibits Cancer Progression through Diverse Molecular Mechanisms. *Nutr. Res.* 2020, 73, 1–14.
- [26] Song, S. B.; Hwang, E. S. High Levels of ROS Impair Lysosomal Acidity and Autophagy Flux in Glucose-Deprived Fibroblasts by Activating ATM and Erk Pathways. *Biomolecules* 2020, 10 (5), 761.
- [27] Zorov, D. B.; Juhaszova, M.; Sollott, S. J. Mitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release. *Physiol. Rev.* 2014, 94 (3), 909–950.
- [28] Sedlackova, L.; Korolchuk, V. I. Mitochondrial Quality Control as a Key Determinant of Cell Survival. *Biochim. Biophys. Acta BBA-Mol. Cell Res.* 2019, 1866 (4), 575–587.
- [29] Gogvadze, V.; Orrenius, S.; Zhivotovsky, B. Multiple Pathways of Cytochrome c Release from Mitochondria in Apoptosis. *Biochim. Biophys. Acta BBA-Bioenerg.* 2006, 1757 (5–6), 639–647.
- [30] Matthews, H. K.; Bertoli, C.; de Bruin, R. A. Cell Cycle Control in Cancer. *Nat. Rev. Mol. Cell Biol.* 2022, 23 (1), 74–88.
- [31] Aborehab, N. M.; Elnagar, M. R.; Waly, N. E. Gallic Acid Potentiates the Apoptotic Effect of Paclitaxel and Carboplatin via Overexpression of Bax and P53 on the MCF-7 Human Breast Cancer Cell Line. *J. Biochem. Mol. Toxicol.* 2021, 35 (2), e22638.
- [32] Kello, M.; Takac, P.; Kubatka, P.; Kuruc, T.; Petrova, K.; Mojzis, J. Oxidative Stress-Induced DNA Damage and Apoptosis in Clove Buds-Treated MCF-7 Cells. *Biomolecules* 2020, 10 (1), 139.
- [33] Behera, Sudhanshu Shekhar, Jayanta Kumar Patra, Krishna Pramanik, Niladri Panda, and Hrudayanath Thatoi. Characterization and evaluation of antibacterial activities of chemically synthesized iron oxide nanoparticles. *WJNSE* 2012, 02(04):196-200.



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