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VOLUME III

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PREFACE

The field of chemical and biological sciences stands at the forefront of human advancement, driving innovation and discovery that touch every aspect of our lives. From the development of life-saving medicines to the design of sustainable materials, the contributions of researchers in these disciplines are profound and far-reaching.

"Advances in Chemical and Biological Sciences" encapsulates the spirit of exploration and progress that defines these fields. In this volume, we bring together a collection of groundbreaking research and insights from leading experts across the globe. Through their dedication and expertise, they have pushed the boundaries of knowledge, unveiling new phenomena, solving complex problems, and laying the groundwork for future breakthroughs.

Within these pages, readers will encounter a diverse array of topics, ranging from fundamental principles of chemistry and biology to cutting-edge applications in medicine, environmental science, and beyond. Each chapter represents a testament to the tireless pursuit of understanding and the relentless drive to make a positive impact on the world.

As editors, it is our privilege to present this compilation to the scientific community and beyond. We believe that the knowledge contained herein will inspire curiosity, foster collaboration, and spur further exploration. May this volume serve as a catalyst for continued advancement in the chemical and biological sciences, ultimately leading to a brighter, healthier, and more sustainable future for all.

Editors:

Dr. Bassa Satyannarayana

Mr. Mukul Machhindra Barawnt

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MICROPLASTIC AND HUMAN HEALTH: A REVIEW EXPLORING THE POTENTIAL LINK TO CANCER

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ABSTRACT

Micro plastic pollution in the environment is increased day by day when humans were exposed to micro plastic by inhalation or ingestion of contaminated food and water it leads to unknown health effects to humans. Micro plastics, which are particles with a size range of 1µm to 5 mm, are formed when plastic waste is subjected to sunshine, wind, and ocean waves. Because they permeate ecosystems such as freshwater and marine systems, agro ecosystems, the atmosphere, food, drinking water, and biota, these micro plastics represent a serious hazard. Some polymers have been shown to be more harmful to people and animals than others, including polyvinyl chloride, or PVC, and polyurethane (PU). According to current studies, micro plastics may have negative impacts on human health such as oxidative stress, immune response regulation, toxicity to the reproductive and developmental systems, inflammation, and the possibility of developing antibiotic resistance. The immediate and long-term impacts, however, are still not fully known, thus further study is required to fully evaluate the dangers and create mitigation plans for human exposure to micro plastics.

KEYWORDS: Cancer, Micro Plastic, Health Effects, Toxicity.

INTRODUCTION

In this modern world to facilitate the needs of over-population we have switches from ecofriendly materials to cheaply available and convenient plastic. The global plastic waste had exceeded 6300 million metric tons by 2015 and will reach 12,000 million metric tons by 2050 with the current production and waste management trends. The continuous exposure to the activity of sunlight, wind, and ocean waves breaks down plastic debris into smaller particles which are micro plastic, which can enter the food chain, ingestion or inhalation and accumulate in organisms, potentially causing harm. The main types of the micro plastic are Polyethylene terephthalate (PET), High density and low-density polyethylene (HDPE, LDPE), Polypropylene (PP) Polystyrene (PS), Polyvinyl chloride (PVC), Polyvinylidene fluoride (PVDF), Polymethyl methacrylate (PMMA), Polyurethane (PU), Polyamide (PA), Polycarbonate (PC). PVC and PU, shown in figure 1, Polymers are more toxicity for humans and animals then other polymers (Lisa Zimmermann *et al.*,).Micro plastics are synthetic solid particles or polymeric matrices with a size ranging from 1µm to 5 mm, originating from primary or secondary manufacturing processes and are insoluble in water. They are ubiquitous in various environments, including marine and freshwater systems, agro ecosystems, atmosphere, food, drinking-water, and biota. Micro plastics can have diverse shapes, polymers, sizes, and concentrations, and they can be transported over long distances by wind or water currents. These particles can also be found in various forms, from thin veils to hard and compact particles. Microplastics can contain hazardous chemicals, including additives and polymeric raw materials from the plastics, as well as chemicals absorbed from the surrounding environment. Due to their physical-chemical properties, micro plastics can serve as vectors for transporting a broad range of hydrophobic and persistent organic pollutants, antibiotics, and heavy metals in ecosystems. This ability to transport toxic chemicals raises concerns about the potential impact of micro plastics on environmental and human health. Furthermore, micro plastics can enter the human system through ingestion of contaminated food, with studies reporting the presence of micro plastics in various food items such as sugar, salt, alcohol, bottled water, and marine species. Additionally, micro plastics have been found in human stools, indicating potential ingestion and excretion of these particles.

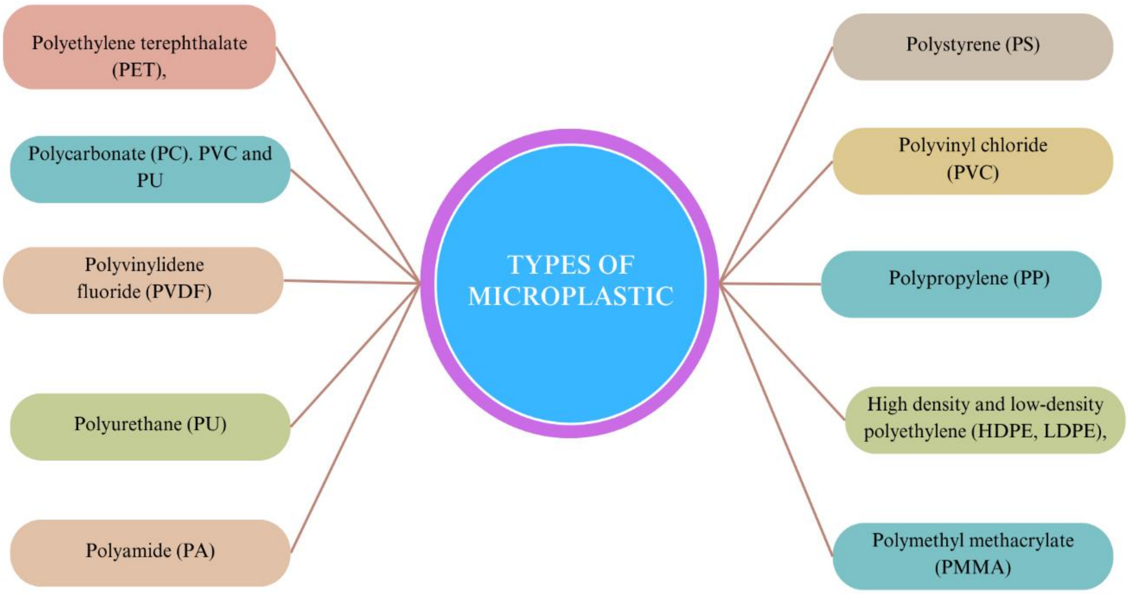

Figure 1: Types of micro plastic

The ecology and human health are both significantly impacted by the presence of micro(nano)plastics in the environment. Among these ramifications are the following:

1. HEALTH OF HUMANS

a. Ingestion: Consuming tainted food or drink can allow micro(nano)plastics to enter the body. This may result in unfavorable health outcomes including genotoxicity, oxidative stress, and inflammation, which may hasten the onset of chronic illnesses like cancer.

b. Inhalation: It is possible for micro(nano)plastics to be breathed and have effects on the respiratory and cardiovascular systems. These particles can induce inflammation and lung tissue damage because of their tiny size, which enables them to enter the lungs deeply.

c. Exposure through Skin: Micro(nano)-plastics may also enter the body through skin contact, potentially leading to skin irritation and other adverse health effects.

d. Bioaccumulation: Over time, micro(nano)plastics may build up in the body and may have long-term negative consequences on health. This is especially problematic for heavy metals and persistent organic pollutants that could be adsorbed onto micro plastics, which can bioaccumulate within the body and have harmful consequences.

2. ECOSYSTEM

a. animals Impacts: Ingesting, enttangling, and bodily harm are just a few of the negative consequences that micro(nano)plastics may have on animals. In the afflicted species, these consequences may result in decreased fitness, decreased reproductive success, and population decreases.

b. Effects on Ecosystem Functioning: Micro(nano)plastics may also have an effect on biodiversity, energy flow, and nutrient cycling in ecosystems. Micro plastics can change the make-up and function of microbial populations in aquatic settings, which may have a domino impact on ecosystem processes.

c. Bioaccumulation: When micro(nano)plastics build up in an organism's tissues, they may have harmful consequences and biomagnify further up the food chain. Human health may be affected by this as tainted fish and other goods may be consumed by people.

All things considered, the ecosystem and human health are significantly impacted by the presence of micro(nano)plastics in the environment. Gaining an understanding of these consequences is essential to creating solutions that effectively reduce the negative impacts of micro plastic pollution and safeguard the health of people and the environment (Deng, Y., *et al.*).

MICROPLASTIC EFFECTS ON HUMAN BEINGS

Human exposure to micro plastics can occur through various sources, including ingestion of food and drink, inhalation, and dermal contact. The main sources of human exposure to micro plastics include:

Ingestion of Contaminated Food and Water: Micro plastics can enter the food chain through contaminated seafood, salt, sugar, honey, and drinking water. Seafood has been identified as a significant source of micro plastic exposure due to the accumulation of micro plastics in marine organisms.

Inhalation: Micro plastics have been found in the atmosphere, and inhalation of airborne micro plastics is considered a potential route of exposure.

Consumer Products: Exposure to micro plastics can also occur using consumer products such as personal care and cosmetic products, which may contain micro plastic particles.

Environmental Contamination: Individuals may be exposed to micro plastics through environmental contamination, including indoor and outdoor environments, as well as through the consumption of contaminated food and beverages.

These sources highlight the potential for widespread human exposure to micro plastics and the need for further research to understand the implications for human health.

The direct effects of micro plastic consumption on human health are still not fully understood, and more research is needed to determine the extent of the risks. However, current research suggests that micro plastics may have several potential health effects on humans, including:

Inflammation: Micro plastics may cause inflammation in the body, which can lead to a range of health problems, including respiratory and cardiovascular diseases.

Oxidative Stress: Micro plastics may cause oxidative stress in the body, which can damage cells and lead to a range of health problems, including cancer, diabetes, and neurodegenerative diseases.

Immune Response: Micro plastics may provoke an immune response in the body, which can lead to a range of health problems, including autoimmune diseases and allergies.

Reproductive and Developmental Toxicity: Micro plastics may cause reproductive and developmental toxicity, which can lead to a range of health problems, including infertility, birth defects, and developmental disorders.

Antimicrobial Resistance: Micro plastics may act as a carrier for antimicrobial-resistant microbes, leading to gut dysbiosis and infection.

While the direct effects of micro plastic consumption on human health are still not fully understood, the potential risks highlight the need for further research to understand the implications for human health and to develop strategies to reduce exposure to micro plastics.

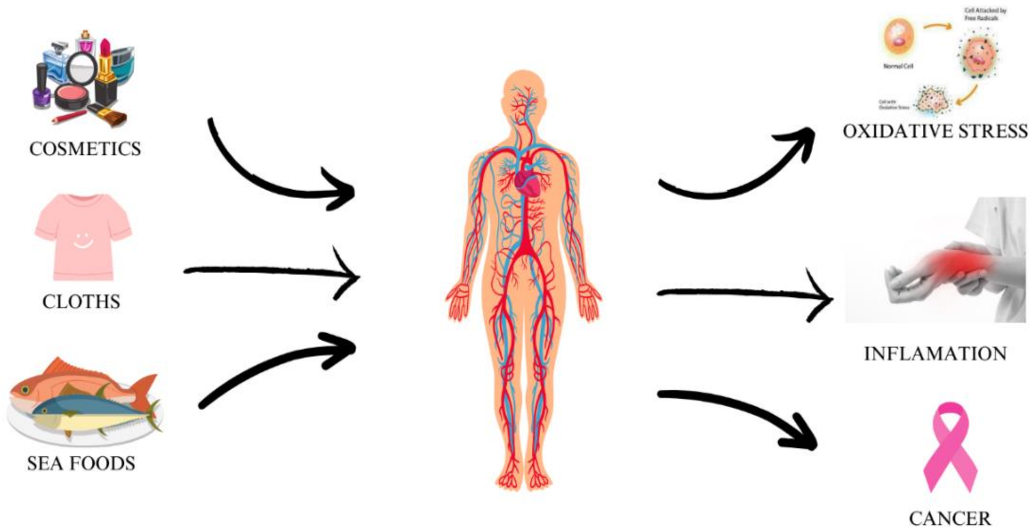

Figure 2: Effects of micro plastic to humans

POTENTIAL LINK TO CANCER

Potential pathways by which micro plastics might cause cancer include oxidative stress, genomic instability, and chronic inflammation, among other intricate biological processes. Comprehending these pathways is crucial in order to appreciate the plausible carcinogenic consequences of micro plastics. One important process connected to the development of cancer is oxidative stress. Oxidative stress can result from the interaction of micro plastics with environmental variables, which can produce reactive oxygen species (ROS). ROS have the ability to cause lipid peroxidation, protein alteration, and damage to DNA, all of which can aid in the development and spread of cancer. Furthermore, heavy metals and persistent organic pollutants that might worsen oxidative stress and encourage carcinogenesis may be carried by micro plastics. Genome instability is yet another important factor linked to the development of cancer. Micro plastics have been connected to genotoxicity and DNA damage, which may result in chromosomal abnormalities and mutations. These genetic changes have the potential to impair regular cellular processes and aid in the development of cancer from healthy cells. Moreover, internalizing micro plastics into the body can disrupt DNA repair pathways, worsening genomic instability and raising the chance of cancer development. Cancer is known to be characterized by chronic inflammation, and micro plastics have been linked to the stimulation of inflammatory reactions. Immune cells and tissues may react with persistent inflammation when exposed to micro plastics. When exposed to micro plastic, the body releases inflammatory mediators and cytokines that can lead to the formation of a microenvironment that supports the growth and survival of cancer cells. Furthermore, persistent inflammation can accelerate the formation of precancerous lesions, cause tissue damage, and eventually aid in the development of cancers. These pathways' combined impacts highlight the possibility that micro plastics may aid in the development of cancer. It is crucial to remember that even while there is growing evidence to support these mechanisms, more investigation is still required to pinpoint the precise mechanisms by which micro plastics may cause cancer. Furthermore, further research is necessary to fully understand the combined influence of micro plastics and other environmental carcinogens on the development of cancer (Alimba, C. G., *et al*).

CONCLUSION

Our dependence on plastic, a cheap and practical substitute for eco-friendly materials, to satisfy the needs of an overpopulated planet has resulted in a worldwide problem. The amount of plastic garbage generated has increased dramatically; it surpassed 6.3 billion metric tons in 2015 and is expected to reach an astounding 12 billion metric tons by the year 2050. Plastic trash weathers into micro plastics when exposed to sunshine, wind, and ocean waves. These particles have the ability to enter the food chain, be consumed or breathed, and build up in organisms, endangering the health of humans and the environment. Concerns over the effects of the wide variety of micro plastic types—including PVC and PU, which are recognized for their increased toxicity—on people and animals are raised. Microplastics are ubiquitous in a variety of settings, including freshwater and marine ecosystems, food, and drinking water. Their sizes range from 1μm to 5 mm. The potential for them to carry dangerous substances, such heavy metals, and persistent organic pollutants, endangers both human health and ecosystems. Micro plastics have been found in commonplace products including salt, sugar, alcohol, bottled water, and even human feces, suggesting the possible degree of exposure. Inflammation, oxidative stress, immunological reactions, and possible harm to reproduction and development are among the effects of this exposure. Microplastics have consequences for ecosystems and human health. Humans can get exposed via breathing in polluted air, touching tainted food or drink, or encountering contaminated environmental or consumer goods. Although the direct consequences of consuming micro plastics on human health are not fully known, they highlight the need for in-depth study to understand the ramifications and create mitigation solutions. It is even more important to comprehend the intricate biological processes including oxidative stress, genomic instability, and chronic inflammation considering the possible correlation between micro plastics and cancer. Although there is evidence that these processes may play a role in the development of cancer, further study is necessary to fully understand how micro plastics may function as a catalyst for the onset of cancer. It is critical to recognize the connection between human and environmental well-being while tackling this complex issue, highlighting the need for sustainable practices and well-informed legislation to lessen the effects of micro plastic contamination.

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Chapter 2

PHARMACOLOGICAL OVERVIEW OF THEVETIA SPECIES

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ABSTRACT

Thevetia species, belonging to the Apocynaceae family, are tropical plants known for their ornamental beauty and significant pharmacological properties. Despite their aesthetic appeal, all parts of these plants contain toxic cardiac glycosides, necessitating careful handling. Thevetia peruviana and Thevetianeriifolia are notable species, with the former being explored for biofuel potential due to its high oil-content seeds and the latter for its medicinal glycosides. Traditional uses include employing Thevetia peruviana's hallucinogenic properties in Andean cultural rituals and its application as a fish poison. Modern research has revealed its alkaloids, flavonoids, and phenolic compounds which have various biological activities, such as antimicrobial, antifungal, and anti-cancer properties, highlighting the plant's potential in medical and agricultural applications. However, the toxicity of Thevetia species poses risks, emphasizing the need for safe practices, and further research is needed to fully understand, harness their benefits effectively and utilize these properties.

KEYWORDS: Thevetia Species, Apocynaceae Family, Biological Activities.

INTRODUCTION

Thevetia species is a member of the Apocynaceae family and is also referred to as the lucky nut or yellow oleander. Thevetia peruviana and Thevetianeriifolia are two of the more common species found in the Thevetia genus. Although these plants are prized for their aesthetic qualities, it's vital to remember that they have hazardous substances and need to be treated carefully (Ahmad, Tabrez & Hamid, Abdulhamid Tahir & Sharma, Anuradha & Bhardwaj, Uma, 2017).

Thevetia peruvianais a plant that originated in South and Central America but has since spread around the world. Its cardiac glycosides create flavonoids with antiviral and phenolic compounds with antibacterial and antineoplastic activities. These chemicals have an inotropic activity akin to digoxin. To improve its chemical synthesis, *T. peruviana* has been cultivated in vitro. (Dary Mendoza, Juan Pablo Arias, Olmedo Cuaspud, Mario Arias, 2020). *Thevetia peruviana* is a medicinal plant with a variety of applications such as treating wounds, ringworm, tumors, etc. It has novel components that possess various therapeutic activities such as cardiovascular, anticancer, antimicrobial, antioxidant, immunomodulatory, and anti-inflammatory effects. However, despite the presence of many research studies on its properties, there is still a lack of systematic information regarding its wound-healing abilities (N. Rahman, H. Rahman, M. Haris, and R. Mahmood. 2017).

Thevetia neriifolia is a notable medicinal plant whose powerful cardiac glycosides have been the focus of numerous studies. Certain glycosides, in particular nerrifoside, thevetin, cerberin, and peruvoside, have demonstrated potential in the management of cardiac conditions. It has long been used in traditional medicine, having been identified for its cardiotonic properties as early as 1863 in ancient India.

Glycosides made of three sugar units and one aglycone unit are found. Due to their complexity, extraction methods have become more dependent on natural sources, which have increased interest in biotechnological methods of production. Thevetianeriifolia in vitro cultures have been investigated as a potential source of these important glycosides. The glycosides in the plant are hydrolyzed by enzymes to produce a variety of chemicals that have been identified and investigated for their potential medicinal uses (Gulati, A., Jain, S.K., & Srivastava, P. 2001). In addition to their profound effects on the cardiovascular system, the glycosides contain other biological functions, like antibacterial and antifungal qualities.

PHARMACOLOGICAL ACTIVITIES AND MEDICINAL IMPLICATIONS

The herb *Thevetia peruviana* has important pharmacological properties and potential uses in medicine. Traditionally, rheumatism, snake bites, and malaria have all been treated using different components. Due to its various pharmacological qualities, scientific research has supported its usage in traditional medicine. Among its many properties include anti-inflammatory, anti-diarrheal, antibacterial, cytotoxic, anti-cancer, anti-coagulant, and anti-fungal properties. For example, in animal models, extracts made with ethanol of *T. peruviana* leaves were found to have significant anti-diarrheal effects. Its antibacterial qualities have been noted against a variety of microorganisms, including *Staphylococcus aureus* and *Shigella flexneri*. Brine shrimp lethality tests have proven the plant's cytotoxic action, suggesting that it may be used to treat cancer (Gaur, R., Rathore, A., Sharma, A. K., Pathak, D., & Kulshreshtha, M. 2023). Moreover, it has been shown that *T. peruviana* latex contains antioxidant, anticoagulant, and anticancer properties.

ANTIMICROBIAL PROPERTIES

Thevetia peruviana's antimicrobial qualities were examined by employing the technique of disc diffusion to examine how the plant's ethanol-extracted leaves fared against different bacterial strains. The findings showed that the extract has weak antibacterial properties against *Salmonella typhi*, *Bacillus* sp., *Shigella flexineri, Klebsiella* sp., and *Staphylococcus aureus*, among other Gram-positive and Gram-negative bacteria. The extract's small zones of inhibition indicate that it has a limited range of antibacterial activity. Nevertheless, the study emphasizes how crucial it is to do more research to separate and pinpoint the extract's active ingredients, which may or may not be responsible for its antibacterial action (Hassan, M. M., Saha, A. K., Khan, S. A., Islam, A., Mahabub-Uz-Zaman, M., & Ahmed, S. S. 2011). The results emphasize how important it is to learn more about the characteristics, security, and effectiveness of herbal remedies like *T. peruviana*, which are widely utilized in traditional medicine, particularly in poor nations.

INSECTICIDAL PROPERTIES

The aqueous extract of *T. peruviana* fruit kernels have been shown in experiments to be harmful to the slug *Laevicaulis alte* and the snail *Achatina fulica*. In the Indo-Pacific area, these pests are serious in the agri-horticultural sectors. All exposed slugs can die in around 981 minutes at even a 1% concentration of the extract. Between 92 and 321 minutes, a 2% solution can eradicate up to 50% of *A. fulica* snails and 100% of *L. alte* slugs. The strongest concentration tested, 20%, quickly eliminated all slugs and snails when added to soil or potato slices used as bait. (Panigrahi, A. and Raut, S.K. 1994).

ANTI-DIARRHEAL PROPERTIES

There may be antidiarrheal qualities to *T. peruviana*. The plant's ethanol-extracted leaves were examined on albino rats that had been given castor oil to induce diarrhea. The extract was shown to help treat diarrhoea as evidenced by the considerable diminished diarrhoeal episodes observed in the results. According to the study, *T. peruviana*'s antidiarrheal properties may be linked to its phytochemical components; however, further research is needed to fully understand these constituents' modes of action. This study advances the investigation of medicinal herbs as a natural remedy for diarrhea, providing an alternative to synthetic medications (Hassan MM, Saha AK, Khan SA, Islam A, Mahabub-Uz-Zaman M, Ahmed SS. 2011). Although the results are encouraging, prudence is suggested because of the plant's recognized poisonous qualities, which call for cautious doses and more research to guarantee safety and effectiveness. The antidiarrheal properties of *T. peruviana* highlight the value of traditional medicine in the search for novel therapeutic agents.

ANTIBACTERIAL PROPERTIES

Significant antibacterial capabilities of *T. peruviana* have been shown in a study presented in the Journal of Coastal Life Medicine. The study focuses on *T. peruviana* leaf extracts' antibacterial potential against bacterial infections linked to food. It demonstrates that the leaf extracts exhibit variable levels of inhibition against common foodborne bacteria like *E. Coli, S. aureus*, and *S. typhimurium* when dissolved in solvents such as acetone, chloroform, methanol, and petroleum ether. These extracts' minimum inhibitory concentrations (MIC), which range from 16.67 to 50.00 mg/mL, show how well they work to stop the development of bacteria. Additionally, the presence of substances with known antibacterial properties such as alkaloids, cardiac glycosides, flavonoids, polyphenols, saponins, and tannins is confirmed by phytochemical screening of *T. peruviana* leaf extracts (Gezahegn, Z., Akhtar, M. S., Woyessa, D., & Tariku, Y. 2015). According to this study, *T. peruviana* presents a promising substitute for synthetic antimicrobials and a natural source for the development of antibacterial agents targeting foodborne pathogens, all while promoting food hygiene and the welfare of the public. The study emphasizes how important it is to investigate the therapeutic potential of herbal remedies like *T. peruviana*, particularly in areas where traditional medicine is still a major component of healthcare.

ANTI-CANCER PROPERTIES

Tumors and other illnesses have long been treated using *T. peruviana*. Its fruit methanolic extract was tested against human cancer cell lines to see whether it has any anticancer properties. Prostate, breast, colorectal, and lung cancer cells were shown to be cytotoxically affected by the extract; the prostate cancer cells exhibited the lowest IC50 value, indicating a high potency. Across all assessed cancer cell lines, the extract also markedly decreased colony formation and cell motility, indicating effective anti-proliferative and perhaps antimetastatic properties. Treatment-induced morphological alterations in cancer cells included membrane blebbing and a decrease in cell size, which suggested that the extract had an impact on the morphology of the cells (Ramos-Silva, A., Tavares-Carreón, F., Figueroa, M. *et al.*, 2017).

Additionally, DNA fragmentation and AO/EB double staining were used to establish that the extract caused apoptotic cell death. The research determined that the active ingredients were secondary metabolites such as cardiac glycosides and thevetia flavone. These results demonstrate the potential of *T. peruviana* as a natural anti-cancer drug that primarily targets human cancer cells, affecting their proliferation, motility, and inducing apoptosis, while having little effect on non-tumorigenic cells. The research recommends more investigation into the bioactive components of *T. peruviana* for the treatment of cancer.

ANTI-COAGULANT PROPERTIES

T. peruviana Latex's Anticoagulant Properties are discussed. Heparin, a well-known anticoagulant, was used to compare the findings of the investigation into the latex's anticoagulant qualities. The anticoagulant activity was measured in the study using traditional coagulation tests, such as prothrombin time (PT) and activated partial thromboplastin time (APTT). The latex had very little anticoagulant activity at 25 μg/mL; the reported durations for PT and APTT were 11.9 and 29.2 seconds, respectively. In contrast, clotting durations for PT and APTT at the same heparin dose were noticeably longer—94.6 and 117.7 seconds, respectively (Al-Rajhi AMH, Yahya R, Abdelghany TM, Fareid MA, Mohamed AM, Amin BH, *et al.*, 2022). This implies that the anticoagulant action of *T. peruviana* latex is not as strong as that of heparin, even though it does have some activity.

ANTI-FUNGAL PROPERTIES

T. peruviana demonstrates noteworthy antifungal characteristics, namely against *Alternaria solani*, a plant pathogenic fungus that significantly reduces the value of potato harvests. *T. peruviana* leaf extract is used as an environmentally safe and efficient biocontrol approach for managing plant diseases. The results of the study suggest that the leaves' alcoholic extracts, both partially refined and crude, had the most inhibitory effects on *Alternaria solani*. Gas Chromatography-Mass Spectrometry (GC-MS) analysis and phytochemical screening were used to identify the secondary metabolites that are responsible for this. Interestingly, chemicals recognized for their antibacterial qualities, benzoic acid, and oxo-benzoate, were present in the extract's active column fraction, which showed the strongest antifungal activity (Meena, B. R., Meena, S., Chittora, D., & Sharma, K. 2021).

The results indicate that creating herbal remedies from *T. peruviana* may offer a sustainable method of controlling agricultural diseases, presenting a viable substitute for traditional chemical fungicides that are hazardous to human health and the environment. The study emphasizes the potential of bioactive chemicals produced from plants as bio-fungicidal products that support sustainable agricultural practices and integrated pest control

BIOLOGICAL ACTIVITIES OF *THEVETIA PERUVIANA*

T. peruviana has important biological activities. The plant is well-known for its white latex and for containing substances like thevetin, which although highly dangerous in their natural state, is utilized as a heart stimulant. Cardiac glycosides, found in *T. peruviana* seeds, directly affect the heart. The plant's fresh blossoms are rich in quercetin, kaempferol, and polyphenols. There have been reports of proteins, glycosides, flavonols, and phenols in *T. peruviana* leaf extract. Because of their photosensitivity, these extracts have been used in the green synthesis of Cadmium Telluride (CdTe) nanoparticles, which find utility in a variety of applications. Furthermore, the leaf extract contributes to the evaluation of power conversion efficiency in indicator-sensitized solar cells (DSSCs) by acting as a natural dye. (Swades Ranjan Bera and Satyajit Saha 2018). *T. peruviana*'s versatility and significance in sustainable and environmentally friendly technological breakthroughs are highlighted by this utilization of the plant in nanotechnology and solar cell applications, which highlights its versatility beyond traditional biological uses.

The hydroalcoholic extract derived from the twigs has demonstrated the ability to quadruple B-cell proliferation in immunomodulation studies, suggesting that it may have potential applications as an immunostimulant. Strong cytotoxic effects against several cancer cell lines, including those from the colon, pancreatic, lung, prostate, and breast, were also noted, indicating potential anti-cancer qualities. While it showed little antimicrobial action, the extract also showed some modest anti-inflammatory effects (Save, S. A., Padmanabhan, U., Kothari, S. T., Lokhande, R. S., & Chowdhary, A. S. 2015). *T. peruviana* is a good option for medication development and bioprospection, especially for ailments such as microbial allergies, cancer, anemia, and arthritis. The results highlight the potential of twigs of *T. Peruviana* to aid in the creation of new medicines.

T. peruviana plant culture suspensions of cells and callus extracts were tested for antibacterial activity against a range of bacterial species. The results show that extracts of these cultures' ethanol, methanol, and hexane have biological activity. The ethanol-suspended cell extract prevents all examined bacteria, especially *S. typhimurium*, but the methanol as the cell suspension extract is active contrary to *B. cereus* and *S. aureus*. The hexane extract has resistance action towards *B. cereus, S. aureus*, and *S. typhimurium* (Echeverri, J. P. A., Ortega, I. C., Peñuela, M., & Arias, M. 2019). The findings highlight the significance of investigating plant-based extracts as substitute antimicrobial agents, particularly considering the rising prevalence of antibiotic resistance.

THERAPEUTIC VALUES

T. peruviana is a plant with several traditional and contemporary medical applications. Its therapeutic potential is well-known. The plant is prized for the variety of chemical components that support its healing abilities. It was previously used to treat many different disorders in allopathy, Ayurveda, ethno medicine, and Unani medicine. Digoxin-like acts have been attributed to *T. peruviana*'s cardiac glycosides, which are responsible for its medicinal advantages. Furthermore, the plant's seeds, leaves, fruits, and roots are thought to be sources of biologically active substances that can be used as fungicides, bactericides, insecticides, and rodenticides (Garima Zibbu and Amla Batra. S, 2011). *T. peruviana* is poisonous, but when administered properly, it has several medical advantages, such as diuretic and cardiotonic properties, The outer layer of bark is utilized as a febrifuge and cathartic, while the leaves are convulsive and purgative. Fruit rind and leaf extracts greatly increase the pace of wound contraction, skin collagen tissue creation, and wound-breaking strength, which promotes quicker and better healing (Nazneen Rahman and Haseebur Rahman and Mir Haris and Riaz Mahmood, 2017). The study observes that treated tissues had higher concentrations of hydroxyproline, hexosamine, and hexuronic acid, suggesting improved connective tissue development, decreased inflammatory markers and free radicals, which aided in the healing process.

This creates opportunities as a possible source of strong wound-healing medications.

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Chapter 3

BIOCONVERSION AND BIOTRANSFORMATION EFFICACY OF WILD MACROFUNGI

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ABSTRACT

There are tremendous uses of fungi especially for food and medicine which dates back a long way in human history but documentation of such knowledge holds a relatively new aspect though many species have been identified. Fungus also plays a significant role in industries, agriculture, pharmaceutical, food, textiles, and in bioremediation process. Mushrooms have a widespread occurrence in nature and are still said to be the earliest form of fungi on earth. They are considered as fleshy fungi with agricultural and biotechnological significance. Due to their high nutritional values and medicinal importance, they are globally recognized as supplementary foods, which includes exhibiting their antioxidant and antimicrobial activities, high immunological potential, and also effective for the treatment of diseases like diabetes and few types of cancers.

KEYWORDS: Bioconversion, Biotransformation, Efficacy, Macro fungi.

INTRODUCTION

There are tremendous uses of fungi especially for food and medicine which dates back a long way in human history but documentation of such knowledge holds a relatively new aspect though many species have been identified. Fungus also plays a significant role in industries, agriculture, pharmaceutical, food, textiles, and in bioremediation process. Mushrooms have a widespread occurrence in nature and are still said to be the earliest form of fungi on earth. They are considered as fleshy fungi with agricultural and biotechnological significance. Due to their high nutritional values and medicinal importance, they are globally recognized as supplementary foods, which includes exhibiting their antioxidant and antimicrobial activities, high immunological potential, and also effective for the treatment of diseases like diabetes and few types of cancers. According to a recent study, extracellular enzymes produced by certain white-rot fungal strains such as Phanerochaete chrysosporium, Pleurotus sajor-caju have shown the ability to decolorize dyes that are very harmful for the environment. Fungi are classified broadly as yeast, moulds and macro fungi or mushrooms.

DIVERSITY OF WILD MACRO FUNGI

Different taxonomic groups of fungi produce conspicuous sporocarps known as macro fungi which includes gilled fungi, jelly fungi, puffballs, bracket fungi, truffles, and bird’s nest. Macrofungal diversity constitutes an essential part of fungal diversity. Ecological classification of macro fungi includes saprophytes, the parasites, and the symbiotic species. Macro fungi are diverse in their uses and several species serve as decomposers as well. They can synthesize nanoparticles useful for the treatment of cancer, gene therapy and DNA analysis that they have become extremely important model for basic biology and commercial manufacture. The diversity of macro fungi serves as an important place both in terms of their ecological and economic value. Macro fungi are found in varied habitats and their distributions of species is low in hot and dry seasons and are abundant in spring and autumn due to favorable climate and abundance of flora. During recent years studies on the taxonomy and diversity of macrofungi have gained much attention, as many macrofungi are facing the risk of extinction due to habitat destruction and also indicates that the distribution of macrofungi is very much dependent on the plant community and the environmental conditions.

Figure 1: Wild Macro Fungi

COLLECTION & PRESERVATION

The ecological habitats like humid soil, wood log, leaf litter, wood, sandy soil, leaf heaps, wheat straw, paddy straw, calcareous soil, wet soil, troops of rotten wood, termite nests, decaying wood log and humus all were taken into consideration for the existence of macrofungi. The collected samples wrapped in wax paper were brought to the laboratory for identification which was made on the basis of macroscopic and microscopic characteristics using relevant literatures.

TRADITIONAL USE

People consumed Wild edible fungi and consumed for thousands of years. Archaeological records reveal the association of edible species with people living 13000 years ago in Chile but it is in China, eating of wild fungi is first reliably noted that is several hundred years even before the birth of Christ. Edible fungi collected from forests in ancient Greek and Roman times were highly valued more by high-ranking people than by peasants. Amanita caesarea said to be Caesar's mushroom reminds of an ancient tradition that still exists in many parts of Italy, dominated today by truffles (Tuber spp.) and porcini (Boletus edulis). China holds a prominent place in the early and later historical record of wild edible fungi. For centuries the Chinese have valued many species for nutrition and for taste and also for their healing properties. Developments in ethno mycology though started with a clear interest in wild edible fungi, later laid its emphasis on hallucinogenic mushrooms and their cultural significance. In the last three decades researchers have substantially increased our knowledge about local traditions in Africa and Asia.

NUTRITIONAL BENEFITS

Mushrooms act as storehouse of nutrition. Among the 2166 worldwide recognized edible species of mushroom around 470 species possess medicinal properties. Interestingly, macrofungi act as bio-indicators of health or age of an ecosystem. Macro fungi plays a pivotal role in the diversification and improvement of human diet. By exploiting low economic value agricultural by-products macrofungi have the potential to act as additional source of income for farmers. One of the most fast-growing commercial sectors is using mushrooms as source for the generation of medicinal compounds and dietary supplements. For example, Pleurotus species are rich in dietary fibres, vitamin B, b-carotene, tocopherol and other valuable nutrients and antioxidants. They also contain a number of biologically active compounds shown to modulate the immune system, inhibit tumour growth and inflammation. They also have hypoglycaemic and antithrombotic activities, lower blood lipid concentration, prevent high blood pressure and atherosclerosis, and present antimicrobial and other activities. The most valuable mushroom fungi in the pharmaceutical industry are Ganoderma spp. This has potent immuno-modulating effects, activation of macrophages, NK and T cells as well as enhanced anti-tumour activity in host cells. As more than 700 mushroom species were reported to possess medicinal properties, it is obvious that this group of organisms represents a powerful spectrum of valuable pharmaceutical products or food supplements. Moreover, truffles are widely accepted as gastronomic treasures of high commercial value, and they are international best-sellers. For many cultures, age groups and families harvesting of truffles is an enjoyable hobby that brings people to forests. The wild macro fungi are consumed by the native population and is said to be an important source of natural dietary product and delicious food supplement since ancient times. Alternative to plant or animal derived food sources edible mushrooms are used. Wild edible mushrooms are the major concern in food security of ethnic and tribal population. Apart from its high nutritive value, wild mushrooms are also employed as nutraceuticals. Mushrooms contain appreciable quantities of crude fibers. Many authors showed that although little information exists on the Total Dietary Fibre (TDF) content of mushroom, the crude fibre contents values suggest that mushrooms are potential sources of dietary fibers. Mushrooms are generally low in fat and oil content. Because of this, they are recommended as good food supplement for patients with cardiac problems. The vitamin content of many mushrooms has been investigated and this shows that they are rich in vitamins C, B1, B2, B3 and vitamins D. The considerable pharmacological activities of mushrooms are made use in pharmaceutical industries for the development of drugs. Thus, many bioactive compounds which play essential role in human and animal physiology have been found in many mushrooms. Auricularia auricular, Pleurotus squarrosulus and Russula sp. has been found to contain alkaloids, phenols, Saponins and Flavonoids. Flavonoids have been reported to be useful in the treatment of some physiological disorders and diseases. The anti-oxidants Flavonones are used to combat carcinogenesis and ageing processes. Antimicrobial and antioxidant activities of mycelia obtained from wild edible mushrooms such as *Armillaria mellea, Meripilus giganteus, Morchella costata, Morchella elata, Morchella esculenta var. vulgaris, Morchella hortensis, Morchella rotunda, Paxillus involutus, Pleurotus eryngii*, and *Pleurotus ostreatus* were investigated. Among the mushroom extracts, *M. elata* showed the most potent radical scavenging activity. This research study has shown that these wild macro fungi have potential as natural antioxidants and antibiotics.

ENZYMES FROMWILDMACRO FUNGI

Mushrooms are a treasure house of several enzymes with biotechnological and industrial potential. Proteases have wide spectrum of applications in detergents, leather processing, silver recovery, medical purposes, food processing, feeds, chemical industry and in waste treatments. Proteases also participate in some major physiological processes, in enzyme modification, nutrition and regulation of gene expression. Amylases and cellulases have extensive application in food, detergents, textile, drinks, animal feed, paper production and fermentation. Cellulases are used in the production of wine, beer and fruit juice and in bioconversion of lignocellulosic biomass. Laccases from mushrooms have found potential applications in paper-pulp bleaching, textile dyes, synthetic dye decolorization, and detoxification of polluted water, bioremediation and chemical synthesis.

BIOCONVERSION & BIOTRANSFORMATION EFFICACY

The Bioconversion Science and Technology group in the USA performs multidisciplinary R&D for the Department of Energy's (DOE) relevant applications of bioprocessing, using biomass. Bioprocessing constitutes the disciplines of chemical engineering, microbiology and biochemistry where it investigates the use of microorganism, microbial consortia and microbial enzymes in bioenergy research. Novel cellulosic ethanol conversion processes have enabled the variety and volume of feedstock that can be bioconverted. Feedstock includes materials derived from plant or animal waste such as paper, tires, fabric, construction materials, municipal solid waste sludge& sewage. Engineered and direct evolution of enzymes have enhanced enzymatic activity and have shown an increase in the number of products obtainable by biotransformation.Macrofungi forms a vital factor for the maintenance of life on earth due to their capacity to biodegrade organic matter.

Macro fungi are extensively used in the bioremediation of industrial waste and in heavy metals accumulation from the environment. Macro fungi are able to efficiently degrade a wide range of substances due to their capacity to produce different enzymes for which they are used as bio detoxification and bio remediation agents. In this aspect it is necessary to explore the efficiency and importance of wild mushroom as bioconversion and biotransformation agents as well as the importance of these fungi as potential decomposers in the soil and finally to explore the tools to understand the mechanism of the process. Initially the fungi need to obtain energy from a nutrient source to create a new product. This occurs by the process of biodegradation where Sugars are produced by the degradation of complex substrates. The process is applied to the disintegration of any matter by biological means. Specialized enzymes are involved in the biodegradation called promiscuous enzymes that has the capability to degrade several analogous substrates. Through the action of these enzymes, the fungi are able to remove some toxic substances by a process of bio detoxification. In such cases if the microorganism in particular the fungal community involve in myco bioremediation which is the elimination of the xenobiotic compounds from contaminated media, including water and soil. Thus, the macrofungi are able to degrade complex organic matter into mineral samples by the process of mineralization.

The bioconversion factor of heavy metals refers to the level of metal concentration in the mushroom body correlated with the metallic element in the soil in which the fungus grows. As stated, earlier bioconversion utilizes sugar from cellulose and hemicellulose and forms the macro fungi's own metabolites and are essential for growth and survival of mushrooms. Thus, wild mushrooms act as a potential source of secondary metabolites and enzymes which are in turn employed in the production of antibiotics, antifungals, nematicides and vitamins production. Macro fungi thus uses the thus produced enzymes to biodegrade and biotransform the lignin of wood to have access to the cellulose and hemicellulose chains. These enzymes degrade lignocellulosic material into sugar monomers for the production of ethanol by fermentation process involving yeast.

Lignocellulosic wastes are used for mushroom cultivation and a bioconversion product can be obtained. The two main aspect of Mushroom cultivation involving industrial wastes results in protein rich mushroom fruiting bodies and also aids in solving pollution related problems to a greater extent.

Potential products from macrofungi are beneficial for human activities. Enzymes are requisite in food, textile, paper and pharmaceutical industries. Wild mushrooms bioconvert the nutrients of dead wood into important bioactive molecules using enzymes. Cell wall components are biotransformed into oligomers and monomers which undergo fermentation process to produce ethanol & organic acids.

Biosorption is the removal of metals or contaminants by mushrooms from aqueous solution. Researchers found that the uptake of heavy metals largely depends on physico-chemical interactions of metallic ions with the cellular compounds of biological species. Biosorption holds the credit of being a very popular method due to maximum uptake capacity and lower cost. Many mushroom species remove pollutants or using biosorption. P. tuber-regium biosorb heavy metals from heavy metal contaminated soil, Fomes fasciatusas biosorption of Cu(II) ions.P. platypus, A. bisporus, Calocybe indica are efficient biosorbent for the removal of Cu, Zn, Fe, Cd, Pb.P.ostreatus removes cadmium and P.sajor-caju biosorb Zn from contaminated site. Mushrooms or macro fungi can uptake pollutants or heavy metals by both bioaccumulation and biosorption process. Studies reveal that compared to plants, vegetables and fruits mushrooms can build up large concentrations of heavy metals in them.

CONCLUSION

More than 2000 species of Wild mushrooms exist in nature but only less than 25 species are widely accepted as food. Besides their high mineral content, low fat, and low calories and the nutritional values, they have been known to have an effect on preventing several diseases such as cancer, hypercholesterolemia, and hypertension. To maintain balanced lipid homeostasis dietary fat, a major constituent of the normal diet is necessary. Lipid content of mushroom species is generally low. It is reported that, fresh mushrooms have more the lipid proportion than dried mushroom since fresh ones contain high amounts of water. Though the edible wild mushrooms hold higher prices than cultivated mushrooms, people prefer to consume them due to their flavour and texture. Since Mushrooms in general holds a place in nature as it helps tackle global challenge such as hunger, it is much needed to record completely the wild edible mushrooms. Also, studies on mushroom pertaining to bioprospecting and nutraceuticals are gaining its importance.

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Chapter
4

EICHHORNIA CRASSIPES: A POLLUTER UNTIL IT BECOMES A SOLUTION

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ABSTRACT

Water hyacinth is one of the most common species in the global decorative plant trade, as well as one of the most invasive. This species is native to Brazil, although it has spread to tropical and subtropical climates all over the world. Water hyacinth is popular among aquarists and water gardeners since it is one of the few floating aquatic plants, reproduces quickly (a population can double its biomass every 6-18 days), and produces a huge number of lovely purple flowers. It is also extremely invasive, forming monospecific mats in infested lakes and rivers and causes problems to the ecosystem. Hence, it has been used in a variety of scientific projects during the last few decades. This review reveals the significance of water hyacinth though it's an invasive species.

KEYWORDS: Water Hyacinth, Antibacterial and Antifungal Properties, Phytoremediation

INTRODUCTION

Water hyacinth is a beautiful aquatic plant that floats on the surface of water bodies, like ponds, lakes, and rivers. Its scientific name is *Eichhornia crassipes*. This plant has vibrant green leaves and lovely purple or blue flowers that add a touch of beauty to any water landscape. It spreads quickly, forming dense mats on the water's surface, which can sometimes cause problems for ecosystems (Elenwo and Akankali 2016).

Despite its beauty, water hyacinth is considered an invasive species in many regions. It can grow rapidly, outcompeting native plants and disrupting the balance of ecosystems. These dense mats can block sunlight from reaching other aquatic plants and prevent oxygen from entering the water, harming fish and other marine life.

However, water hyacinth also has some beneficial uses. Its thick mats can help control erosion, and its roots can filter and purify water, improving water quality. Some people even use water hyacinth to make crafts and furniture, as its stems and leaves can be woven into useful and beautiful products (Sharma *et al.*, 2016)

POLLUTION OF WATER HYACINTH IN WATER BODY

Water hyacinth itself is not a source of pollution in water bodies. However, its rapid growth and dense mats can contribute to certain water quality issues if left uncontrolled. One-way water hyacinth can indirectly contribute to pollution is by blocking sunlight from reaching other aquatic plants. This can hinder their growth and lead to reduced oxygen levels in the water, potentially harming fish and other organisms that rely on oxygen.

Additionally, when water hyacinth mats decompose or die off, the decaying organic matter can deplete oxygen levels in the water. This process, known as eutrophication, can result in a decrease in water quality and can lead to the formation of harmful algal blooms.

It's worth noting that the presence of water hyacinth itself is not a pollutant, but it can exacerbate existing pollution issues in water bodies if left unmanaged. Regular monitoring, control measures, and proper waste management practices are crucial to minimize the negative impacts of water hyacinth on water quality (Navarro *et al.,* 2000).

Additionally, it's crucial to consider the environmental impact of water hyacinth as an invasive species. Water hyacinth can disrupt aquatic ecosystems, so it is important to manage its growth responsibly and in accordance with local regulations (Egaga 2018).

A solution lies within While the plant is rightfully regarded an aquatic pest, numerous beneficial elements of it have been uncovered over time. The use of water hyacinth in wastewater treatment has been shown to be highly efficient and cost-effective. Growing water hyacinth in a sewage lagoon system reduces BOD5 up to 95%, TSS (Total suspended solids) up to 90%, nitrogen, phosphorus, heavy metals, and pesticides, according to one study titled 'Use of Water Hyacinths in Wastewater Treatment' authored by Gian C Gupta and published in the Journal of Environmental Health. (Haque2023; Mujere 2016).

Eichhornia crassipes is known to produce various secondary metabolites, some of which have been of interest for their potential applications in different fields. One such secondary metabolite found in water hyacinth is allantoin. Allantoin is a nitrogenous compound that has been identified in water hyacinth. It is known for its ability to promote wound healing and has been used in various skincare and cosmetic products. Allantoin has moisturizing, soothing, and anti-irritant properties, making it a desirable ingredient in creams, lotions, and other skincare formulations (Su *et al.,* 2018).

Water hyacinth is also known to contain other secondary metabolites such as phenolic compounds, flavonoids, and alkaloids. These compounds are known for their antioxidant properties, which may have potential therapeutic benefits (Akinwande *et al.,* 2013).

It has the following secondary metabolites:

Flavonoids: Water hyacinth contains a variety of flavonoids, including quercetin, kaempferol, apigenin, and luteolin. Flavonoids have antioxidant, anti-inflammatory, and antimicrobial properties.

Alkaloids: Water hyacinth contains alkaloids like vasicine and vasicinone. These compounds possess antifungal, antiviral, and anti-inflammatory activities.

Phenols: Water hyacinth is a good source of phenolic compounds such as caffeic acid, p-coumaric acid, and ferulic acid. Phenols have antioxidant and anti-inflammatory effects. Phenolic compounds, which are a diverse group of organic compounds found in plants, have been extensively studied for their effects on fungal contamination. These compounds possess a range of biological activities, including antifungal properties that can help combat fungal growth and contamination.

Phenolic compounds can inhibit fungal growth by disrupting various cellular processes. They have been shown to interfere with the synthesis of fungal cell walls, leading to structural damage and reduced fungal viability. Additionally, phenolic compounds can interfere with fungal enzymes and proteins, disrupting essential metabolic pathways and inhibiting fungal growth.

Furthermore, phenolic compounds can induce oxidative stress in fungi. They generate reactive oxygen species that can damage fungal cells and impair their survival. This oxidative stress can lead to the inhibition of fungal growth and even induce programmed cell death. Interestingly, phenolic compounds also have the ability to modulate the expression of fungal genes involved in pathogenicity and virulence. By regulating these gene expressions, phenolic compounds can potentially disrupt the ability of fungi to cause infections and colonize host tissues.

It's worth noting that the efficacy of phenolic compounds against fungal contamination can vary depending on the specific compound and the target fungal species. Additionally, factors such as concentration, exposure time, and environmental conditions can influence their effectiveness.

Terpenoids: Water hyacinth contains various terpenoids, including limonene, α-pinene, and β-pinene. Terpenoids have antimicrobial, antifungal, and anti-inflammatory properties.

Terpenoids, which are a diverse group of organic compounds commonly found in plants, have indeed been found to have interesting effects on fungal contamination. Terpenoids exhibit a wide range of biological activities, including antifungal properties that can help combat fungal growth and contamination. The use of terpenoids as natural antifungal agents has gained attention due to their potential effectiveness and environmentally friendly nature. Research has shown that terpenoids can inhibit the growth and development of various fungal species by interfering with their cellular processes.

Terpenoids can disrupt fungal cell membranes, affecting their integrity and permeability. This disruption can lead to leakage of cellular contents, ultimately inhibiting the growth and survival of the fungi. Some terpenoids have also been found to interfere with fungal enzymes and proteins essential for their survival. Moreover, terpenoids can act as repellents or deterrents, preventing fungi from colonizing certain surfaces. This property makes them useful in protecting various materials susceptible to fungal contamination, such as wood, textiles, and even food products.

It's worth mentioning that the effectiveness of terpenoids against fungal contamination may vary depending on the specific terpenoid compound and the target fungal species. Ongoing research is continuously exploring the potential of terpenoids as natural alternatives to synthetic antifungal agents.

Saponins: Water hyacinth contains saponins, which have antibacterial and anticancer properties.

Tannins: Tannins are present in water hyacinth and have antioxidant and antibacterial properties (Zah *et al.*, 2015)

WATER HYACINTH EXTRACT TO CONTROL BACTERIAL GROWTH

Water hyacinth (*Eichhornia crassipes*) extract has shown promising potential in controlling bacterial growth due to its bioactive compounds. The extract contains secondary metabolites that possess antimicrobial properties, which can inhibit the growth of certain bacteria.

Studies have demonstrated that water hyacinth extract exhibits antibacterial effects against various pathogenic bacteria, including both Gram-positive and Gram-negative strains. The specific mechanisms by which water hyacinth extract exerts its antimicrobial effects are still being explored, but it is believed to involve the disruption of bacterial cell membranes, interference with cell signaling pathways, or inhibition of essential enzymes (Haggag *et al.*, 2017).

It's worth mentioning that while water hyacinth extract shows antibacterial activity, further research is needed to determine its efficacy against a wide range of bacterial species and to understand its potential applications. It is always recommended to consult with professionals in the field, such as microbiologists or environmental scientists, for specific guidance on the use of water hyacinth extract or any other natural products for bacterial control.

Additionally, it's important to consider the ecological impact of water hyacinth as an invasive species. Water hyacinth can negatively affect aquatic ecosystems, so it is crucial to manage its growth responsibly and in accordance with local regulations.

WATER HYACINTH EXTRACT TO CONTROL FUNGAL CONTAMINATION

Water hyacinth (*Eichhornia crassipes*) extract has shown potential in controlling fungal contamination due to its bioactive compounds. The extract contains secondary metabolites that possess antifungal properties, which can inhibit the growth of certain fungi.

The antifungal effects of water hyacinth, technically known as *Eichhornia crassipes*, have been discovered. Various studies have explored the potential antifungal activity of water hyacinth extracts and have shown promising results. This plant contains bioactive compounds such as flavonoids, alkaloids, phenols, and terpenoids, which contribute to its antifungal potential.

Water hyacinth extract has demonstrated inhibitory effects against a range of fungal strains, including Candida albicans, *Aspergillus* niger, *Fusarium*oxysporum, and Trichophytonmentagrophytes, among others (Kumar *et al.,* 2014).

Research suggests that water hyacinth extract exhibits antifungal effects against various pathogenic fungi, including both yeasts and molds. The specific mechanisms by which water hyacinth extract exerts its antifungal effects are still being investigated, but it is believed to involve the disruption of fungal cell membranes, interference with cell signaling pathways, or inhibition of essential enzymes.

It's important to note that while water hyacinth extract shows antifungal activity, further research is needed to determine its efficacy against a wide range of fungal species and to understand its potential applications. It is always recommended to consult with professionals in the field, such as mycologists or microbiologists, for specific guidance on the use of water hyacinth extract or any other natural products for fungal control (Aravind *et al.*, 2013).

Water hyacinth's antifungal effect can be linked to its phytochemical composition, which includes chemicals with fungicidal or fungistatic activities. Water hyacinth has the potential to be a natural antifungal agent, but further research is needed to completely understand the mechanisms of action and optimise extraction methods.

PHYTOREMEDIATION CAPACITY OF WATER HYACINTH

water hyacinth can act as a sink for pollutants present in the water. It can absorb heavy metals, pesticides, and other contaminants, potentially leading to the accumulation of these pollutants in the plant tissues. If not properly managed, the removal of water hyacinth from the water body can release these pollutants back into the environment, causing further pollution. Water hyacinth has the ability to absorb heavy metals, pesticides, and other contaminants present in water bodies. This process is known as phytoremediation, where the plant takes up and accumulates these pollutants in its tissues.

The roots of water hyacinth have been found to be particularly effective in absorbing heavy metals such as lead, cadmium, and mercury. The plant's leaves and stems can also absorb pesticides and other organic contaminants (Priya and Selvan, 2017)

The absorption of these pollutants by water hyacinth can help in reducing their concentration in the water, potentially improving water quality. Water hyacinth has been found to have the ability to absorb heavy metals from water bodies. The roots of water hyacinth are particularly effective in this process.

They have the capability to take up heavy metals such as lead, cadmium, mercury, and others from the surrounding water.

The absorption of heavy metals by water hyacinth occurs through a combination of physical and biochemical processes. The roots of the plant have specialized structures that allow them to uptake and accumulate heavy metals. These metals can bind to various cellular components within the plant, including cell walls and vacuoles.

The effectiveness of water hyacinth in absorbing heavy metals can vary depending on factors such as the concentration of metals in the water, the duration of exposure, and the overall health and growth of the plant. Additionally, the presence of other substances in the water, such as organic matter, can influence the absorption process (Lart *et al.*, 2019).

However, it is important to note that while water hyacinth can absorb pollutants, it does not eliminate them entirely. The pollutants remain stored in the plant's tissues, and if not properly managed, the disposal of water hyacinth can lead to the release of these contaminants back into the environment. Therefore, the use of water hyacinth for phytoremediation purposes should be accompanied by proper disposal methods to prevent the recontamination of the water body.

The invasive aquatic plant *Eichhornia crassipes* is very successful in phytoremediation, particularly in the rhizofiltration of heavy metal-contaminated effluents. Laet *et al.* (2019) showed that it is also a good bioindicator of water polluted by harmful organic pollutants including endocrine disruptors and neonicotinoids. After a brief period of exposure, di-n-hexylphthalate, pentabromodiphenyl ether, nitenpyram, acetamiprid, and bis (3-tert-butyl-4-hydroxy-6-methylphenyl) sulphide were clearly detected by UHPLC-HRMS or GC-MS in the root system of E. crassipes. These findings bring up new avenues for the remediation of water polluted by dangerous organic contaminants.

CONCLUSION

Eichhornia crassipes (Mart.) Solms., also known as water hyacinth, is a warm water aquatic plant that is the world's worst aquatic weed. It has the ability to grow swiftly and totally close water bodies. It has the potential to spread swiftly, completely shutting water bodies and significantly harming fisheries and related commercial activity. Water hyacinth contains compounds that have therapeutic properties. This plant's leaf extract contains flavonoids, alkaloids, tannins, and phenols, all of which have biological properties such as antifungal and antibacterial agents. It's important to note that the exact composition of secondary metabolites in water hyacinth can vary depending on factors such as environmental conditions, geographical location, and plant age. Research in this area is ongoing, and scientists continue to explore the potential uses and benefits of these secondary metabolites found in water hyacinth.

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Chapter
5

DETECTION OF MYCOTOXINS BY ADVANCED AND EMERGING ANALYTICAL METHODS

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ABSTRACT

Mycotoxins are toxic compounds produced by molds that can contaminate food and feed products, posing a risk to human and animal health. Various fungal genera, such as *Aspergillus*, *Penicillium*, and *Fusarium*, are known for producing mycotoxins like aflatoxins, ochratoxin A, patulin, and deoxynivalenol (DON).Advanced analytical methods like Liquid Chromatography-Mass Spectrometry (LC-MS/MS), Gas Chromatography-Mass Spectrometry (GC-MS), and High-Resolution Mass Spectrometry (HRMS) offer improved sensitivity and efficiency in mycotoxin detection.Control measures such as Good Agricultural Practices (GAPs) and proper storage techniques are crucial to mitigate mycotoxin contamination in food and feed products. Regulations and monitoring methods have been established to control mycotoxin levels in food and feed, contributing to improved food safety and public health.

KEYWORDS: Mycotoxins, Liquid Chromatography-Mass Spectrometry, GC-MS.

INTRODUCTION

Mycotoxins are toxic compounds produced by certain molds (fungi) that can contaminate various agricultural products, including crops, grains, and animal feed, posing a significant risk to human and animal health. These naturally occurring toxins have been a concern in the food and agricultural industries for decades, as they can lead to food safety issues, economic losses, and public health problems.

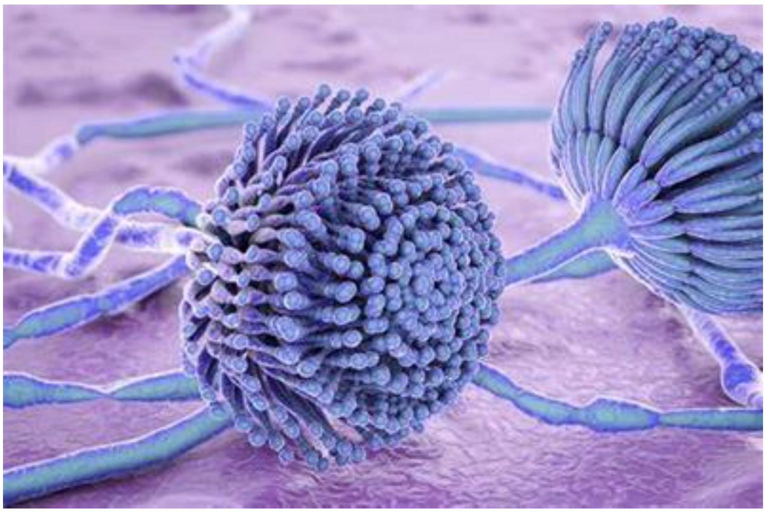

Figure 1: Micotoxins Producing Fungi

Mycotoxins are primarily produced by molds belonging to genera such as *Aspergillus*, *Penicillium*, and *Fusarium*. These molds can grow on crops both in the field and during storage, depending on factors like temperature, humidity, and substrate composition. As they grow, they may produce mycotoxins, which can persist even after the molds are killed or removed.

The presence of mycotoxins in food and feed can have various adverse effects:

- **Health Risks:** Mycotoxins can cause a range of health problems in humans and animals when ingested, inhaled, or absorbed through the skin. These effects can include acute poisoning, chronic health issues, and even carcinogenicity.
- **Economic Impact:** Mycotoxin contamination can lead to significant economic losses in the agriculture and food industries. Infected crops and products may be discarded, leading to decreased yields and financial setbacks.
- **Trade Barriers:** Many countries have established strict regulations regarding mycotoxin levels in imported and domestically produced food and feed. Non-compliance with these regulations can result in trade barriers and product rejections.
- **Food Quality:** Mycotoxins can affect the sensory qualities of food products, including taste, odor, and appearance, making them less appealing to consumers.

Common mycotoxins include aflatoxins, ochratoxin A, fumonisins, deoxynivalenol (DON), and zearalenone, among others. Each of these mycotoxins is associated with specific mold species and conditions, and they vary in their toxicity and health effects.

To mitigate the risks associated with mycotoxin contamination, various strategies are employed, including good agricultural practices (GAPs), good manufacturing practices (GMPs), and mycotoxin management throughout the supply chain. Additionally, advanced analytical methods and regulations have been established to monitor and control mycotoxin levels in food and feed products, contributing to improved food safety and public health. Understanding mycotoxins and their impact is essential for ensuring the safety and quality of the global food supply.

Mycotoxins are produced by various fungal species, and the type of mycotoxin produced can vary depending on the specific fungal strain and the environmental conditions in which it grows. Here are some common fungal genera known for producing mycotoxins:

ASPERGILLUS

- Aflatoxins: *Aspergillus flavus* and *Aspergillus parasiticus* are well-known producers of aflatoxins, which are highly toxic and carcinogenic mycotoxins. Aflatoxins are primarily found in nuts, grains, and legumes.
- Ochratoxin A: *Aspergillus ochraceus* is a major producer of ochratoxin A, which can contaminate a variety of food products, including cereals, coffee, and wine.
- Sterigmatocystin: This mycotoxin is a precursor of aflatoxins and is produced by several *Aspergillus* species, including *Aspergillus* versicolor.

PENICILLIUM

- Patulin: *Penicillium expansum* is known to produce patulin, which is commonly found in decaying apples and apple-based products like apple juice and cider.
- Penicillin Acid: *Penicillium verrucosum* produces penicillic acid, which is associated with moldy grains and wheat products.

FUSARIUM

- Deoxynivalenol (DON): *Fusarium graminearum* and *Fusarium culmorum* produce DON, also known as vomitoxin, which contaminates wheat, barley, and corn.
- Zearalenone: *Fusarium* species produce zearalenone, a mycotoxin that can be found in grains like maize, wheat, and barley. It has estrogenic effects and affects animals and humans.
- Fumonisins: *Fusarium verticillioides* and related species produce fumonisins, which contaminate maize and maize-based products.
- T-2 Toxin and HT-2 Toxin: These mycotoxins are produced by various *Fusarium* species and can be found in grains like oats, wheat, and barley.

CLAVICEPS

- Ergot Alkaloids: The ` genus produces ergot alkaloids, which can infect cereal grains and grasses. Consumption of contaminated grains can lead to ergotism, characterized by symptoms like hallucinations and convulsions.

ALTERNARIA

- Alternariol (AOH) and Alternariol Monomethyl Ether (AME): These mycotoxins are produced by *Alternaria* species and can contaminate a variety of fruits, vegetables, and grains.

TRICHOTHECIUM

- T-2 Toxin and HT-2 Toxin: These mycotoxins are produced by several *Trichothecium* species and can contaminate grains like wheat, barley, and oats.

***PENCILLIUM* (BLUE MOLD)**

- Roisin: This mycotoxin is produced by *Pencillium roqueforti* and *Pencillium glaucum* and is commonly associated with blue cheese.

It's important to note that the specific mycotoxin produced by a fungus can depend on factors such as the fungal species, the substrate it grows on, environmental conditions (temperature, humidity), and other factors. These mycotoxins can have significant health implications when present in food and feed, which is why monitoring and control measures are essential in the agricultural and food processing industries.

DETECTION METHODS OF MYCOTOXINS

The detection of mycotoxins by advanced and emerging analytical methods is a crucial aspect of food safety and quality control. Traditional methods like high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assays (ELISA) have been widely used for mycotoxin analysis. However, advanced and emerging analytical techniques offer several advantages in terms of sensitivity, speed, and precision. Here are some of these methods:

- Liquid Chromatography-Mass Spectrometry (LC-MS/MS):
  LC-MS/MS is a powerful technique for mycotoxin detection due to its high sensitivity and specificity. It allows for simultaneous quantification of multiple mycotoxins in a single analysis, reducing the time and cost of testing.
- Gas Chromatography-Mass Spectrometry (GC-MS):
  GC-MS is used for mycotoxins that can be derivatized into volatile compounds. It offers excellent separation and quantification capabilities and is commonly used for analyzing aflatoxins and some other mycotoxins.

- High-Resolution Mass Spectrometry (HRMS):
  HRMS provides superior mass accuracy and resolution, enabling the identification and quantification of mycotoxins and their metabolites with high confidence. It is valuable for identifying unknown or emerging mycotoxins.
- Ultra-High-Performance Liquid Chromatography (UHPLC):
  UHPLC systems provide faster separations and higher resolution compared to traditional HPLC, reducing analysis time and solvent consumption.
- Imaging Mass Spectrometry (IMS):
  IMS allows for the spatial distribution analysis of mycotoxins on food surfaces. It is valuable for understanding how mycotoxins are distributed within food matrices.
- Biosensors and Immunosensors:
  Biosensors and immunosensors use antibodies or aptamers to detect mycotoxins selectively. They offer rapid and on-site testing capabilities, making them suitable for field applications.
- Nanotechnology-Based Methods:
  Nanomaterials can enhance the sensitivity and selectivity of mycotoxin detection methods. Nanosensors and nanoparticles are used in various techniques, including electrochemical sensors and surface-enhanced Raman spectroscopy (SERS).
- Microfluidic Devices:
  Microfluidic platforms enable miniaturized and high-throughput mycotoxin analysis. They are particularly useful for point-of-care testing and remote monitoring.
- Nuclear Magnetic Resonance (NMR) Spectroscopy:
  NMR spectroscopy can be employed for quantitative and qualitative mycotoxin analysis, It is non-destructive and can provide valuable structural information.
- Machine Learning and Data Analytics:
  Advanced data analysis techniques, including machine learning algorithms, can help in the interpretation of complex analytical data. They can improve the accuracy of mycotoxin quantification and pattern recognition.
- Spectroscopic Techniques:
  Techniques like Fourier-transform infrared spectroscopy (FTIR) and near-infrared spectroscopy (NIRS) are explored for their potential in rapid mycotoxin analysis.

These advanced and emerging analytical methods offer improved sensitivity, specificity, and efficiency in mycotoxin detection, helping to ensure food safety and regulatory compliance in the food industry. Researchers and food safety professionals continue to explore and develop these techniques to address the challenges associated with mycotoxin contamination in food products.

CONTROLLING METHODS OF MYCOTOXINS

Controlling mycotoxins in food and feed is crucial to ensure food safety and protect human and animal health. Mycotoxins are produced by molds and can contaminate various agricultural commodities both in the field and during storage. Here are some key control measures and strategies to mitigate mycotoxin contamination:

- Good Agricultural Practices (GAPs):
  Properly manage and maintain crops in the field to prevent fungal infection and mycotoxin production. Implement crop rotation to reduce the risk of mycotoxin buildup in soil. Use disease-resistant crop varieties when available.
- Pre-Harvest Monitoring:
  Regularly monitor crops for signs of mold growth and mycotoxin contamination. Harvest crops at the appropriate maturity to minimize the risk of mycotoxin development.
- Post-Harvest Handling:
  Dry harvested crops promptly to reduce moisture levels to safe levels. Use appropriate storage conditions, such as temperature and humidity control, to prevent mold growth and mycotoxin production. Store grains and other commodities in clean, well-ventilated, and pest-free facilities. Implement proper cleaning and maintenance of storage facilities to prevent mold contamination.
- Sorting and Cleaning:
  Use equipment such as air screen cleaners, gravity separators, and color sorters to remove contaminated grains or seeds from batches. Visually inspect and sort grains to remove moldy or damaged kernels.
- Mycotoxin Testing:
  Regularly test agricultural commodities for mycotoxin contamination using reliable analytical methods, such as HPLC, LC-MS/MS, or ELISA. Implement a sampling plan to ensure representative testing.
- Processing and Milling:
  Properly process and mill grains to remove mycotoxin-contaminated outer layers. Some mycotoxins can be reduced through thermal processes like cooking and baking.
- Biocontrol Agents:
  Explore the use of biocontrol agents, such as competitive non-toxigenic strains of fungi, to outcompete mycotoxin-producing molds.
- Chemical Control:
  Use approved mycotoxin binders or sequestering agents in animal feed to reduce mycotoxin absorption in the digestive tract of animals. Consider the application of safe fungicides or preservatives during storage, ensuring compliance with regulations.
- Education and Training:
  Educate farmers, food handlers, and consumers about mycotoxin risks and safe handling practices. Train personnel involved in the production and processing of food and feed on mycotoxin prevention and control.
- Regulations and Standards:
  Adhere to mycotoxin regulations and standards set by national and international authorities. Establish and enforce maximum allowable limits for mycotoxins in food and feed products.
- Integrated Pest Management (IPM):
  Implement IPM strategies to control pests that may damage crops and increase the risk of mycotoxin contamination.

- Monitoring and Surveillance:
  Establish monitoring and surveillance systems to track mycotoxin contamination trends and respond promptly to outbreaks.
- Research and Innovation:
  Support ongoing research to develop new and innovative methods for mycotoxin control and mitigation. Effective mycotoxin control requires a multi-faceted approach involving collaboration across the entire food supply chain, from agricultural production to food processing and distribution. By implementing these control measures, the food industry can reduce the risk of mycotoxin contamination and ensure the safety of food and feed products.

CONCLUSION

The detection and control of mycotoxins in food and agricultural products. Mycotoxins are toxic compounds produced by molds that can contaminate crops and pose a risk to human and animal health. Advanced and emerging analytical methods, such as NMR spectroscopy, machine learning, and spectroscopic techniques, are being explored for mycotoxin analysis. These methods offer improved sensitivity, specificity, and efficiency in detecting mycotoxins, ensuring food safety and regulatory compliance. Controlling mycotoxins requires implementing good agricultural practices, pre-harvest monitoring, and post-harvest strategies to prevent fungal infection and mycotoxin production. Overall, the article emphasizes the importance of addressing mycotoxin contamination to protect public health and ensure food safety.

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Chapter 6

HARNESSING THE COLOUR OF THE SEA: EXPLORING MELANIN PRODUCTION IN MARINE ACTINOMYCETES

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ABSTRACT

Marine actinomycetes represent a unique group of bacteria thriving in marine environments, displaying remarkable adaptability to extreme conditions such as high salinity, pressure, and temperature. Marine actinomycetes are found in a variety of marine habitats, including deep-sea sediments and shallow coastal waters. Because of the abundance of organic matter they contain, they are frequently linked to marine sediments. Salinity, temperature, and the availability of nutrients all have an impact on where they are found. When compared to their terrestrial counterparts, marine actinomycetes have distinctive traits that are partly attributed to the particularities of the marine environment. Recent years have seen a major increase in interest in marine actinomycetes because of their potential for biotechnology. Bioactive chemicals such as antibiotics, antifungals, antivirals, and anticancer drugs are produced in large quantities by them. These substances have commercial and pharmacological uses, and research into marine actinomycetes has produced new bioactive chemicals with potential medical uses. Actinomycetes are known for their diverse secondary metabolite production, and recent studies have shed light on their potential to produce melanin—a pigment of significant interest due to its multifaceted biological activities.

KEYWORDS: Melanin, Actinomycetes, Biological Activities, Sediments.

INTRODUCTION

Marine actinomycetes represent a unique group of bacteria thriving in marine environments, displaying remarkable adaptability to extreme conditions such as high salinity, pressure, and temperature.Marine actinomycetes are found in a variety of marine habitats, including deep-sea sediments and shallow coastal waters. Because of the abundance of organic matter they contain, they are frequently linked to marine sediments. Salinity, temperature, and the availability of nutrients all have an impact on where they are found. When compared to their terrestrial counterparts, marine actinomycetes have distinctive traits that are partly attributed to the particularities of the marine environment. Recent years have seen a major increase in interest in marine actinomycetes because of their potential for biotechnology. Bioactive chemicals such as antibiotics, antifungals, antivirals, and anticancer drugs are produced in large quantities by them. These substances have commercial and pharmacological uses, and research into marine actinomycetes has produced new bioactive chemicals with potential medical uses. Actinomycetes are known for their diverse secondary metabolite production, and recent studies have shed light on their potential to produce melanin—a pigment of significant interest due to its multifaceted biological activities.

In marine habitats, marine actinomycetes play a role in the nutrient cycle. They contribute to the breakdown of organic matter, which replenishes the environment with vital nutrients. Their participation in the cycle of biogeochemical processes emphasizes their ecological importance in preserving the equilibrium of marine habitats. Marine actinomycetes are an intriguing class of microorganisms having applications in biotechnology, ecology, and medicine. To fully utilize their benefits to a variety of businesses and to further our understanding of marine microbial ecosystems, it is imperative that we comprehend their ecology, adaptability, and biotechnological potential.

MELANIN PRODUCTION IN ACTINOMYCETES

Melanin are high-molecular-weight pigments with broad applications in various fields, including medicine, industry, and environmental protection. Traditionally associated with fungi and animals, melanin production has been less explored in bacteria, especially marine actinomycetes. The skin of humans and the majority of other mammals contains pheomelanin and eumelanin. Since ancient times, skin coloration has been a topic of interest. The ability of marine actinomycetes to produce melanin has garnered attention for several reasons. Melanin's role in protecting organisms from environmental stress, such as UV radiation and oxidative damage, has led researchers to investigate its production in actinomycetes as a potential adaptive response to harsh marine conditions.

The most of melanin-producing marine organism is the actinomycetes. This is particularly the case for the genus Streptomyces, from which most compounds with known biological activity have been isolated. All Streptomyces strains are reported to use tyrosinases in the synthesis of melanin pigments. Another important melanin-synthesizing actinomycetes is Marinomonasmediterranea, which produces black eumelanin from L-tyrosine.

BIOSYNTHESIS OF MELANIN

Melanin synthesis can be produced by random polymerization of a few building blocks, including homogentisate, cysteinylopa, dopamine, dopamine-o-quinone, L-tyrosine metabolites of indole-5,6-quinone, 5,6-dihydroxyindole carboxylic acid (DHICA), and some phenolic precursors. The mechanism of melanin synthesis is dependent on the radical formation. The classes of melanin that are eumelanin, pyomelanin, pheomelanin, neuromelanin, and allomelanin are determined by the polymerization pathways, building blocks, and enzymes.

Microorganisms can produce various types of melanin, including eumelanin and pheomelanin. Eumelanin is a dark pigment responsible for brown and black colours, while pheomelanin is responsible for lighter colours like red and yellow. The specific type produced depends on the strain of actinomycetes. The eumelanins and pheomelanins are produced by two distinct biosynthetic routes. L-tyrosine is converted to 3,4-dihydroxyphenylalanine (DOPA) by oxidation, and tyrosinase then produces dopaquinone, which starts both routes. The latter product is converted to either eumelanin with intermediate leucodopachrome, dopachrome (red), 5,6-dihydroxyindole, and 5,6-indolequinone (yellow) production or to pheomelanin by interacting with cystein and creating an intermediate S-cysteinyldopa and benzothiazine. Thendopachrome, which nonenzymatically polymerizes to create melanin (Fig 1).

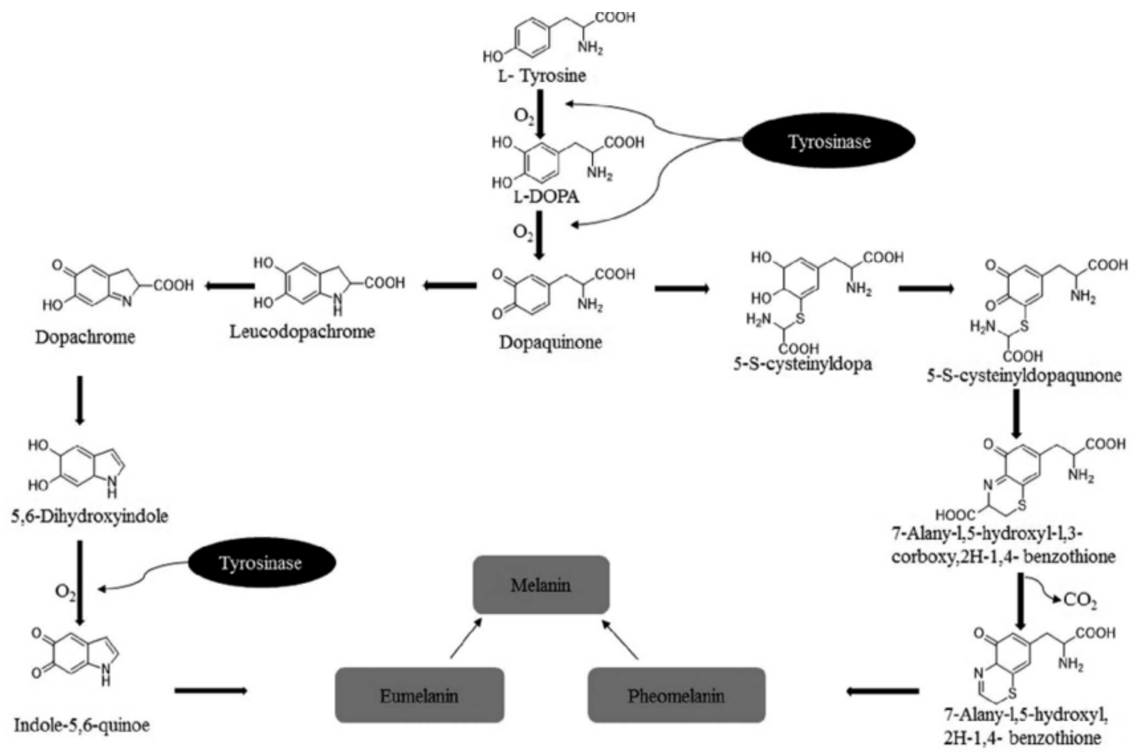

Figure 1: Melanin production pathway

The generation of pigment is a crucial characteristic of actinomycetes. Prodigiosin, melanin, carotenoids, quinones, and other bioactive pigmented substances have been studied from marine actinomycetes. These are widely utilized in a variety of industries, including food, paint, cosmetics, textiles, and medicines. Although melanin is not necessary for an organism's growth and development, it does play a significant role in enhancing its competitiveness and survival. Melanin is a type of polymer derived from the oxidative polymerization of phenolic and indolic chemicals. It has a variety of molecular configurations and is usually regarded as black or brown. It has a negative charge, is hydrophobic, insoluble in organic or aqueous solvents, resistant to strong acids, and vulnerable to oxidizing agents' bleaching. However, little research has been done on the use of marine actinomycetes' melanin.

BIOTECHNOLOGICAL SIGNIFICANCE

The biotechnological potential of melanin from marine actinomycetes is substantial. Melanin has antioxidant properties and can scavenge free radicals, suggesting potential applications in medicine and cosmetics. Additionally, melanin's ability to absorb various wavelengths of light, including UV radiation, makes it a promising material for sunscreens and other protective coatings.

- Microbial melanin protects bacteria from the harmful effects of UV radiation. It acts as a shield by absorbing and scattering UV light, preventing damage to cellular components, including DNA.
- Microbial melanin exhibits antioxidant properties, helping to neutralize free radicals. This contributes to the protection of microbial cells from oxidative stress, which can be induced by factors like UV radiation and environmental pollutants.
- Microbial melanin can bind to and sequester metal ions, contributing to the detoxification of heavy metals. This is especially significant in environments with high metal concentrations.

- Microorganisms producing melanin are often found in extreme environments such as deserts, high altitudes, and deep-sea environments. Melanin aids these microorganisms in surviving the harsh conditions prevalent in such environments.
- Some microbial melanins exhibit antimicrobial properties, inhibiting the growth of other microorganisms in their vicinity. This can provide a competitive advantage to melanin-producing microbes.
- Microbial melanin's metal-binding capabilities have been explored for bioremediation purposes. Melanin-producing microbes can be used to reduce environmental pollution by immobilizing and sequestering heavy metals.
- Melanin from some microbes has been investigated for potential pharmaceutical applications due to its antioxidant and anti-inflammatory properties.
- Microbial melanin can contribute to the formation and structure of biofilms. Biofilms play important roles in microbial communities, providing protection and facilitating interactions between microorganisms.
- Melanin production is associated with increased resistance to various environmental stressors, including oxidative stress, desiccation, and extreme temperatures.
- Microbial melanin has been implicated in biomineralization processes. Some microbes use melanin to facilitate the deposition of minerals, contributing to the formation of complex structures.
- Melanin in certain microorganisms has been suggested to protect DNA from damage caused by ionizing radiation, making them more resilient in radiation-rich environments.

ISOLATION OF MELANIN PRODUCING MARINE ACTINOMYCETES

Isolating melanin-producing marine actinomycetes involves a series of steps to collect samples from the marine environment, cultivate the microorganisms, and selectively isolate those capable of melanin production.

Site Selection: Choose a marine environment with potential actinomycete populations. Common sources include marine sediments, seawater, and marine organisms.

Selective Media: Use selective media that encourage the growth of actinomycetes. Common selective media for actinomycetes include Starch Casein Agar, Glycerol Yeast Extract Agar, and Actinomycete Isolation Agar.

Incorporate Inhibitors: Some media may include antibiotics to inhibit the growth of other microorganisms and promote the isolation of actinomycetes.

Inoculation: Spread or streak the diluted samples onto the selective media and incubate at appropriate temperatures (typically 25-30°C) for several days.

Observation of Colonies: Actinomycetes colonies are often characterized by a powdery or filamentous appearance.

Gram Staining: Perform Gram staining to identify Gram-positive bacteria, which is a characteristic of actinomycetes.

Microscopic Examination: Examine colonies under a microscope to observe the characteristic filamentous morphology of actinomycetes.

Molecular Identification: Use molecular techniques like PCR and DNA sequencing to confirm the identity of the isolated strains as actinomycetes.

SCREENING TECHNIQUES

Screening for melanin-producing marine actinomycetes involves specific techniques to identify strains capable of synthesizing melanin. Melanin production can be assessed through both qualitative and quantitative methods.

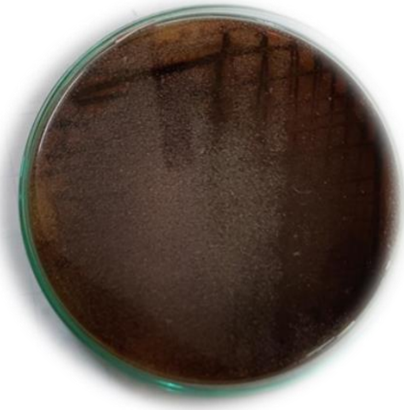

Figure 2: Screening plate: Melanin produces by marine actinomycete

Microscopic Observation of Pigmentation: Examine bacterial cells under a microscope to observe pigmentation. Melanin production may be visible as dark granules within the cells.

Fontana-Masson Stain: This stain is specific for melanin and can be used to visualize melanin granules in bacterial cells. It involves using silver nitrate to stain melanin black.

Melanin-Inducing Agar: Cultivate actinomycetes on media specifically designed to induce melanin production. These media may contain precursors such as L-tyrosine to stimulate melanin synthesis (Fig 2).

L-Tyrosine Utilization Test: Actinomycetes capable of melanin production often utilize L-tyrosine as a substrate. This can be assessed by monitoring growth and color changes in media containing L-tyrosine.

Tyrosinase Activity Assay: Tyrosinase is an enzyme involved in melanin synthesis. An assay measuring tyrosinase activity can be used to identify melanin-producing strains. The assay may involve detecting the conversion of tyrosine to melanin or the oxidation of a substrate like L-DOPA (L-3,4-dihydroxyphenylalanine).

Melanin Extraction and Quantification: Extract melanin from the bacterial culture and quantify it using colorimetric assays. This may involve measuring the absorbance of melanin at specific wavelengths.

Absorbance Measurement: Measure the absorbance of bacterial cultures at specific wavelengths associated with melanin absorption. Melanin typically absorbs light in the UV and visible spectra.

Detection of Melanin Biosynthesis Genes: Use PCR to detect the presence of genes involved in melanin biosynthesis. Genes such as tyrosinase (melC) and other melanin pathway genes can be targeted for amplification.

Identification of Melanin Compounds: Use Liquid Chromatography- Mass Spectrometry (LC-MS) to identify specific melanin compounds produced by actinomycetes. This method provides detailed information about the chemical composition of melanin.

Quantification of Melanin Precursors: Use High Performance Liquid Chromatography (HPLC) to quantify melanin precursors, such as L-tyrosine, as an indirect measure of melanin production.

Transcriptomics and Proteomics: Study the expression of genes and proteins involved in melanin biosynthesis under different conditions using transcriptomic and proteomic approaches.

APPLICATIONS OF MELANIN PRODUCING MARINE ACTINOMYCETES

Melanin, a pigment with diverse properties, has the potential for various biotechnological applications. Few of the potential applications are:

Heavy Metal Removal: Melanin produced by marine actinomycetes can bind to heavy metals, facilitating their removal from contaminated environments. This application is relevant in bioremediation efforts aimed at cleaning up metal-contaminated sites.

Antioxidant and Anti-Inflammatory Agents: Melanin possesses antioxidant properties, and some studies suggest anti-inflammatory effects. Marine actinomycetes-derived melanin could be explored for the development of pharmaceuticals with applications in treating conditions related to oxidative stress and inflammation.

Protection Against Ionizing Radiation: Melanin has been investigated for its potential as a radioprotective agent. Marine actinomycetes-derived melanin may find applications in developing agents to protect living organisms from ionizing radiation.

Biopolymeric Materials: Melanin can serve as a biopolymer with applications in materials science. The extraction and utilization of melanin from marine actinomycetes may lead to the development of biocompatible and biodegradable materials for various purposes.

UV Protection: Due to its ability to absorb and dissipate UV radiation, melanin from marine actinomycetes could be incorporated into cosmetic and skincare products for UV protection, contributing to sunscreen formulations.

Textile Industry: Melanin can be explored for its potential in the textile industry as a natural dye. Incorporating melanin-producing marine actinomycetes into dyeing processes may offer a sustainable and eco-friendly alternative to synthetic dyes.

Antibacterial and Antifungal Properties: Melanin has been associated with antimicrobial properties. Extracts or compounds derived from melanin-producing marine actinomycetes may be investigated for their potential as antimicrobial agents.

Detection of Environmental Changes: Melanin-based biosensors may be developed using marine actinomycetes-derived melanin. These sensors could be designed to detect changes in environmental conditions, such as metal concentrations or radiation levels.

Contrast Agents: Melanin's ability to absorb light could be exploited in medical imaging as a contrast agent. This application may be explored for its potential in enhancing imaging techniques such as photoacoustic imaging.

Biosignatures: Melanin production in marine actinomycetes may serve as a biological marker for environmental conditions. Monitoring melanin levels could provide insights into changes in microbial communities and ecosystem health.

FUTURE PROSPECTS

While progress has been made in elucidating melanin production by marine actinomycetes, challenges remain. Further research is needed to uncover the underlying genetic and biochemical mechanisms of melanin biosynthesis in these actinomycetes. The development of melanin-based biosensors for environmental monitoring may become more sophisticated. These biosensors could offer real-time detection of environmental changes and pollutants, contributing to environmental monitoring and remediation efforts. Continued research may uncover new biomedical applications for melanin from marine actinomycetes. This could involve exploring its potential in drug delivery, wound healing, or as an imaging agent in medical diagnostics. Melanin from marine actinomycetes may find increased use in cosmetic and skincare products, particularly in the formulation of natural and UV-protective skincare solutions. Future prospects may include the development of biodegradable materials using melanin from marine actinomycetes. This aligns with a growing emphasis on sustainable practices and eco-friendly alternatives in various industries. Additionally, exploring the diversity of melanin types produced by different strains and their functional properties can open avenues for novel applications.

CONCLUSION

In conclusion, the investigation of melanin production in marine actinomycetes represents an exciting frontier in microbiology with potential implications for biotechnology, medicine, and our understanding of microbial ecology in marine environments. Ongoing research in this field may unveil new insights into the biochemistry and applications of melanin produced by these intriguing microorganisms.

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Chapter
7

PHARMACOLOGICAL POTENTIAL OF PLANTS

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ABSTRACT

Since ancient times, plants have served as the primary source of therapeutic agents, contributing significantly to healthcare practices worldwide. Medicinal plants not only play a crucial role in healthcare but also form a vital component of international trade. In recent years, the trade of medicinal plants has surged due to the availability of affordable herbal drugs with fewer side effects, making them popular.

The therapeutic efficacy of medicinal plants is attributed to the intricate chemical compounds they contain, each with distinct compositions and biological functions. Herbal medicine, known for its effectiveness and affordability, enjoys widespread practice across the globe. Notably, there has been a growing emphasis on utilizing eco-friendly and bio-friendly plant-based products to combat various human diseases, leading to a substantial rise in global pharmaceutical demand.

However, the escalating demand for plants as raw materials in the pharmaceutical industry poses a significant threat to plant biodiversity. Consequently, there is an urgent need for advanced research aimed at developing and characterizing natural drugs derived from plants and other natural sources. This research should incorporate improved screening methods to ensure the sustainable utilization of medicinal plants. Furthermore, the validation of medicinal plant properties through scientific methods is essential to identify safe and potent natural drugs for effective disease management.

KEYWORDS: Medicinal plants, healthcare, trade, herbal drugs, biodiversity conservation, eco-friendly, pharmaceutical industry, natural drugs, scientific validation.

INTRODUCTION

Man has been inextricably linked to nature and has relied on it for survival from the beginning of time. Man depends on the environment for fundamental requirements (food, fibres, shelter, clothing, and gum) as well as for healthcare, sustenance, and livelihood. Plants not only supplied his basic needs but also his need for medicine. In addition to plants, man has begun to prepare medicine utilizing animal products and other naturally occurring bioresources. As a result, many traditional medical systems have developed depending on the social and cultural context, as well as the ethnic group, in various nations [Daniel, 1984].

Plants play a significant role as vital natural resources in both traditional and contemporary medicinal practices worldwide. The healing properties of plants and their derivatives have been recognized for millennia. Knowledge about the medicinal advantages of plants, in conjunction with other therapeutic methods, has been documented and preserved in various historical records from civilizations such as Babylonia, Egypt, China, Greece, and Rome.

In today's context, herbal medicine has regained considerable attention and holds promise from both practical and scientific perspectives. Herbal remedies consist of complex mixtures derived from single herbs or multiple herbs, which can sometimes result in synergistic effects, enhancing their therapeutic potential [CK, 2014]. It is essential to identify the biologically active compounds responsible for their medicinal properties and to ensure quality control. Accurate identification and quality assessment are crucial in maintaining the quality, safety, and efficacy of herbal medicine. The therapeutic effectiveness of herbal formulations relies on their phytochemical constituents. Conducting phytochemical investigations of medicinally important plants is necessary, as it aids in standardization, quality assessment, and the overall efficacy of herbal drugs. Consequently, pharmacognosy is regarded as a vital tool for studying medicinal plants to identify, validate, and standardize them [Kumar *et al.,* 2011].

BIOPROSPECTING OF PLANTS

Bioprospecting of medicinal plants is the process of discovering, identifying, and harnessing the potential therapeutic compounds and properties found in various plant species. Medicinal plants have been used for centuries in traditional medicine systems worldwide, and they continue to be a valuable source of drugs and natural remedies. Here's an overview of the bioprospecting process for medicinal plants:

EXPLORATION AND SELECTION

Bioprospecting begins with the exploration and selection of target areas rich in biodiversity and potential medicinal plants. Indigenous knowledge and ethnobotanical studies often guide the initial selection of promising plant species [Bodeker and Kronenberg, 2002]. Collaborations with indigenous communities play a vital role in identifying the most promising plant resources for further investigation.

COLLECTION AND DOCUMENTATION

Field expeditions are organized to collect plant specimens from the selected areas. Accurate documentation, including GPS coordinates, habitat descriptions, and photographs, is crucial for later reference and research purposes [Cunningham, 1993]. Proper documentation ensures that collected plant material is traceable and allows for the correct identification of species.

TAXONOMIC IDENTIFICATION

Taxonomic experts are enlisted to identify and classify the collected plant specimens accurately. This step is essential to avoid misidentification and ensure the correct characterization of the plant species [Heywood, 1993]. Accurate taxonomy is fundamental to understanding the biological diversity of the collected plants.

CHEMICAL AND PHARMACOLOGICAL SCREENING

Extracts from the collected plant specimens are subjected to chemical analysis and pharmacological screening to identify bioactive compounds and assess potential medicinal properties [Fabricant and Farnsworth, 2001]. This stage aims to uncover compounds with therapeutic potential, which can serve as leads for further research.

BIOASSAYS AND IN VITRO TESTING

Bioassays and in vitro experiments are conducted to evaluate the biological activity of plant extracts. Researchers assess properties such as antimicrobial, anti-inflammatory, or anticancer activity [Edeoga *et al.,* 2005]. These assays help determine the therapeutic potential of the plant materials.

TOXICOLOGY AND SAFETY ASSESSMENT

Toxicology studies are carried out to assess the safety of plant extracts and potential side effects [Benzie and Wachtel-Galor, 2011]. Ensuring the safety of any potential medicinal applications is critical to the development process.

ISOLATION AND CHARACTERIZATION OF ACTIVE COMPOUNDS

Active compounds identified during earlier stages are isolated from plant extracts and characterized using various analytical techniques. This step provides a deeper understanding of the chemical nature of the bioactive compounds.

PRECLINICAL AND CLINICAL STUDIES

Promising compounds undergo preclinical studies to assess their safety and efficacy in animal models, and if successful, progress to clinical trials to evaluate their safety and efficacy in humans [Benzie and Wachtel-Galor, 2011]. These trials are crucial for bringing potential plant-derived drugs to market.

INTELLECTUAL PROPERTY RIGHTS AND BENEFIT SHARING

Ethical and legal considerations regarding intellectual property rights and benefit-sharing agreements with indigenous communities are addressed [Laird and Wynberg, 2008]. Ensuring equitable benefit-sharing is essential for respecting traditional knowledge and fostering collaboration.

CONSERVATION AND SUSTAINABLE USE

Efforts are made to conserve plant species and their habitats, and sustainable harvesting practices are developed to protect biodiversity [Dobson, 2005]. Sustainable practices are vital to ensure the long-term availability of valuable plant resources.

PHARMOCOGNOSY OF MEDICINAL PLANTS

The term pharmacognosy was originally coined by the Austrian physician Schmidt in 1811. A "crude material" refers to a natural substance of plant, animal, or mineral origin that is dried and unprocessed and is used for medicinal purposes. The term "pharmacognosy" is derived from the Greek words "pharmakon," meaning drug, and "gnosis," meaning knowledge. Pharmacognosy is the examination of medicinal substances obtained from natural sources, primarily plants, which can lead to the creation of novel pharmaceuticals.

Phytochemicals (where "Phyto" pertains to plants) are naturally occurring chemical constituents in plants, including substances like sugars, amino acids, proteins, chlorophyll, alkaloids, flavonoids, steroids, and tannins. These phytochemicals are active compounds possessing therapeutic properties, often considered as medicines or drugs. Over 4,000 phytochemicals have been identified and categorized based on their protective functions and physical and chemical characteristics, with detailed studies conducted on approximately 150 of them [Sharma and Kumar, 2013].

Findings indicate that a significant portion of phytochemicals exhibit advantageous activities, such as antimicrobial, anti-malarial, anti-diabetic, antiarthritic, and anti-cancer properties. The medicinal, biological, and pharmaceutical value of phytoconstituents has led to increased interest in exploring and utilizing plant resources in recent years. Combining chemical information about plants with their medicinal attributes, along with other biological effects, enhances the potential for the development of valuable herbal medications [Kumar *et al.,* 2011]. During pharmacognostic investigations, the analysis of physico-chemical properties is also regarded as a crucial parameter in the assessment and identification of raw drugs.

It is imperative to perform both macroscopic and microscopic analyses to detect any adulterants, herbal drug contaminants, and to evaluate quality before proceeding with further examinations. The determination of extractive value and solubility value proves valuable in gauging the dry yield of specific chemical constituents when different solvents are employed. The analysis of ash value serves the purpose of identifying extraneous materials (such as sand and soil) adhering to the plant's surface [Vivekraj *et al.*, 2017].

Assessing moisture content is indispensable for appraising the stability of the raw drug. Fluorescence analysis stands as a dependable method for standardizing raw drugs. When suitably illuminated, various chemical constituents present in the plant extract exhibit characteristic fluorescence. In instances where certain chemical substances do not possess inherent fluorescence, they can be treated with different reagents to induce fluorescence [Roy *et al.*, 2018].

PHYTOCHEMICALS FROM MEDICINAL PLANTS

Medicinal plants can be described as plants commonly utilized for the treatment and prevention of specific ailments and diseases that are generally considered harmful to humans [Akerele, 1993]. These plants fall into two categories: "Wild Plant Varieties," which grow naturally in self-sustaining ecosystems, and "Cultivated Plant Varieties," which have been selectively bred by humans and rely on human care for their survival [Srivastava *et al.*, 1996].

Metabolites are compounds produced by plants for essential functions like growth and development, as well as specialized functions such as defence against herbivores or attraction of pollinators [Pagare *et al.*, 2015]. Metabolites are organic compounds generated by organisms through enzyme-mediated chemical reactions known as metabolic processes [Calixto, 2000]. Primary metabolites serve essential roles in growth and development and are found in all plants, while secondary metabolites are more scattered throughout the plant kingdom and have specific functions unique to the plants in which they are found [Hermsmeier *et al.*, 2001].

Secondary metabolites are often colourful, aromatic, or flavourful compounds that mediate interactions between plants and other organisms, including herbivores, pathogens, and pollinators [Harborne, 2014]. Due to their sessile nature, plants face various challenges, including managing their own pollination and seed dispersal, responding to fluctuations in essential nutrient availability, and coping with herbivores and pathogens in their vicinity [Bernays, 2017].

Consequently, plants have evolved secondary biochemical pathways to produce a range of chemicals, often in response to environmental stimuli such as herbivore induced damage, pathogen attacks, or nutrient deficiencies. These secondary metabolites can be specific to certain species or genera and serve roles unrelated to the plant's primary metabolic needs, enhancing their ability to interact with their environment [Wink, 2003]. The substantial energy invested in producing these secondary metabolites far exceeds what is required for primary metabolites and underscores their significance in plant survival.

Secondary metabolites play diverse roles, including protective functions (e.g., antioxidant, free-radical scavenging, UV light absorption, and antiproliferative activities) that defend the plant against microorganisms like bacteria, fungi, and viruses. They also regulate interactions between plants, acting as allelopathic agents to safeguard the plant's growing space against competing plants [Verpoorte and Alfermann, 2000]. Their primary function is to deter feeding by predators, often through bitterness or toxicity, which extends to direct interactions with the herbivore's nervous system [Cragg *et al.*, 1997].

However, for their own survival, plants also engage in various symbiotic associations, such as attracting pollinators and other symbiotic partners through colour, fragrance, or by indirectly defending against attackers by luring their natural enemies. This may involve creating a chemical signal for the predator or responding directly to tissue damage caused by herbivores, resulting in the synthesis and release of a blend of phytochemicals that attract the herbivore's natural predators.

PLANT SECONDARY METABOLITES

Plant secondary metabolites are a diverse group of organic compounds produced by plants that are not directly involved in their primary metabolic processes but serve various ecological and physiological functions. These compounds are essential for plant survival, playing roles in defence against herbivores and pathogens, attraction of pollinators, and adaptation to environmental conditions. Additionally, they have significant implications for human health, industry, and agriculture.

ALKALOIDS

These are a diverse group of naturally occurring organic compounds characterized by the presence of one or more nitrogen atoms in a heterocyclic ring. These compounds are often alkaline and have a wide range of pharmacological activities. They are synthesized from amino acids, primarily phenylalanine and tyrosine, and serve as potent defensive chemicals in plants.

Some well-known alkaloids include caffeine (found in coffee beans), nicotine (present in tobacco leaves), morphine (derived from opium poppies), and quinine (found in the bark of the cinchona tree and used to treat malaria). Alkaloids often act as deterrents against herbivores and pathogens due to their toxic or bitter properties. They also have cultural and economic significance, with some alkaloids being used for medicinal and recreational purposes [Dey *et al.*, 2020].

PHENOLIC COMPOUNDS

These are a large and diverse group of secondary metabolites characterized by the presence of phenol rings. They are ubiquitous in the plant kingdom and play essential roles in plant defence, UV protection, and antioxidative processes. Phenolic compounds are synthesized from phenylalanine and tyrosine through the phenyl propanoid pathway. Flavonoids, one subgroup of phenolic compounds, include quercetin, catechins, and anthocyanins, and are responsible for the vibrant colours of many fruits and flowers. Lignans are another subgroup with antioxidant properties. Tannins are phenolic compounds that can form complexes with proteins, making them astringent and often involved in plant defence. Resveratrol, found in grapes and red wine, has gained attention for its potential health benefits.

Phenolic compounds have attracted significant interest due to their antioxidant properties and potential health benefits for humans. They are found in various plant-based foods and beverages and have been studied for their role in preventing chronic diseases [Santos-Buelga and Scalbert, 2000].

TERPENOIDS

Terpenoids, also known as isoprenoids, are a vast and structurally diverse class of secondary metabolites derived from isoprene units. They are responsible for the characteristic scents and flavours of plants and play essential roles in various ecological and physiological processes. Essential oils, which are commonly extracted from plants, are composed primarily of terpenoids. For example, lavender oil contains linalool, while tea tree oil contains terpinen-4- ol. Carotenoids, another group of terpenoids, are responsible for the yellow, orange, and red pigments in fruits and vegetables and have important roles in photosynthesis.

Terpenoids are not only responsible for the aromatic qualities of plants but also serve as defensive compounds against herbivores and pathogens. They can also attract pollinators and seed dispersers through their fragrances [Gershenzon and Dudareva, 2007].

GLYCOSIDES

These are compounds in which a sugar molecule (glycone) is attached to a non-sugar molecule (aglycone or genin) through a glycosidic bond. They are widely distributed in the plant kingdom and serve multiple functions, including energy storage and defence against herbivores. Cyanogenic glycosides, such as amygdalin found in bitter almonds, release toxic hydrogen cyanide when broken down, serving as a deterrent against herbivores. Cardiac glycosides, like digitoxin from foxglove, affect the heart and are used in medicine to treat heart conditions. Some glycosides have sweet tastes and are used by plants to attract animals that aid in seed dispersal. Others are involved in plant-pollinator interactions, where they contribute to the scent and colour of flowers [Harborne and Grayer, 2017].

ESSENTIAL OILS

Essential oils are volatile, aromatic compounds found in various plant parts, including leaves, flowers, bark, and roots. They are responsible for the characteristic scents and flavours of many plants and have long been used for medicinal, cosmetic, and culinary purposes. Lavender oil, extracted from lavender flowers, is known for its calming properties, and is often used in aromatherapy. Peppermint oil has a refreshing aroma and is used to alleviate digestive discomfort. Tea tree oil has antiseptic and antimicrobial properties, making it a popular ingredient in skincare products. These oils are obtained through processes such as steam distillation or cold pressing and have a wide range of applications, including perfumes, flavorings, and herbal remedies [Bakkali *et al.,* 2008].

TANNINS

Tannins are polyphenolic compounds that can bind to and precipitate proteins, resulting in astringency. They are found in various plant parts, including leaves, bark, and fruits. Tannins have roles in plant defence against herbivores and pathogens. There are two primary types of tannins: condensed tannins (proanthocyanidins) and hydrolyzable tannins. Condensed tannins are found in plants like grapes and have antioxidant properties. Hydrolyzable tannins, found in oak trees, are used in the aging of wines and spirits. Tannins also have applications in traditional medicine, as they can have anti-inflammatory and antimicrobial effects. Additionally, they are used in the tanning of leather [Haslam, 1998].

RESINS

These are amorphous, semi-solid substances produced by plants, often in response to injury or stress. They can contain a mixture of secondary metabolites, including terpenoids and phenolic compounds. Resins play roles in plant defence, wound healing, and protection against pathogens. Frankincense and myrrh are well-known resins that have been used for centuries in religious ceremonies, traditional medicine, and perfumes. These resins contain a variety of bioactive compounds, including terpenoids and essential oils, which contribute to their therapeutic properties. Resins are valued for their antimicrobial and anti-inflammatory properties and continue to be used in various cultural and medicinal practices [Langenheim, 2003].

Plant secondary metabolites, including alkaloids, phenolic compounds, terpenoids, glycosides, essential oils, tannins, and resins, are a diverse and fascinating group of compounds. They serve multiple functions in plants, ranging from defence mechanisms to attracting pollinators, and have significant implications for human health and industry. Understanding these metabolites and their roles in plants is essential for both ecological research and the development of novel applications in fields such as medicine, agriculture, and cosmetics.

THE STATUS OF HERBAL MEDICINE

The status of herbal medicine reflects a dynamic and evolving landscape in the field of healthcare. Over the past few decades, there has been a resurgence of interest in herbal medicine worldwide, driven by several factors. First, there is a growing awareness of the limitations and potential side effects of conventional pharmaceuticals, leading many individuals to seek natural and plant-based alternatives for their health and wellness needs. Second, scientific research has advanced our understanding of the bioactive compounds present in medicinal plants, providing a solid foundation for the development of evidence-based herbal therapies.

In many countries, including the United States and European nations, herbal medicine has gained recognition and is increasingly integrated into mainstream healthcare systems. Regulatory agencies have established guidelines and quality standards for herbal products to ensure safety and efficacy. This has led to the standardization and quality control of herbal remedies, making them more reliable and accessible to consumers.

Furthermore, traditional knowledge about herbal remedies from various cultures is being documented and studied, allowing for a better understanding of their therapeutic potential. Indigenous practices and traditional herbal wisdom are being respected and integrated into modern herbal medicine practices. The market for herbal products, including dietary supplements and herbal remedies, continues to grow substantially, reflecting the demand for natural and holistic approaches to health. This growth has led to increased research, investment, and innovation in the herbal medicine industry.

Despite these positive developments, challenges persist, such as the need for more rigorous clinical trials to establish the safety and efficacy of herbal treatments, as well as addressing issues related to quality control, standardization, and potential herb-drug interactions. However, the status of herbal medicine suggests a promising future, with the potential to provide complementary and alternative healthcare options that can enhance overall well-being and healthcare outcomes for individuals around the world.

CONSERVATION OF MEDICINAL PLANTS

The conservation of medicinal plants is an urgent and multifaceted endeavour that stands at the intersection of healthcare, biodiversity preservation, and cultural heritage protection. Medicinal plants have been a cornerstone of traditional healing systems for centuries, offering a vast array of natural remedies for various ailments. However, escalating human activities, including deforestation, habitat destruction, over-harvesting, and climate change, have placed many of these valuable plant species at risk of extinction. This has far-reaching consequences, not 8 only for the communities that rely on these plants for their healthcare needs but also for global biodiversity.

Efforts to conserve medicinal plants encompass a range of strategies, from documenting and inventorying plant species to safeguarding their natural habitats. Protecting these habitats is paramount, as many medicinal plants are intricately linked to specific ecosystems. Sustainable harvesting practices and the controlled cultivation of popular medicinal plants can help alleviate pressure on wild populations. Furthermore, ethical wild harvesting, which respects both the plants and the knowledge of indigenous communities, is integral to conservation. Ongoing research is essential to deepening our understanding of medicinal plant biology and pharmacology, enabling us to make informed conservation decisions.

International cooperation and the enactment of laws and regulations to control the trade of endangered species are also crucial aspects of conservation. Engaging local communities is equally vital, as their traditional knowledge and practices often hold the key to sustainable plant use. Furthermore, raising awareness about the importance of medicinal plant conservation among policymakers and the public can garner support and drive change. Ultimately, the conservation of medicinal plants is not just about protecting a valuable resource; it's about preserving cultural traditions, safeguarding biodiversity, and ensuring that future generations can continue to benefit from the natural remedies offered by these remarkable plants.

CONCLUSION

Medicinal plants have been used for healing since ancient times and are not only vital for healthcare but also a significant part of global trade. In recent years, the demand for medicinal plants has risen due to the availability of affordable herbal drugs with fewer side effects. These plants contain complex chemical compounds that offer therapeutic benefits. Herbal medicine is widely practiced globally for its effectiveness and affordability, with a growing focus on eco-friendly solutions for treating diseases. This increased demand for natural remedies has put plant biodiversity at risk. To address this, there is a need for advanced research to develop and characterize natural drugs from plants and other sources, subjecting medicinal plants to scientific validation for safe and effective disease treatment.

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Chapter
8

UNRAVELING THE ROLE OF ANTIOXIDANTS IN MITIGATION OF SYNTHETIC PYRETHROID (CYPERMETHRIN) INDUCED MALE REPRODUCTIVE TOXICITY

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ABSTRACT

Cypermethrin is a Type II Synthetic Pyrethroid, widely distributed in the environment as a contaminant due to its high usage as a pesticide in agriculture, in veterinary medicine and for household applications to control insects as Insecticide. Despite widespread worries about Cypermethrin's toxicity, Since its accumulation and contamination in food and environment is rapidly increasing, humans are readily encountered with its toxicity and increasing the chance of risk to develop reproductive anomalies. There is currently no antidote for Cypermethrin poisoning, in fact this synthetic Pyrethroid can have devastating effects on the body. Cypermethrin exposure inhibits sodium channel activity in addition to magnesium and ATPase. It binds to open sodium channels and markedly prolong axonal sodium channel depolarization, causing a prolonged sodium current to flow which leads to hyper excitation of the nervous system and delays the closure of voltage-sensitive sodium channels. Many studies reported the toxic effects of cypermethrin like reprotoxicity, neurotoxic effects, Hepatotoxicity and genotoxicity in laboratory animals. Cypermethrin is as hazardous in nature whether it is ingested orally or through skin contact, though animals generally have a higher tolerance for it than humans. In this study, we dug through the literature and analyzed a large number of studies for the better understanding of how natural antioxidants can help to reduce oxidative stress and repair the damaged reproductive system from Cypermethrin intoxication. This current research seeks to answer these questions by analyzing the effects of Cypermethrin on malerats and their subsequent recovery of reproductive function after exposure to various natural antioxidants such as vitamin E and C, the spice curcumin, Resveratrol and the extract of the plant Tribulus terrestris.

KEYWORDS: Cypermethrin, Oxidative Stress, Reproductive toxicity, Antioxidants.

INTRODUCTION

One of the most popular classes of pesticides are synthetic pyrethroids. Because of their excellent efficiency against a wide range of insects, rapid biodegradation, minimal mammalian toxicity, and target orientated method of action, these insecticides have seen an upsurge in use in recent years at the expense of organochlorines, organophosphates, and carbamates. Cypermethrin is widely-used synthetic pyrethroids with use in agriculture, forestry, public and animal health. Recent investigations in laboratory animals have indicated that Cypermethrin has a deleterious effect on the neurological system (Singh et. al., 2012), hepatic and renal system (Sushma, 2010) and male reproductive system (Wang, 2010).

Because human spermatogenesis may be sensitive to continuous exposure to chemicals at extremely low levels, reports of cypermethrin's reproductive toxicity are very concerning. As a result, this chapter is aimed to investigate whether or not Cypermethrin exposure in male Wistar rats causes any kind of reproductive damage.

There is a complex network of antioxidant enzymes and free radical scavengers in testicular tissue that protects spermatogenic and steroidogenic processes from the effects of long-term xenobiotic exposure. Because of oxidative damage is thought to be the primary cause of diminished testicular function, antioxidant defense systems are crucial. Excessive lipid peroxidation (LPO) and oxidative damage may result from continuous exposure to xenobiotic like Cypermethrin, despite the testes' abundant endogenous antioxidants for free radical scavenging. In order to reduce oxidative stress in the testes and promote healthy regulation of the spermatogenic cycle and steroidogenic function, exogenous antioxidants are required.

Every day, more and more people are turning to plant-based remedies to help with their health problems. Phytochemicals are used to treat a wide variety of illnesses, yet unlike many pharmaceuticals, they don't have any unwanted side effects. Red wine contains a naturally occurring polyphenol component called resveratrol (3,5,4'-trihydroxy-trans-stilbene), Grape seed extract, grape juice and grape skin are rich source of resveratrol. Among the many known physiologic effects of resveratrol is its ability to inhibit LPO, making it a powerful antioxidant (Gulcin, 2010) and anti-inflammatory agent (Collodel, 2011). Among the many well-known cellular and molecular effectors that resveratrol activates, the estrogen response systems stand out as particularly noteworthy. Because of its ability to influence estrogen-response pathways, resveratrol may play a role in male reproduction (Bhat KPL, 2001).

Male fertility involves a series of physiological processes, depends on the combined effects of genetic, physiological and environmental factors. Intact structural and functional integrity of the male reproductive system enables the effective fertility. Male reproductive system mainly comprises of testis, ductus deferens, epididymis, accessory glands and structurally external penis and scrotum and involved in the continuous production, maturation, storage and the release of male gametes (spermatozoa) through ejaculation during the sexual act.

PYRETHROIDS INDUCED TOXICITY

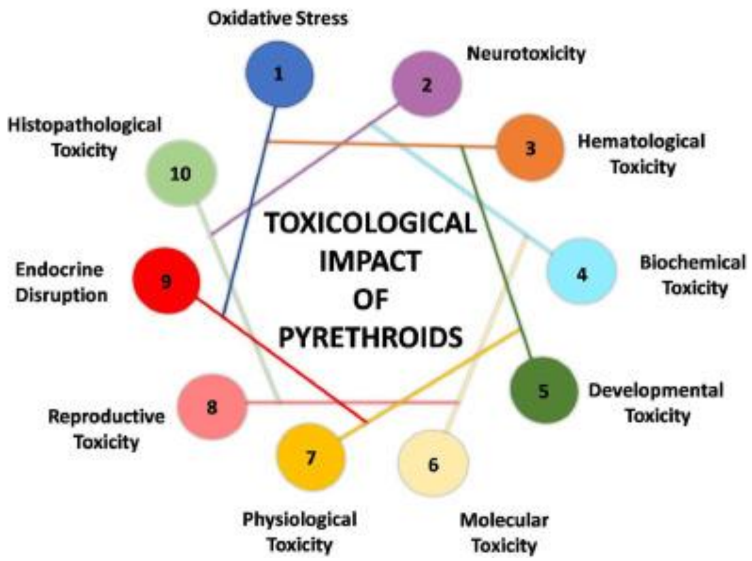

Figure 1: Toxicological impacts of pyrethroids

For more than 40 years, pyrethroids are the pesticides used in agriculture and public health programmes, constitute 25% of the insecticidal market worldwide (Shafer *et al.,* 2005). Pyrethroids derived from pyrethrin and isolated from Chrysanthemum plant Cineria folium (Casida, 1980) were extensively used due to non-persistence, decreased photostability and easy biodegradability over organophosphates, carbamates and organochlorides. Synthetic pyrethroids were introduced into the market as allethrin in 1949. Pyrethroids can be recognized by the suffix-thrinand most common pyrethroids include allethrin, deltamethrin, chyfluthrin, permethrin and cypermethrin, etc. These pesticides were used to control agricultural pest, veterinary, household and horticultural practices. As per EPA, atleast18 different pesticides were registered for use on agricultural crops for human consumption (Elbert *et al.,* 2008). Researchers have shown that via ingestion, inhalation and through dermal routes these pyrethroid pesticides were incorporated into humans (Saillenfait *et al.,* 2015). Based on absence and presence of cyano group the pyrethroids further sub-divided into two groups namely type-I and type II and claimed to pose relatively low mammalian toxicity. Type-II pyrethroids were proved to be more potent insecticide as compared to type-I due to alpha-cyano group in its structure (Tabarean and Narahashi, 1998).

Pyrethroids were unlikely to be induced acute toxicity with symptoms like epigastric pain, fatigue, nausea, increased stromal secretion, disturbance in consciousness, convulsive attacks and pulmonary edema. The symptoms of acute occupational pyrethroid include burning, itching sensation, dizziness, sneezing, broncho spasm, respiratory failure and corneal damage. Pyrethroids were also found to induce oxidative stress that alters the antioxidant system of an organism. In mice brain, in vivo permethrin exposure resulted in cell stress due to reduced mitochondrial function (Kale *et al.,* 1999; Karen *et al.,* 2001). Some pyrethroids act as endocrine disruptors altered the hormone, mimic and block their action (Ankley *et al.,* 1997), thereby; testicular and prostate cancer, miscarriages and even breast, ovarian and uterine cancer (Hunter *et al.,* 1997). In addition, pyrethroids also induced sister chromatin exchange, chromosomal aberrations and formation of micronuclei in mammals (Chauhan *et al.,* 2016).

In various studies the pyrethroid pesticides and male infertility has been found to closely associated. Post pyrethroid administration reduced sperm count was observed in mice, rat and rabbits. Pyrethroids also damage quality of semen and sperm DNA. Testosterone concentration also found to be decreased in rabbits treated with pyrethroids, rats, and mice. Elbetieha *et al.* (2001) revealed that testes showed atrophy and haemorrhages surrounding seminiferous tubules post pyrethroids treatment. In addition, pyrethroids reduced the expression level of peripheral benzodiazepine receptor and StAR protein, involved in cholesterol transfer via steroidogenesis

CYPERMETHRIN TOXICITY

Cypermethrin is manufactured pyrethroid that are broadly utilized as a pesticide for break, fissure, and spot treatment in horticulture, family, and creature farming. All eight chiral isomers are present in this compound (Valles SM, Koehler PG, 1997). (See Fig.2)

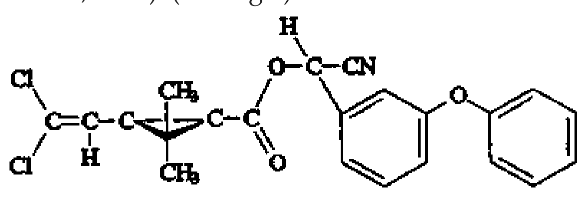

Figure 2: Formula of Cypermethrin (Baldwin, M.K., 1977a)

Molecular formula: C22H19Cl2NO3

Molecular weight: 416.3

Cypermethrin is a somewhat hazardous substance whether either orally or absorbed topically. The most widely recognized responses to dermal openness are disturbance, tingling at the skin and eyes, deadness, shivering, and tingling, a consuming inclination, incontinence, ungainliness, spasms, and even passing. Inebriation with Cypermethrin and other manufactured pyrethroids principally influences the anxious and muscle frameworks. Acute oral dosages of 10-100mg are potentially deadly in mammals (Olsen KR, 1994), while chronic oral doses of 100-1000mg/kg are hazardous. Inhaling lethal concentrations can result in respiratory paralysis and death. Cypermethrin's intense neurotoxicity is interceded generally by expanded movement in the focal sensory system. Cypermethrin also causes neurotoxicity by altering the concentration of GABA. In addition, Cypermethrin's capacity to stimulate free radical production contributes to its neurotoxicity.

Deltamethrin is engineered pyrethroids that can be poisonous through both cutaneous contact and ingestion. It's one of the most widely utilized pesticides in industrial settings. As the most poisonous pyrethroids, it is also the most effective (Shrivastava, 2011). As synthetic pyrethroids, flumethrin finds application in veterinary medicine. Parasites and ticks on pets and livestock can be eliminated by dousing them in a 10% flumethrin solution in hot water. Skin contact with these pyrethroids has not been found to be dangerous, but ingestion has been shown to be toxic properties exhibited by this substance. The cell reinforcement chemicals superoxide dismutase, catalase, glutathione peroxidase, and diminished glutathione were undeniably kept at levels like those in control rodents when high-fat eating regimens were joined with dark pepper or piperine, as revealed by (Vijaya kumar *et al.* 2004).

Cell reinforcement and hepato protective impact of piperine against benzo(a)pyrene and aflatoxin-prompted toxicity in mice and rodents has been exhibited in past examinations from different research facilities. In this manner, we set off on a mission to perceive how well piperine proceeded as a cell reinforcement against CYP-prompted oxidative stress in rodents.

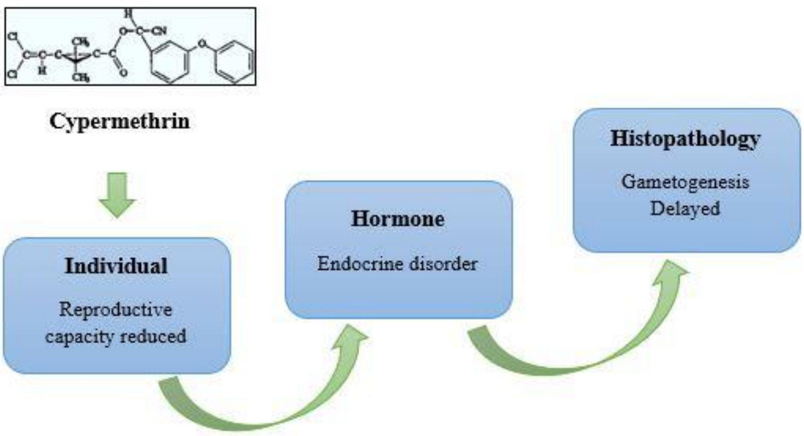

Figure 3: Reproductive toxicity induced by Cypermethrin

In addition, CYP is a potent inducer of apoptosis that draws the attention towards its role in inducing testicular germ cells apoptosis. Few reports suggest its involvement in inducing apoptosis human neuroblastoma cells where CYP in combination with chlorpyrifos triggered cell death either by causing genomic DNA fragmentation (Raszewski *et al.*, 2015) or by affecting Bcl-2, Bcl-xL and caspase-3 activation (Raszewski *et al.*, 2015). CYP administration through intra peritonial or oral resulted in ROS generation

(Giray *et al.,* 2001). Altered pituitary-gonadal hormones, steroidogenic enzymes (Sharma *et al.,* 2018) or modulating apoptotic and anti-apoptotic pathways may be the mechanism of CYP toxicity. All these studies suggest that CYP toxicity on functional and physiological aspect of reproductive system is a great matter of concern which further needs to be explored.

MECHANISM OF CYP

CYP arbitrate via complex mechanisms. It acts like endocrine disruptor, anti-androgen, xenoestrogen and depends upon target gene expression or cellular growth effects. It consists of an alpha-cyano group attached to the benzylic carbon which enhances the insecticidal properties which cause toxic signs of choreoathetosis with salivation (CS-syndrome) in the rat and bursts of spikes in the cerebral motor nerve of the cockroach

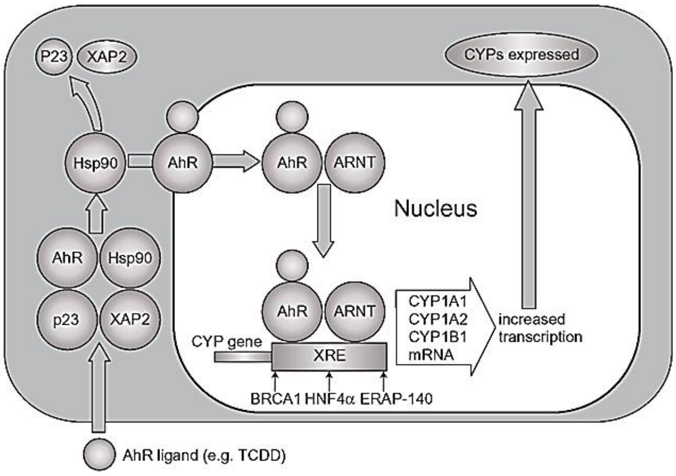

Figure 4: Mechanism of CYP

Androgens mediate a wide range of developmental and physiological responses in the male rat and are vital for testicular, accessory sex gland development and function, pubertal sexual maturation in multiple organs, maintenance of spermatogenesis, maturation of sperm, male gonadotropin regulation through feedback loops and various male secondary characteristics such as bone mass, musculature, fat distribution and hair patterning. CYP interferes with androgen action and has a greater impact on male developmental programming, reproductive tract maturation and one of the major pathways through which it operates is AR mediated signaling. Primary androgenic hormones, testosterone and it metabolite DHT (5-α-dihydrotestosterone) mediate their biological effects predominantly through binding to the AR and is expressed in many end-organs including the hypothalamus, pituitary, liver, prostate and testis (Matsumoto *et al.,* 2008). CYP can interfere with androgen-dependent mechanisms and affect male reproductive tract health and these include androgen synthesis, metabolism and clearance, feedback regulation, AR expression in target organs and direct AR binding. CYP is known to be anti-androgen, inhibits synthesis of testosterone binding to AR and sex hormone binding globulin. CYP elicits anti androgenic activity by interfering with interleukin-6 (IL-6) -induced ligand-independent AR signaling through signal transducer and activator of transcription 3 (Zhou *et al.,* 2017).

METABOLISM OF CYP

In mammals synthetic pyrethroids are generally metabolized through ester hydrolysis, oxidation and conjugation. After absorption in the gastrointestinal tract, CYP is metabolized via cleavage of the ester bond to phenoxybenzoic acid (PBA) and cyclopropane carboxylic acid (CPA). The PBA moiety is excreted as conjugate differential in different animal species. PBA is further metabolized to a hydroxy derivative and conjugated as glucuronate or sulphate and CPA moiety is mainly excreted as a glucuronate. Due to the lipophilic nature of CYP, the maximum tissue concentrations were recorded in body fat, kidneys, liver, skin, ovaries and adrenals.

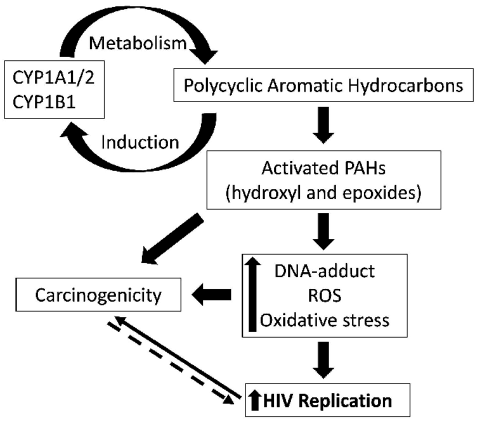

Figure 5: Metabolism of CYP

The elimination of CYP from fat is approximately 3 to 4 times slower than in other tissues (WHO, 1989). Studies related to half-life of CYP have shown 8 weeks in soil, almost 100 days in water and it persists about three months in household treatment for pests. Human volunteer studies has shown that urinary metabolite profile by oral and dermal routes submit that CYP might be significantly metabolised in the skin before systemic circulation occurs and major urinary metabolites of CYP are a variety of conjugates of cis and trans 3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylic acid (DCVA), 3-phenoxybenzoic acid (3PBA), and 3-(4'-hydroxyphenoxy) benzoic acid (4OH3PBA)

OXIDATIVE STRESS

Cypermethrin's neurotoxicity is believed to be brought about by oxidative stress. Expanded arrangement of receptive oxygen species (ROS) and responsive nitrogen species in cells or tissues presented to Cypermethrin, or diminished degrees of parts of the cancer prevention agent hardware, are key supporters of oxidative stress. When Cypermethrin is given orally or intraperitoneally, it causes oxidative stress in the nervous system (Rodriguez, 2009). After moderate doses and long-term exposures, there was no discernible change in overall glutathione-S-transferees (GST) activity (Giray B, Gurbay A, Hincal F, 2001); despite considerable changes in the expression of a few GST isoforms. One of the central members in Cypermethrin digestion that produces responsive oxygen species (ROS) and oxidative stress by means of blended capability oxidase is cytochrome P450 2E1 (CYP2E1). Based on Nernst formula, the way back in year 2011 by Sies, oxidative stress generated within the body is an imbalance in their redox coupling. As per Lushchak (2014), transient and chronic elevation in oxidative stress might be due to production of ROS that adversely affects the homeostatic balance between normal cellular metabolism and its regulatory processes. However, in the biological system ROS might be produced due to cellular metabolism at moderate concentration and play a pivotal role in physiological processes; however, its high concentration directly or indirectly targets the DNA, lipids and proteins (Birben *et al.,* 2012; Weidinger and Kozlov, 2015). Aerobic organisms counteract ROS mediated toxicity by the antioxidant defence system present in their body. However, lack of these antioxidants to scavange the ROS generated creates a stressful condition in the favour of oxidants termed as 'Oxidative Stress' that leads to adverse effects (Birben *et al.*, 2012). This stressful condition possess severe acute to chronic illness and also induces various diseases such as aging, genetic abnormalities, cardiovascular and respiratory diseases, carcinogenesis, autoimmune diseases, cataract, stroke and septic shock and neurodegenerative disorders like Parkinson's and Alzheimers disease (LukaszewiczHussain, 2008)

EFFECTS ON REPRODUCTIVE SYSTEM

Cypermethrin has been shown to have a negative impact on the seminal gland, either immediately or over time. Furthermore, an expansion in the level and multiplication of cells in the Secretory epithelium of this organ was viewed because of the poisonous intense activity of Cypermethrin, while the rising development of pole cells was evidence of both the intense and ongoing effects. According to the findings of (Rodriguez *et al.* 2009), this behavior may reflect an asymptomatic inflammatory condition that, in fact, may impair male infertility. Increased usage of Cypermethrin, whether for an acute or chronic condition, has the potential to reduce male fertility in the future.

IMPACTS ON ANIMALS

In most cases, Cypermethrin prevents the sodium channel's ion gates from closing during re-polarization via acting on the cell membrane (Mun JY, Lee WY, Han SS, 2005). This significantly interferes with nerve impulse transmission, leading to membrane depolarization or discharges that occur repeatedly (Nasuti, 2007). Insects and other arthropods become overly active at even trace amounts. They get immobilized and eventually die at high quantities. Particularly sensitive are the cells of the neurological and sensory systems. Cypermethrin is poorly absorbed by the skin when applied to animals because of their hair coat. Despite its poor blood-brain barrier penetration, glucuronidase speeds up its liver conversion to non-toxic metabolites that are then eliminated in the urine (Bhattacharyya, 2005). Cypermethrin and other synthetic pyrethroids are not metabolized by several chordate species, including cats, since they lack the glucuronidase enzyme. As a result, these animals are more susceptible to the harmful effects of Cypermethrin and other synthetic pyrethroids. Cypermethrin products can be used on dairy animals, but there is evidence that it can cause reproductive damage if ingested by humans.

PIPERINE PREVENTS CYPERMETHRIN-INDUCED OXIDATIVE DAMAGE

Estimating free radical production and antioxidant defense has emerged as a critical area of study in mammals in recent years. Antioxidants such vitamin E, isoflavones, and L-ascorbic acid have been demonstrated to protect rats against the oxidative damage brought on by CYP induced toxicity (Raina, 2009). The antioxidant defense mechanism is known to be modulated and scavenged by free radicals, which is how plant compounds achieve their protective benefits. Both long pepper (Flute player longum Linn.) and dark pepper (Flautist nigrum Linn.) contain the alkaloid piperine. Cell reinforcement, bioenhancer, mitigating, and hepatoprotective are only not many of the pharmacologic.

ANTIOXIDANTS

The living system is well equipped with the substances capable of counteracting the damage caused by the ROS in response to oxidative stress known as 'antioxidants defence system' including antioxidants and antioxidant enzymes. However, ROS mediated oxidative stress induces alterations in lipids, proteins and DNA which was found to be controlled by the antioxidant system of the body by maintaining the balance between ROS and antioxidants (Schieber and Chandel, 2014). In addition, to provide maximum protection to the cell, various scavengers such as antioxidant enzymes are compartmentalized in sub-cellular organelles leading to decline in ROS level; but, failure in this defence system results in oxidative stress mediated cell death.

As per Halliwell and Gutteridge (1984), these antioxidant defences are categorized as primary antioxidants (involved in the impediment of oxidants formation), secondary antioxidants (exhibit scavengers of ROS) or tertiary antioxidants (through sources such as dietry and consecutive antioxidants and repair the oxidized molecules). Various factors disturbing the efficiency of antioxidants include activation energy of antioxidants, oxidation, reduction capability, solubility and pH stability (Noori, 2012). The antioxidant defence system consists of enzymatic and non-enzymatic components including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPx), glutathione reductase (GSR), glutathione-s-transferase (GST) etc., and non-enzymatic components includes vit C, vit E, zinc, n-acetyl-l-cysteine, glutathione etc. (Birben *et al.,* 2012).

To offset the ROS mediated oxidative stress the antioxidant defence system assists the cells to produce antioxidant enzymes for diminishing their action. The antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPx), glutathione reductase (GSR), glutathione-s-transferase (GST). Superoxide Dismutase (SOD), an inducible metalloenzymes that catalyzes the dismutation of the superoxide radical (O2-) into hydrogen peroxide H2O2 and oxygen (O2), and act as a first line of defence (Gupta *et al.,* 2008). The reaction catalyzed by SOD maintains the level of O2- in the tissue and is highly efficient.

MITIGATION OF CYPERMETHRIN-INDUCED REPRODUCTIVE TOXICITY WITH ANTIOXIDANTS

Antioxidants are substances that can help mitigate oxidative stress by eliminating reactive oxygen species (ROS). Researchers have looked into whether or not antioxidants can reduce the reproductive toxicity of Cypermethrin. Some promising antioxidants for mitigating cypermethrin's effects on reproduction are listed below.

Vitamin E: Vitamin E is a powerful antioxidant that has been shown to reduce the reproductive toxicity of Cypermethrin in experimental animal models. It can protect the reproductive system by lowering oxidative stress.

Vitamin C: The antioxidant vitamin C has been demonstrated to reduce the harmful effects of Cypermethrin on reproduction. Reactive oxygen species (ROS) can be scavenged, protecting reproductive tissues from oxidative injury.

Curcumin: The Curcuma longa plant produces a brilliant yellow substance known as curcumin. Turmeric (Curcuma longa), a plant in the ginger family Zingiberaceae, has this compound as its primary curcuminoid. It's on the market as a dietary supplement, a component in cosmetics, a flavoring agent, and a coloring agent for foods. Antioxidant activity of curcumin aids in ROS scavenging and protects reproductive organs from oxidative stress. The reproductive toxicity-related inflammation can also be mitigated by its anti-inflammatory properties.

Tribulus: Spine-covered fruit producer native to the Mediterranean called "Tribulus terrestris." The pierce vine name is another name for this plant. The tribulus plant is used medicinally for its fruit, leaves, and roots. There may be additional components in some formulas. It's rich in antioxidant-rich bioactive substances like flavonoids and steroidal saponins.

EFFECT OF VARIOUS NATURAL ANTIOXIDANTS ON CYPERMETHRIN INDUCED REPRODUCTIVE TOXICITY

CURCUMIN

The antioxidant defense mechanism is known to be modulated and scavenged by free radicals, which is how plant compounds achieve their protective benefits. A radiant orange color called curcumin (CMN) (diferuloylmethane) is separated from the rhizomes of the turmeric plant, Curcuma longa (Maheshwari, 2006). Curcumin is an antioxidant and anti-inflammatory that has been shown to be very effective at preventing the production of reactive oxygen species (Biswas, 2005). Researchers found that CMN effectively removed both superoxide anion and hydroxyl radicals from their respective samples. CMN treatment has been displayed to safeguard rodents from oxidative stress created by arsenic, gentamicin, and acetaminophen (Ambali, S. 2011). Similarly, CMN inhibits free radical production in paraquat-induced lung damage and rat models of myocardial ischemia (Fatma, 2009). The current investigation aimed to find out if CMM could prevent or lessen the serum biochemical changes and oxidative stress caused by the sub-acute dose of CYP in rats.

Blood biochemical markers and tissue cell reinforcement chemical levels were modified by Cypermethrin in rodents; however curcumin forestalled these modifications. Six groups of rats were employed for the study, with groups I and II serving as controls and groups III as vehicle controls. Curcumin (100 mg/kg body weight), Cypermethrin (25 mg/kg body weight), and Cypermethrin in addition to curcumin were given orally to bunches IV, V, and VI for 28 days. The serum was tried for biochemical pointers, and the liver, kidney, and mind were dissected for lipid peroxidation and cell reinforcement compound movement. Expanded serum levels of biochemical pointers and lipid peroxidation in the liver, kidneys, and mind were seen after Cypermethrin treatment. Superoxide dismutase was the main enzymatic cell reinforcement whose movement wasn't diminished in liver, kidney, and mind tissues. Curcumin's co-organization with Cypermethrin brought about impressive decreases in blood biochemical markers and lipid peroxidation, as well as expansions in diminished glutathione, catalase, and glutathione peroxidase, and the upkeep of ordinary liver, kidney, and mind histology. Based on our findings, curcumin has the potential to act as a powerful preventative agent against the metabolic changes and oxidative damage caused by Cypermethrin in rats (Sank, 2012).

Male Wistar rats' reproductive systems were negatively affected by Cyp and Del exposure. Curcumin and quercetin reduced the reproductive toxicity of these pesticides. Testicular and epididymis weights were lower after exposure to either Cyp or Del alone or in combination than they were after exposure to the control. Previous research in animals has shown that both Cyp and Del are harmful to male reproductive systems. The direct cytotoxic activity of these insecticides on testicular tissue may explain the reduction in testicle and epididymis weight seen in the present investigation. Single and combined exposure to Cyp and Del resulted in a substantial drop in testicular sperm head counts compared to the control. Serum testosterone levels, which were observed in the present experiment and reported in previous studies, may also be responsible for the decrease in sperm head counts, which may have been caused by the accumulation of insecticides in the testicular tissue. Sperm counts have been shown to drop when ROS levels rise. After being exposed to Cyp and Del, the study participants saw increases in LPO and decreases in enzymatic and non-enzymatic antioxidants. Thus, elevated lipid peroxidation and ROS production may contribute to low sperm counts. The use of dichlorvos has been linked to lower sperm motility and an increase in sperm abnormalities. In our investigation, we found that exposure to Cyp and Del increased sperm abnormalities and decreased sperm motility, which may have been caused by an increase in ROS generation and a drop in testosterone. Del and Cyp have been found to disturb the endocrine system, although the method by which they do so is unclear. The consequences of this review propose that openness to Cyp and Del lessens testosterone levels, which might be owing to a blend of direct impacts on androgen creation pathways in the testicles and changes in gonadotropin levels. We found that Cyp and Del exposure decreased steroid genic enzymes (3-HSD and 17-HSD). This decrease in 3-HSD and 17-HSD may be attributable to Leydig cell injury or the direct action of these insecticides on gene expression of 3-HSD and 17-HSD because of the considerable increases in oxidative stress in the testicular tissue.

VITAMIN E

This study was embraced in view of mounting proof connecting receptive oxygen species (ROS) to the toxicity of various pesticides; explicitly, Cypermethrin, Sort II pyrethroids. Raised degrees of thiobarbituric corrosive receptive substances in the testes of rodents were noticed 4 and 24 hours after a solitary (170 mg/kg) or rehashed (75 mg/kg each day for 5 days) oral organization of Cypermethrin, separately. Expanded lipid peroxidation in the testes after Cypermethrin organization in rodents is steady with free revolutionary intervened tissue harm; this was alleviated by pretreatment with allopurinol and vitamin E (Giray, B., 2001).

With regards to Cypermethrin (CYP) - prompted oxidative stress in the testes of rodents, vitamin E (VitE), selenium (Se), or both have defensive and additionally upgraded impacts. Subsequent to regulating VitE (100 mg/kg sc), Se (0.1 mg/kg sc), and VitE in addition to Se (0.1 mg/kg sc) to rodents for three days, the creatures were given 50 mg/kg (1/4 ED50) of CYP in corn oil orally once every day for five days. Tissues and testes were investigated for their degrees of malondialdehyde (MDA), as well as their GSH-Px. CYP treated bunch had essentially more elevated levels of MDA in all organs aside from plasma, as well as higher GSH-Px and Feline movement in the liver and erythrocytes, separately. Vitamin E treatment before CYP organization diminished aversion to CYP-actuated oxidative stress. supplementation was related with raised degrees of receptive oxygen species (ROS) and the cell reinforcement framework was essentially modified by Vit. E because of oxidative stress actuated by CYP. As indicated by the discoveries, CYP can prompt oxidative stress, while VitE can assist with alleviating the adverse consequences by changing CYP digestion (Ateşşahin, 2005).

VITAMIN C

Vit C, as an important water soluble antioxidant, mitigated the oxidative stress generated; thereby counteracting the ROS, by easily reacting with them in extracellular body fluids. Vit C causes reduction in abnormal sperm (Greco *et al.*, 2005), several apoptosis characteristics attribute in the testicular germinal epithelium; thereby maintaining the physiological integrity of the testes. In addition, Vit C also exhibited effective attenuation on endosulphan and atrazine induced goat testicular toxicity (Sharma *et al.*, 2010b, c). Supplementation of Vit C also reduced MDA level (as a by-product of lipid peroxidation) in the tissues after chronic exposure to organophosphates (Ambali *et al.*, 2011). A previous study marked the cadmium induced toxicity in thyroid gland that was found to be ameliorated by ascorbic acid. However, Vit C also modulated the genotoxicity induced by various pesticides in the primary spermatocytes (Khan and Sinha, 1994b). Along with this, Vit C also decreased the lipid per-oxidation and increased antioxidant enzymes such as CAT, SOD and GST and thereby decreasing methomyl toxicity resulting in modulation of key stress and apoptosis related four genes such as Cas-3, Cas-9, Tp53 and Bcl-2 in the rats (Heikal *et al.*, 2014). Vit C efficiently protects the cell against lipid peroxidation by its antioxidative action of chain breaking, where the Vit C is transformed to weak free radicals which converted back to its active state by the ameliorative action of Vit C. Supplementation to Vit C ameliorated the DEHP induced male reproductive toxicity by modulating the peroxisome proliferator-activated receptor (PPAR)-dependent mechanisms (Wang *et al.*, 2017). In addition to their independent effects, studies have also showed the synergistic effects of Vit C and Vit E. Therefore, employing Vit C and Vit E as ameliorants against testicular cells apoptosis due to pesticide poisoning has high potential.

CONCLUSION

Our research centered on whether antioxidants could mitigate the reproductive damage caused by Cypermethrin. We have conducted an extensive literature review to determine the role of several antioxidants, including Vitamin E, Vitamin C, Curcumin, and plant extracts like Tribulus, play in the restoration of reproductive function in Cypermethrin-exposed rats and other species. Based on our research, it appears that antioxidants are crucial in protecting against the reproductive toxicity of Cypermethrin. Proving their worth, these antioxidants mitigate oxidative stress, safeguard cell integrity, and revive reproductive capabilities.

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Chapter
9

LUMPY SKIN DISEASE- AN OVERVIEW

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ABSTRACT

It is a viral disease which affects cattle. This disease originated from Africa. It is caused by LSDV (Lumpy Skin Disease Virus). Nodular skin disease, fever is the symptoms of LSD disease. Till now, there is no proper vaccination for LSD disease. It can be controlled by keeping the infected cattle away from the herd. Mortality rate in LSD is very high. LSD leads to economic losses to the farmers. Strict bio-security protocols to limit the spread of the virus.

KEYWORDS: LSD, Mortality, Economic loss.

INTRODUCTION

LSD is believed to be originated in 1929, Zambia, Africa. From there, it has been spread to various regions like middle-east, Asia, Europe. The vector of this disease is insects, biting flies which transmit the virus from infected to other animals.

Here are some impacts of LSD on cattle:

Skin Lesions:

Nodular skin lesions are found on the body of infected cattle. The animals feel pain, itching, behaviour change and reduced the body activity. Even nodules are found in digestive and reproductive tract in severe cases.

Fever and General Malaise:

Animal feels general malaise and fever. This decreases the feed intake, weight loss.

Secondary Infections:

Severe case of LSD may become cause of other secondary infections and it increases the potential of mortality.

Impact on Reproduction:

LSD is not directly associated with reproductive issues. But the stress and physiological changes induced may affect the reproductive performance.

Economic Losses:

Due to decrease in milk production, disease treatment costs, the farmers face huge economic losses.

CAUSATIVE AGENT OF LSD

It is cause by virus and known as LSD virus. The virus is a member of the genus Capripoxvirus. Family of virus is Poxviridae. This virus is responsible for the appearance of nodular skin lesions over the body of infected cattle which is a big concern for the livestock sector.

Family: Poxviridae

Subfamily: Chordopoxvirinae

Genus: Capripoxvirus

This virus infects hoofed animals with different genus. Other viruses in this genus are sheep-pox virus and goat-pox virus which affects sheep and goats respectively.

Study of genetic and structural characteristics of the virus helps in developing effective measures and strategies against these viruses.

BEHAVIOUR OF THE LSD VIRUS

Transmission:

It is transmitted through blood sucking insects like mosquitoes, ticks and flies. It can also be transmitted by direct contact of infected animals with other animals or farm equipments like feeder and drinker.

Infection:

After entering the host animal's body the virus starts replicating itself in the host cells. Further the characteristic symptoms of the virus appear on the skin of the animal in the form of nodules or lumps.

Clinical Manifestations:

The major symptoms are fever, nodules on the skin and swelling. This affects the overall health and activities of the animals.

CONTROL OF LSD VIRUS

By implementing so many strategies, spread of LSD can be control. Here are some following strategies-

Vaccination:

Vaccination for LSDV has been developed. This vaccine gives immunity against this virus. Massive vaccination campaign must be launched in the disease outbreak region.

Quarantine:

Infected animals must be kept in quarantine for at least 30 days and during the quarantine period regular monitoring should be done by the experts and veterinary experts.

Control of Vectors:

Mosquitoes, ticks, flies and other bood sucking insects are the vectors in the transmission of the disease. Use insecticides, phenyl and other ways to control the growth of vectors in the surrounding.

Bio-security measures:

- Prohibit the entry of outsiders in the farm.
- Foot bath ($KMnO_4$) must be made at the entry gate.
- Isolation of infected animals.
- Expert advices
- Early detection and reporting to farmers and veterinary authorities.

EDUCATION AND AWARENESS

Educating farmers about LSD virus is very important. Training program must be organised and educate the farmers. KVK, agricultural colleges, research institutes should organise the training program and create awareness among the farmers.

CONCLUSION

Lumpy skin disease is a viral disease and it spreads through blood sucking insects, contaminated water, feed and saliva. Nodules or lumps are found all over the body of the animal. Even lumps are found on udder. And later on, these nodules get convert into lesions. Even the pregnant cow could have abortion. Keep infected people isolated. There should be no physical contact among the animals. Mortality is not very high but still world health organization of animals have issued the advisory to report the organization as soon as possible if this case is found anywhere of the world.

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Chapter
10

LARVICIDAL ACTIVITY OF *ACACIA LEUCOPHLOEA* (ROXB.) WILLDMETHANOLIC LEAF AND BARK EXTRACT AGAINST *AEDES AEGYPTI* LINN. LARVAE

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ABSTRACT

The plant *Acacia leucophloea* (Roxb.) Willd is reported to have immense medicinal value in Indian medicine. The plant bark used to treat inflammation, thirst, vomiting, bronchitis and effective in biliousness, burning sensation and an astringent. The bark is used as blood purifier, antihelmentic, antimicrobial and expectorant. Bark extract showed significant antipyretic activity against the yeast induced pyrexia model. It is also used in ulcer, gum bleeding, mouth ulcer, fever, dry cough, dysentery, skin diseases treatment (leprosy), diabetes, and Snake-bite. Bark decoction and gum is used for menstrual complaints and contraception. Tannins and dyes are manufactured from inner bark of the plant. It also shows antimicrobial and antioxidant activity

The present investigation is carried out to reveal the larvicidal activity of the *Acacia leucophloea* (Roxb.) Willdmethanolicbark and leaf extract against *Aedes aegypti* Linn. Larvae. It showed that the bark extract has morelarvicidal activity than the leaves extract. As the source of the plant is very common and the formulation is not costly. The present study may open new approach towards the eco-friendly, biodegradable and effective larvicidal against *Aedes aegypti* Linn. larvae.

KEYWORDS: Larvicidal Activity, *Acacia leucophloea*, *Aedes aegypti*

INTRODUCTION

Plants are most important parts to our wellbeing, not only as food, but also as key components of our religions, cultures and medicines. This can be observed in way that tribal peoples collect plant materials for medicinal used or for religious practices. We do not just get nutrients and nourish-ment from plants, they are central key aspects to our societies (Schaal, 2019). Plant is the abundant source of the natural product which play animportant role as a source of drug compounds and currently, a number of modern drugs which are derived from traditional herbal medicine are used in modern pharmacotherapy (Marrelli, 2021). Presently the research is much more focused on the investigation of these bioactive molecules occurs in the plants and their effective isolation for treatment of and protection from the several diseases to the human beings.

Acacia leucophloea (Roxb.) Wild. (Syn. *Mimosa leucophloea*) (Mimosoideae) is a large thorny tree reaching heights of around 35 m and diameters at breast height is 100 cm (Kaul, 1963; Naik, 1998; YadavandSardesai, 2002). Its native is range from South and Southeast Asia is noncontiguous. It is majorly distributed in arid India through Bangladesh, Burma, Sri Lankaand much of Thailand (Nielsen, 1992). In India it is mainly occur in Cental India, Deccan, Rajputana and Punjab etc. (Anonymous 1948).

The plant bark cures burning sensation, inflammation, bronchitis useful in biliousness, vomiting, thirst and an astringent (Kirtikarand Basu, 1975; Nadkarni and Nadkarni, 1976). The plant bark is used blood purifier, asantihelmentic, antimicrobial and expectorant. Bark extract shows substantial antipyretic activity against the yeast induced pyrexia model. (Gupta, *et al.*, 2012). It is also used in ulcer, gum bleeding, mouth ulcer, fever, skin diseases treatment (leprosy), dry cough, dysentery, diabetes, and Snakebite (Jhade, *et al.*, 2012). Bark decoction and gum is used for menstrual complaints and contraception. Tannins and dyes are manufacture from inner bark of the plant. Bark and leaf are used for the treatment of gonorrhea (Jitin, *et al.*, 2013). It also shows antimicrobial and antioxidant activity (Anjaneyalu, *et al.*, 2010).

Plant products are offer as a promising substitute for synthetic chemical agents for insect management and control. Phytochemicals belonging to different chemical classes such as terpenes, steroids, alkaloids and phenolic constituents were examined earlier for insect control and are potential and found promising. There is a need for an alternate source with least risk to environment and human health, and the present investigation has intended to recognize potential plant-based mos-quitolarvicides against the mosquito vectors.

MATERIALS AND METHOD

COLLECTION OF PLANT MATERIALS

Bark and Leaves of *Acacia leucophloea* (Roxb.) Willd were collected from Mahur forest ranges (N.19^0 42.548′ E 078^0 13.256′) in Nanded district of Maharashtra. Harbarium, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad identified and authenticated the collected specimen (Accession No.-17394). Freshly collected stem bark and leaves of the plants were dried in shade and pulverized to coarse powder. The powder was stored in an airtight container and kept in a dark, cool, and dry place (Hassan, *et al.*, 2014; Das, *et al.*, 2014).

Figure 1: ***A. leucopholea*** **plant branch**

Figure 2: ***A. leuopholea*** **inner and outer bark**

METHOD OF PREPARATION OF METHANOL EXTRACT

The extraction was carried out by hot continuous method using Soxhlet apparatus. The 25 gm powder of bark and leaves were extracted using 250 ml methanol for 72 hours. The methanolic extract of leaves and bark of the plants were used for the further study (Vijayalakshmi, *et al.*, 2012).

LARVICIDAL ACTIVITY

Larvicidal activity was done according to "WHO Guideline for laboratory and field testing of Mosquito Larvicides" (Anonymous, 2005) with some modification. Batches of 25 third and fourth instar larvae of *Aedes aegypti* Linn. were used for the present larvicidal activity (Mathalaimuthu, *et al.,* 2015,Kamaraj, *et al.,* 2011).

Larvae Mortality percentage was calculated with the help of following formula.

$$Mortality\ (\%) = \frac{X - Y}{X} \times 100$$

Where, X = percentage survival in the untreated control and Y =percentage survival in the treated sample.

RESULTS AND DISCUSSION

LARVICIDAL ACTIVITY OF BARK AND LEAVES EXTRACT OF *ACACIA LEUCOPHLOEA* (ROXB.) WILLD.

Table 1: Larvicidal activity of leaves and bark extract of *Acacia leucophloea* (Roxb.) Willd.

Name of the plant	% mortality (500 PPM)	LC 50 PPM	95 % Confidence LFL -UFL	Regression equation ($Y= \alpha + \beta X$)	Chi-square (X^2)
Acacia leucophloea bark	61.33 ±1.63	336.68 ± 14.55	314.06-354.3	Y= 2.34 + 1.05 X	0.12
Acacia leucophloea leaf	52 ±2.83	577.33± 34.45	481.21-572.42	Y= 2.49 + 0.92 X	0.17

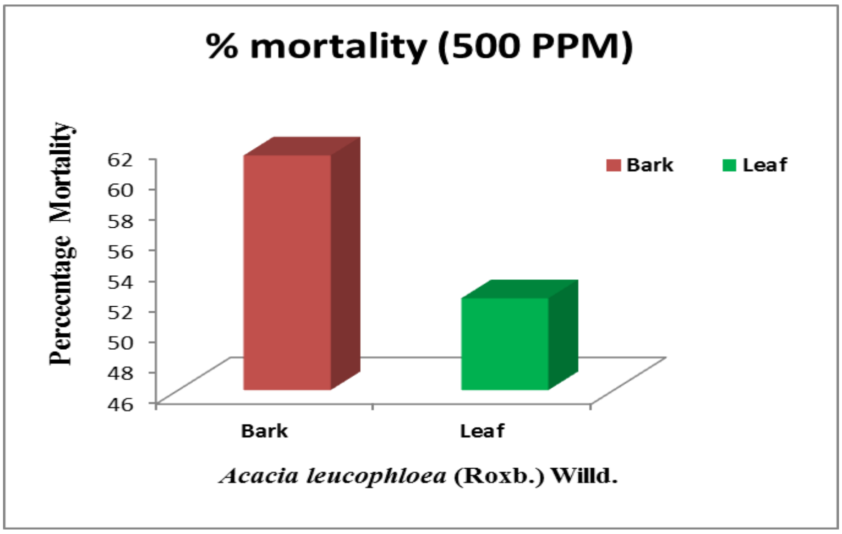

Figure 3: Larvicidal activity of leaves and bark extract of *Acacia leucophloea*(Roxb.) Willd.

Larvicidal activity of the plant extract decreased with decrease in the concentration from 500 PPM to 100 PPM. Larvicidal activity of the plant extracts revealed that bark extract (61.33 ±1.63 %) has more % mortality than the leaf extracts (52 ± 2.83 %) (Fig. No. 31). LC 50 value for the bark extract was 336.68 ± 14.55PPM and for the leaf extract value was 577.33± 34.45PPM. Similarly, 95% confidence LFL-UFL (LFL: Lower fiducidal limit, UFL: Upper fiducidal limit.) lower for the bark extract was 314.06-354.3PPM and 481.21-572.42PPM for the leaves extract. It showed from the present research that the bark extract has more potent larvicidal activity than that of the leaves extract (Table No.1). Methanolic leaves and bark extract of *Acacia leucophloea*Roxbshas different types of phytochemicals, bioactive molecules and these are rich in the flavonoid contain (Wankhade and Mulani, 2015). The natural product and bioactive molecules which act as the larvicidal drug are obtained from the bark and leaves methanolic extracts of the *Albizia julibrissin* Durazz. (Wankhade and Mulani, 2016).

CONCLUSION

The present investigation suggests that the methanolic bark extract of the *Acacia leucophloea* (Roxb.) Willd shows the potential larvicidal activity against the third and fourth instar larvae of *Aedes aegypti* Linn. The plant leaf and bark extract contain effective larvicidal bioactive principles which may be needed for further purification to obtained natural product larvicidal drug. The present research work is further used to prepare eco-friendly, environmentally safe, biodegradable, and low-cost natural products larvicidal which can be applied to relatively small areas where larvae are concentrated.

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Chapter
11

AVIAN INFLUENZA – AN OVERVIEW

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ABSTRACT

Avian Influenza is a contagious disease which is also known as bird flu. It is a contagious viral infection. The mortality rate is very high in Avian Influenza. Even it is transmitted to humans. Bird flu causes huge economic loss to the poultry farmers. In severe condition, the flock dies within two-three days. There are various subtypes of avian influenza virus. H1N1, H5N1 are deadly viral forms of avian influenza. Ducks, geese and migratory birds are the primary cause of the transmission of avian influenza. Human get affected by the direct contact with the infected birds or their droppings.

KEYWORDS: bird Flu, Viral, Economic, Avian Influenza, H1N1 and H5N1.

INTRODUCTION

The bird flu is commonly referred to as avian influenza. Various subtypes of avian influenza are identified by the combination of proteins hemagglutinin(H) and neuraminidase(N). H1N1, H5N1 are the types of avian influenza. H1N1 is more dangerous than H5N1 as H5N1 primarily infects birds. These viruses are of two types which are low pathogenic avian influenza(LPAI) and Highly pathogenic avian Influenza(HPAI). HPAI is more fatal than LPAI. The virus gets transmitted by respiratory droplets or just dust particles from infected birds. The infected birds face coughing, watering of eyes, respiratory disorders. In birds, according to the strain of the virus, bird flu varies. Nasal discharge also happens in this.

SYMPTOMS OF BIRD FLU

Figure 1: Swelling Symptoms

(Source:https://cluckin.net/bird-flu-faq-and-some-practical-advice-for-avian-influenza.html)

Respiratory problems

Infected birds show respiratory signs such as coughing, sneezing, nasal discharge and abnormal breathing rate.

Swelling

Swelling on head, neck, and eyes are found in severe cases. Birds feel continuous restlessness and body activities hindered by these symptoms.

Drop in Egg Production

Laying hens produces lesser eggs and the quality of the eggs is not good. Egg shell would be thinner.

Edema of wattles and comb

Comb and wattles are the prominence of the head may become swollen too.

Sluggishness and Depression

Reluctance to eat and drink can be seen in the birds. Under the depression, birds become inactive or sluggish. Body weight falls down.

Mortality

Rapid outbreak could wipe out the entire flock within 2-3 days. There is no proper way to control the outbreak once it starts. Prevention is better than cure; this must be in the mind.

TRANSMISSION TO HUMANS

Human to human transmission is rare but from infected birds to the humans is possible by droppings, litter, share of respiration. Severe conditions may lead to pneumonia and other respiratory complications. Once the human gets infected, he could have following symptoms:

- **Fever:** High fever is the initial symptoms.
- **Cough:** Painful cough and irritation and pain in the throat can be experienced.
- **Muscles ache:** Sluggishness, pain in the muscles and fatigueness can be felt.
- **Breathing disorder:** Abnormal respiration rate, deep breathing.
- **Headache and conjunctivitis**: Eye infection, headache may also occur.

It's important to know that everyone will not face the same symptoms of avian influenza as it varies with many subtypes.

MODE OF TRANSMISSION

The virus is typically present in the respiratory and digestive secretions of the infected birds.

WILD BIRDS

Migratory waterfowl are the natural transmission of avian influenza as they migrate from once place to another place according to the climatic condition.

DOMESTIC POULTRY

When new birds are introduced in the farm and if it is not vaccinated then a chance of disease outbreak is possible. Infection can be spread by direct contact or exposure to the contaminated surfaces. When one bird gets infected then other birds will automatically get infected if proper guidelines are not followed.

HUMAN ACTIVITIES

Sometimes, poultry birds for making profit, sell infected and sick birds to other poultry farmers and into the market without telling any facts about the disease. This selfish behaviour could become a cause of the spread of avian influenza.

GLOBAL IMPACT

Avian influenza outbreaks have huge affect on poultry sector. Poultry meat is easily affordable by every section of the society. Rise of this disease compel poultry farmers to do culling of the birds, which give them huge economic loss and also decreases the poultry meat and egg production.

PREVENTION AND CONTROL

Bio- Security

Strict bio-security guidelines must be followed. Prohibit the entry of outsiders into the farm. Foot bath ($KMnO4$) must be made at the entry gate.

Vaccination

Vaccination of poultry birds gives immunity to the birds. Vaccination schedule must be followed. It is very important for the birds. Vaccine of Marex, Ranikhet, IBD, etc must be done.

Early detection

Regular monitoring of the birds is very essential. Any bizarre activities of the birds must not be ignored. The farmer must make a round in the poultry farm twice a day. Any symptoms if noticed then that bird must be removed from the flocks for further monitoring. Keep the bird in isolation. If the symptoms are related to bird flu, then immediately contact to the experts.

Expert advices and the awareness

The poultry farmers must take the advice of the KVK (Krishi Vigyan Kendra) experts regarding the health of the birds and the farm conditions. The farmers must be aware with the new schemes of the vaccination, etc.

GLOBAL CONCERNS

Bird flu strains mutate and spread rapidly. So, it's a global concern. Thousands of birds are culled when disease outbreak and this cause a huge financial loss.

CONCLUSION

This article is helpful in understanding the causes, symptoms and preventive measures of the avian influenza. Bird flu is crucial for both poultry sector and public health. Early detection of the infection can save the flock and minimize the spread of the disease. Regular monitoring, vaccination and suggestions from the progressive farmer, KVK experts, Veterinarian can help in keeping the poultry farmer safe from the Avian influenza.

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Chapter
12

BIO TECHNOLOGY APPLICATIONS IN VARIOUS FIELDS

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ABSTRACT

Biotechnology is the use of biology to grow new products, methods, and organisms intended to improve human health and society. Biotechnology also called biotech, has introduced the commencement of civilization with the domestication of vegetation, creatures, and the detection of fermentation (Chukwuma *et al.,* 2012). Biotechnology started six thousand years before with the agricultural rebellion. This early era was categorised by exploiting existing organisms in their usual forms. Humans were cultured to harness the biological method of fermentation to introduce bread and alcohol. The manufacture is modifying the genetic change of domesticated plants and animals through respective breeding.

KEYWORDS: Biotechnology, Vegetation, Fermentation.

INTRODUCTION

BIOTECHNOLOGY

Biotechnology is the use of biology to grow new products, methods, and organisms intended to improve human health and society. Biotechnology also called biotech, has introduced the commencement of civilization with the domestication of vegetation, creatures, and the detection of fermentation (Chukwuma *et al.,* 2012).

Development of biotechnology from olden days

Biotechnology started six thousand years before with the agricultural rebellion. This early era was categorised by exploiting existing organisms in their usual forms. Humans were cultured to harness the biological method of fermentation to introduce bread and alcohol. The manufacture is modifying the genetic change of domesticated plants and animals through respective breeding.

Discriminating breeding works by breeding paternities with the necessary characteristics to express or abolish certain genetic features in their own offspring. Past period, selective species are farmed evolve to be dissimilar from their wild ancestors. In the cultivated revolution, wheat was farmed to stay on its branch when harvested instead of dropping to the place as wild wheat. Dogs were produced with greater docile than their wolf ancestors.

In biotech methods, the changes may take long duration for corresponding breeding of species. The biotechnology process were inadequate to these leisurely, agricultural approaches until the nineteen century, once biologist Gregor Mendel exposed the elementary philosophies of heredity and genetics. Similarly, during that period, inventers Joseph Lister and Louis Pasteur exposed the microbial procedures of fermentation. This invention laid the basics for biotechnology companies, where scientists interrelate more directly with the molecular and genetic methods of organisms.

In the year 1973, genetic engineering made a major development in the world. This method is the basis of modern biotechnology's performance and recent advances. It empowered the first direct guidance of plant and animal genomes, which is the whole set of genetic factors existing in a cell (www. coursehero.com).

DISCOVERIES AND ADVANCEMENTS OF BIOTECHNOLOGY

Biotechnology has made more discoveries in the last 100 years, as shown in Table 1.

Table 1: Biotechnology discoveries and advancements

Sl. No.	Year	Discoveries and Advancements
1	1919	Hungarian inventor Karl Ereky changes the term biotechnology.
2	1928	Alexander Fleming invented penicillin, the primary true antibiotic.
3	1943	Oswald Avery proved that DNA carries genetic details.
4	1953	Francis Crick and James Watson determined the double - helix structure of DNA.
5	1960	Insulin is synthesised to resist diabetes, and produce vaccines for measles, and rubella are established.
6	1969	The primary synthesis of an enzyme external the body is conducted.
7	1973	Stanley Cohen and Herbert Boyer developed genomic engineering with the first supplement of DNA a primary bacterium into another.
8	1980	The initial biotechnology drugs to recover cancer were developed.
9	1982	A biotechnology - established form of insulin becomes the initial genetic engineer invention agreed upon by the Unites States Food and Drug Administration.
10	1983	The first genetically altered plant was introduced.
11	1993	Genetically modified creatures were implemented into agriculture with the Food and Drug Administration's endorsement of growing hormones that produce more amount of milk in cows.
12	1997	The first mammal was cloned.
13	1998	The initial draft of the Human Genome Project was formed, giving researchers access to over thirty thousand human genes and enabling research on the treatment of illnesses like cancer and alzheimer's.
14	2010	The primary synthetic cell was created.
15	2013	The initial bionic eye was created.
16	2020	A MRNA vaccine and monoclonal antibody technique were utilized for the treatment of the SARS-CoV-2 virus.

MODERN PRACTICE OF BIOTECHNOLOGY

The primary applications of biotech are directed towards the development of products like bread and vaccines. The discipline has changed expressively over the past century in ways that operate on the genomic structures and biomolecular procedures of living organisms.

The recent days of biotechnology comes from numerous castigations of science and technology, as indicated in Figure 1.

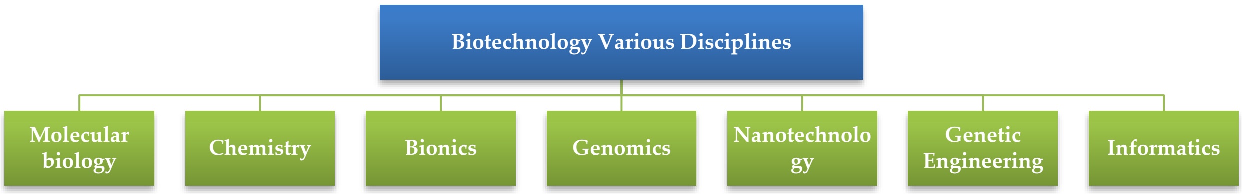

Figure 1: Biotechnology in numerous castigations of science and technology

This modern approach has resulted in inventions and breakthroughs in the subsequent areas:

- ❖ Medicines and therapeutics that avoid and treat the diseases; medical diagnostics like pregnancy tests; and biofuels are maintainable, decreasing waste and pollution.
- ❖ Genetically modified organisms under go procedures that lead to more effective and profitable cultivation.
- ❖ Modern applications of biotechnology are most often done through genomic engineering, which is similarly called recombinant DNA technology.
- ❖ Animals and plants contain cells that produce proteins, which determine the features of the creature. Biotechnology field of genetic engineering modifies the genetic cell struture, hence, it can change the character of organism.
- ❖ The scientist can able to change the character of organisum and create the new organisum, which will create better crops and increase resistance to drought.

VARIOUS TYPES OF BIOTECHNOLOGY

The science of biotechnology is fragmented into sub disciplines that are colour-coded based on common usages. The types of biotechnology are shown in Figure 2.

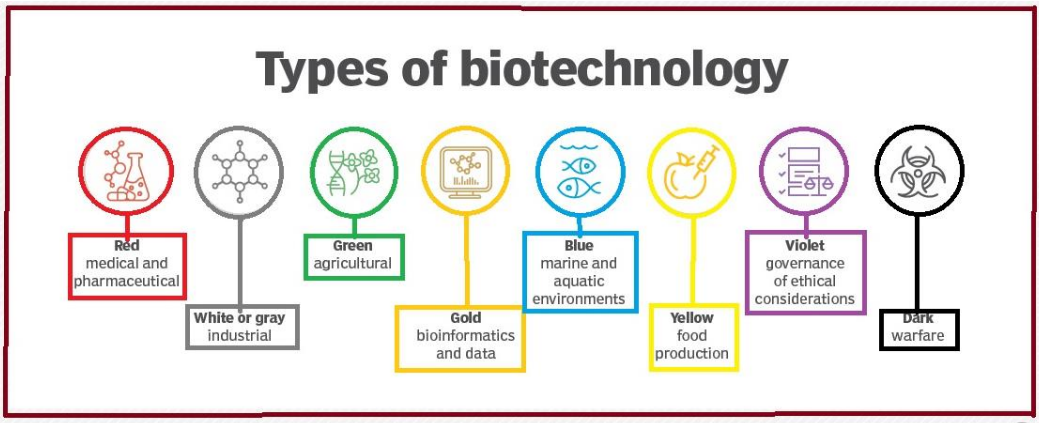

Figure 2: Types of Biotechnology

1. Red biotechnology contains medical methods, like consuming organisms to yield innovative drugs and stem cells to redevelop injured human tissues and breed, regrow complete organs.
2. White or grey colour refers to industrial progressions, the development of innovative compounds, or new biofuels for automobiles.
3. The green colour indicates agricultural procedures, such as manufacturing pest-protective crops, disease-protective animals, and ecologically friendly agricultural practices.
4. Gold, also recognised as bioinformatics, is a combination between biological procedures and informatics. It indicates the approaches healthcare employers utilised to collect, store, and investigate biological data for delicacy patients.

5. Blue incorporates processes in marine life and aquatic atmospheres, like altering aquatic biomass into gases and pharmaceuticals.
6. Yellow denotes processes that aid food manufacture; the most popular use is the fermentation of liquor and a variety of cheese.
7. The Violet colour indicates the preparation of biotechnology is in compliance with the laws and moral standards leading every field.
8. The dark colour represents the use of biotechnology for warfare.

BIOTECHNOLOGY USES AND APPLICATIONS

The usage and commercialization of recent biotechnology frequently fall into four different fields, which are shown in Figure 3.

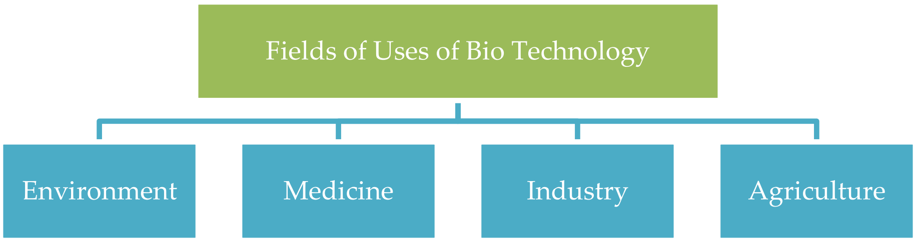

Figure 3: Application of Bio Technology in Various Fields

ENVIRONMENT

The objective of ecological biotechnology is to progress sustainable environmental practices that decrease pollution and waste. Examples of environmental biotechnology:

- Phytoremediation is used in the field of genetically engineered microorganisms to clean soils of different heavy metals and other types of pollutants.
- The bioremediation process introduces microorganisms into waste locations in order to break down non recyclable waste.
- Plastic-eating bacteria were used to break down waste plastic in soil and water.
- GMO type of foods live fresher, long period, and decrease food waste.
- Genetic renovation attempts to return an endangered species, the American chestnut tree.
- Cover-crops, and corn are utilised as biofuels, substituting traditional fuel sources that generate greenhouse smoke releases when extracted and utilised.

MEDICINE

Biotechnology in the medical field is also known as biopharma, which fights and avoids disease and expands health benefits. In the field of Biotechnology and biomedical research work are the foundation of the recent pharmaceutical industry. Uses of bio technology in the medicine field include the following:

- Stem cell research work helps replace or repair defective cells; antibiotics progress; gene therapies for sicknesses such as leukaemia; research work into hazardous pathogens and the antibodies that fight or react to them.
- 3D printing procedures or budding of body part and bones in laboratory; and mRNA injections, monoclonal antibody actions, and research for COVID-19.

INDUSTRY

Industrial biotechnology includes using microorganisms to generate industrial goods. Bio technology industry field examples include the following:

- Fermentation and the utilisation of enzymes and microbes to modernise chemical manufacturing and decrease operative charges and chemical discharges.

- Biofuels are used in renewable harvests like corn to harvest combustible fuel instead of ordinary, nonrenewable fossil fuel resources, petroleum and oil.
- Biodegradable products includes garments and textiles manufactured from the proteins of existing organisms, like silk proteins of spiders.

AGRICULTURE

Agricultural field of biotechnology causes plants and animals to harvest more efficiently. It increases the nutritional value and decreases food insecurity. Agricultural biotechnology has the following examples:

- Biologically formed pesticides and herbicides creates minimum harmful to humans than organic ones; drought-resistant harvests; and minimally space-resilient crops.
- The meat product is grown in labs or by using 3D printers.
- Gluten-free grains responsive to sufferers of celiac disease; important breeding that genetrates healthier, superior livestock and crops.
- Nutrient supplementation that imbues nourishment with additional nutrients to progress diets and medical treatments.

ENVIRONMENTAL BIO TECHNOLOGY APPLIED TO PHOSPHATE REMOVAL METHODS

Environmental biotechnology refers to the usage of microorganisms to develop ecological value, and it has to pay attention to the growth of technology to clean up the aquatic environment (Leila Fahmideh, *et al.,* 2014). The total phosphate removal methods are

Phosphorus recovery by algae and macrophytes

Algae are universally used for treatment in variation and greater rate aerobic ponds because of their ability to assist in the cost-effective treatment of organic carbon and pathogenic pollutants. In phosphorus removal, it shows a quite low result. Aquatic plant species, and floating macrophytes such as water hyacinth (Eichhornia crassipes) and duckweed (Lemnaceae minor) cultivate on the surface of ponds. These are placed floating in the water column; all nutrient necessities for the plants must be taken from the surface water (Andrew N *et al.,* 2012).

Chemical Dosing to Promote Phosphate Precipitation

The chemical Phosphate removal involves dosing metal salts into pond water. The mechanism is to add the salt for, example trivalent metal salts, and ferric chloride, it precipitates phosphate in the water, and the solid residuals are removed by settling by gravity or filtration (Joshua T *et al.,* 2018).

Phyto remediation

Plants can play an important role in purifying polluted pond water. Total phosphate removal involves the pathway of plant origins and microorganisms, some other ways via microbial activity, and fewer amounts engrossed directly by plants (Feng Su *et al.,* 2019).

Activated sludge process

The biological phosphate subtraction in the activated sludge process is an ancient method. The first effort was to recognize microorganisms involved in phosphate removal, and, Acinetobacter was the first bacterium proposed to be responsible for phosphate removal in polluted water (Akpor O. B. and Muchie M, 2010).

ADVANTAGES OF BIOTECHNOLOGY

Biotechnology creation has the following advantages:

- Pollution and waste were reduced to reverse catastrophic climate variation and ecological damage.
- To create well, tougher, and greater-sustainable food crops that enhance nourishment and combat food uncertainty.

- The diseases were treated for children before they were born by changing their genomes; medicine was designed to enhance the health and permanence of people, animals, and plants.
- Cutting costs of farm provisions pesticides while growing crop yields and profits.

DISADVANTAGES OF BIOTECHNOLOGY

Biotechnology has disadvantages including the following:

- Biological warfare has the potential to cause the growth of pathogens and epidemics that might be utilised in a struggling region to contaminate populations.
- A decrease in biodiversity causes the farming of a minor quantity of hereditarily engineered crops to shrink the normal genetic field of species and making them in a less quantity resilient and adjustable to unexpected changes in the environment.
- Loss of soil fertility needs Bio-enhanced plants necessitate more nutrients from the soil and harvest more crops. This can deplete the soil of fruitful nutrients, devastate woodland, and necessitate the use of environmentally injurious fertilisers to create a healthy deficit.
- Biotechnology products are often more expensive than old goods and have the potential to increase pricing structures in numerous industries.

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Chapter
13

CURLED TOE PARALYSIS IN CHICKEN

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ABSTRACT

Curled toe disease in chicken is a health issue in which chicken toes become weak and curled. The chicken becomes unable to walk and stay at one place. This condition is because of mineral deficiencies and also because of genetic factors. The body weight of the chicken falls down within 2-3 days as chicken is unable to consume the feed and drink water. Regular monitoring of the bird is essential for providing the feed and water which is not easy and so chicken dies after few days.

KEYWORDS: Curled Toe, Mineral Deficiencies, Body Weight, Monitoring.

INTRODUCTION

Curled toe paralysis is a difficult situation in chicken in which due to mineral deficiencies in the feed, their toes become weak and curled. The chicken becomes immobile and stays at one place for longer period of time. The body weight of chicken falls down rapidly and this becomes a traumatic situation for the poultry farmers. In worst condition, the chicken dies within a week but if this situation is with 2-3 weeks old chicks, then they die within 4-5 days. Minerals and vitamins supplements are very essential for the chickens. In the deficiencies of these, chicken faces this situation.

There are following reasons which have been mentioned behind this-

GENETIC FACTORS

Some breeds are easily affected by curled toe paralysis by genetic factors which is not easy to improve.

Figure 1: Chicken with curledtoes (Source: https://htrchickens.com/2020/08/06/odd-health-conditions-chicken-with-curled-toes/)

Figure 2: Vitamin B1 deficiency in chickens (Source: https://bitchinchickens.com/2021/01/21/thiamine-vitamin-b1-deficiency-in-chickens/)

MINERALS AND VITAMINS DEFICIENCIES

Inadequate amount of nutrition in the feed during incubation and growing stage can become a reason for curled toe paralysis. Chicken needs vitamin B- complex, Methionine, Lysine, Vitamin E, Calcium, biotin, etc in adequate amount (Riboflavin)Vitamin B2, B6 and B12 are very essential for the chicken.

INCUBATION PERIOD

During incubation time, the temperature must be set appropriately. During incubation period, the room temperature should be 90-to-97-degree Fahrenheit. Inappropriate temperature and humidity level can become a cause of curled toe paralysis. So, make ensure that according to the age and weather conditions, temperature should be set.

Treatments

- Firstly isolate the birds from the flock. If you are keeping the birds in the cage then put the paralyzed birds on the floor to make it more comfortable.
- Put the drinker, feeder nearer to the chicken so that it can easily reach and consume.
- Vitamin supplements like Ambiplex must be given orally to the chicken through dropper 5 ml/dose twice a day in concentrated form. Ambiplex contains b- complex vitamins which is very essential against the curled toe paralysis.
- Ensure the feed quality. If the feed is manual then the farmer must mix all the feed ingredients in right amount and mineral mixture of recognized brand must be added and if the feed is market based then it should be of recognized brand only.
- Ambiplex must be given orally unless the concerned bird feel healthy.
- Gentle massage can be done on the shank of the chicken.
- Take veterinarian, expert advices immediately to tackle this condition.
- The regular treatment will take almost 7-10 days to recover completely.

Figure 3: Treatments

CONCLUSION

Curled toe paralysis is a problem related to mineral and vitamin deficiencies. If the feed is not containing riboflavin and other essential b-complex then the birds will face curled toe paralysis. This disease is more dangerous if it is happening in starting of 2-3 weeks as this cause mortality and all the birds will be underweighted. Proper care and management can save the birds and recovering time is almost 7-10 days.

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Chapter 14

PHARMACOLOGICAL POTENTIAL OF CNIDOSCOLUS SPECIES-A COMPREHENSIVE OVERVIEW

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ABSTRACT

Throughout ancient times, people have utilized plants for food preservation and flavouring, medical treatment, and disease prevention, including epidemic control. Over the ages, both within and between human communities, the knowledge of their therapeutic qualities has been passed down. Some plant species have biological traits that are used globally for a variety of purposes, including the treatment of infectious diseases. These traits are typically attributed to active compounds created during secondary vegetal metabolism. One new plant source with potential medical uses is Cnidoscolus spp. A few among these specimens have therapeutic uses as phytotherapies, including analgesic, anti-inflammatory, antibiotic, diuretic, and anticancer effects. Among other substances, terpenoids, alkaloids, coumarins, flavonoids, and phenolic compounds may be related to these effects. This work aimed to assess the literature on the phytochemical composition and biological activity of the two most frequently cited species, *Cnidoscolus aconitifolius* and *Cnidoscolus chayamansa*, which possess advantageous pharmacological properties such as antioxidant, anti-inflammatory, and possibly cytotoxic activity.

KEYWODS: *Cnidoscolus aconitifolius*, *Cnidoscolus chayamansa*, Biological Activity, Phytochemicals.

INTRODUCTION

The plant kingdom with its diverse chemical structures and capacity to ward off disease, is the home to a vast reservoir of biologically active compounds. These phytochemicals, which tend to be secondary metabolites and are found in higher plants in smaller amounts, include tannins, alkaloids, terpenoids, flavonoids, and many more. Investigating the phytochemistry of higher plants that are associated with ethnobotanical knowledge has been the main focus of current research. The separated phytochemicals are subsequently examined for various forms of biological activity [1].

ABOUT THE GENUS CNIDOSCOLUS

The term "chaya" refers to any group of plants in the genus Cnidoscolus, which belongs to the Euphorbiaceae family and has between 50 and 75 species. [2]. They can be found throughout tropical and subtropical regions, mostly in areas with deciduous forests and xerophytic and shrub fields. The nutritional and/or medicinal potential of certain Cnidoscolus species is worth considering [3]. Cnidoscolus plants are thought to provide food for the animals in the Caatinga biome, especially during drought conditions [7]. With a wide range of therapeutic uses, these plants are still employed as herbal remedies. Some of these uses include treating acute appendicitis and acute rheumatism, as well as acting as an antitumor, genitourinary system, antiseptic, hematomas, fractures, wounds, and warts. Because of their nutritional potential, some species of Cnidoscolus are worth considering. Two of the most popular species are *Cnidoscolus aconitifolius* and *Cnidoscolus chayamansa*, which are used for both traditional medicinal and ornamental plant purposes and consumption (5).

Cnidoscolus species- *C. aconitifolius* and *C. chayamansa*

MORPHOLOGY OF PLANT

The *Cnidoscolus aconitifolius* is an evergreen shrub that can grow up to 6 meters tall and resist drought. It has milky sap, lobate or alternate leaves, and small white flowers on dichotomously branched cymules. It can reach a height of 5 to 6 meters as shrubs [6]. On the other hand, *C. chayamansa*is a semi-woody, semi-perennial succulent shrub with a maximum height of two meters. It has leaves that are similar to those of a sagittate base, with glands present, smooth lobed edges, short petiole length, and white flowers [7]. These two species are perennial shrubs with rapid growth that originated in the Yucatán region of southern Mexico.

Nutritional value of *C. aconitifolius* and *C. chayamansa*

Plants nutritional value is correlated with their use as food ingredients, whereas their secondary metabolite content is associated with their use in traditional medicine [8]. Compared to spinach (Amaranthus sp.), cabbage (Brassica sp.), and lettuce (Lactuca sp.) [9], *Cnidoscolus aconitifolius* has two to three times the amount of nutrients. Fresh leaves have the following composition: 4.38% protein, 1.20 percent fat, 2.39% fibre, and 85.36% carbohydrates. [10] or nutrients (mg/100 g), specifically carbohydrates (41.895), protein (7.68), crude fat (1.145), and crude fiber (31.165) [9]. Furthermore, *C. aconitifolius* has important vitamin content, including thiamine, carotene, niacin, and riboflavin, as well as essential amino acids [9]. Iron, zinc, copper, magnesium, sodium, and potassium are among the minerals present [9, 11]. Vitamin A, vitamin B2, vitamin B1, vitamin B9, vitamin C, vitamin D, vitamin E, and vitamin K are abundant in *C. aconitifolius* leaves [9,12], as are vitamin B3, vitamin B6, and vitamin B12.

The leaves of *C. chayamansa* are rich in amino acids, minerals such as sulphur, manganese, zinc, calcium, sodium, iron, phosphorus and copper, as well as vitamins, terpenoids including thiamine, niacin, riboflavin, retinol, ascorbic acid, and β-carotene. The species is regarded as having high nutritional value because of the presence of these organic compounds, minerals, and amino acids [13], [14], and [15].

Table 1: Chemical composition of fresh leaves of *C. aconitifolius* and *C. chayamansa*

Chemical composition (mg/100g)	*C. aconitifolius*	*C. chayamansa*
Carbohydrate	41.895	49.91
Fat	1.145	5.63
Fibre	31.165	17.14
Protein	7.68	34.02
Sodium	41.47	6.57
Potassium	33.88	67.2
Iron	14.83	1.39
Calcium	29.16	15.53
Phosphorus	24.68	92.77

PHYTOCHEMICAL CONSTITUENTS

Plant species medicinal properties are attributed to their phytochemical components. In line with the findings of Peixoto *et al.* [16] and Price *et al.* [17], the qualitative phytochemical screening and nutritional evaluation of the *Cnidoscolus aconitifolius* leaf revealed the presence of phenols, flavonoids, alkaloids, terpenoids, and saponins. The plant has the potential to treat cancer because of its phenolic content. Because terpenes are effective against bacteria and act as an anti-diarrheal agent, the presence of terpenoids further validates and supports the use of *Cnidoscolus aconitifolius* in the treatment of bacterial infections [18]. A significant amount of saponins were present in the leaves of *Cnidoscolus aconitifolius*. This is consistent with findings published by Awoyinka *et al.* [19] Saponins have antimicrobial, anti-inflammatory, and protective effects against hyperglycemia, hypercholesterolemia, and hypertension [18,17]. A study published by Araújo *et al.* [20] found a trace amount of tannin content. Its existence is correlated with the healing and anti-inflammatory properties.

According to Mariana Perez *et al.* two major terpenoids with strong antimycobacterial and antiprotozoal properties were identified from *C. chayamansa* leaf extract: moretenol and moretenyl acetate. Furthermore, two polyphenols, 5-hydroxy-7-3′,4′-trimethoxyflavanone and kaempferol-3,7-dimethyl ether, were discovered to have significant antiprotozoal properties [21]. Although toxic metabolites have been identified, such as cyanogenic glycosides (linamarin), they are readily eliminated through cooking [22].Terpenoids have potent anti-inflammatory properties. Because of their high phenolic content, leaves have a greater capacity for antioxidants. Additional compounds that were found to be significant included polyphenols (such as chlorogenic acid), phenolic acids (such as protocatechuic acid), flavonoids (such as quercetin, kaempferol, amentoflavone, astragalin, naringenin, rutin, catechin, and dihydromyricetin), coumarins, phenolic polymers (such as tannins and lignin), and saponins [23,24,25].

PHARMACOLOGICAL ACTIVITY OF CNIDOSCOLUS SPECIES

Various biological activities were observed in extracts, fractions, or compounds (primary and secondary metabolites) derived from Cnidoscolus species investigated. Cnidoscolus is a genus with analgesic, antibiotic, diuretic, anti-inflammatory, anticancer [26] and anti-free radical properties [27]. *C. aconitifolius* possesses hematopoietic [28], antitumor, antidiabetic, and antimicrobial properties andcan therefore be used as an antimicrobial agent to treat infections [29].

ANTIOXIDANT ACTIVITY

Gallic acid, vanillic acid, vanillin, chlorogenic acid, caffeic acid, ferulic acid, rosmarinic acid, p-coumaric acid, luteolin, apigenin, and resveratrol are among the phenolic compounds found in the hydroalcoholic extract of *C. aconitifolius* leaves. Compared to raw leaves, boiled *C. aconitifolius* leaves have more antioxidants. Boiled leaves provide an antioxidant source because of their increased polyphenol content [32]. The amount of phenolic compounds present has an impact on the antioxidant's bioactivity [31, 32].

The primary phenolic compounds found in methanolic extract of *C. chayamansa* were hydroxybenzoic acid, caffeic acid, and sinapic acid [30]. These compounds are members of a phenolic family that exhibits remarkable antioxidant potential, including the capacity to scavenge highly reactive hydroxyl radicals and other free radicals, including hydroperoxyl radicals, nitric oxide radicals, and peroxynitrite, by inhibiting lipid peroxidation. This study found that cooked leaves have a significant decrease in the individual phenolic and flavonoid compounds from the raw leaves.

ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory properties of *C. aconitifolius* extract, both ethanolic and aqueous, were found to reduce TNF-α and IL-6 by 46 and 48.38%, respectively, in macrophages stimulated by lipopolysaccharide [33]. The ethanolic extract and the ethyl acetate [34]of *C. aconitifolius* contain quercetin flavonoids, which target prostaglandins involved in the late phase of acute inflammation [35].

Applying *C. chayamansa* to the topical and systemic acute inflammation model exhibits good anti-inflammatory activity; on the chronic inflammation model, however, it has a significant positive impact. β-amyrin acetate and lupeol acetate are primarily responsible for the anti-inflammatory action [36–41]. Lupeol acetate has a significant in vivo anti-inflammatory effect through regulating TNF-α and IL-2, which involves the opioid system.

ANTI-HYPERCHOLESTEROLEMIA ACTIVITY

The leaf extract of *C. aconitifolius* exhibits potential antihypercholesterolemic properties [42]. The administration of 200-800 mg/kg BW aqueous and ethanol extract of *C. aconitifolius* leaves to rats resulted in a dose-dependent reduction in total cholesterol (TC), low density lipoprotein (LDL), and triglyceride (TG), as well as an increase in high density lipoprotein (HDL), which is beneficial for the treatment of peripheral vascular disease [43].

Consuming Chaya may have hypolipidemic effects because of its impact on polyphenols. Polyphenols may have this lipid-lowering effect through activating AMP-activated protein kinase (AMPK) and deactivating acetyl-CoA carboxylase, which in turn favors decreased fatty acid synthesis and increased fatty acid oxidation [41]. Studies conducted in vitro and in vivo have demonstrated that polyphenols, like quercetin and kaempferol, lower plasma TG through controlling the synthesis of triglycerides and the mobilization of fatty acids [43–45]. Moreover, activation of AMPK's energetic sensors results in decreased lipogenesis, increased energy expenditure, decreased fat mass, and decreased oxidative stress [46–48].

ANTI-DIABETIC ACTIVITY

The oral administration of *C. aconitifolius* leaf aqueous extract resulted in a reduction of blood sugar levels in diabetic rats [49]. Because of its insulinogenic qualities, *C. aconitifolius* might promote the secretion of insulin from dormant cells. A group of mice given *C. aconitifolius* treatment had their islets of Langerhans preserved [50]. The ethyl acetate fraction of *C. aconitifolius* leaves inhibited the activity of phosphodiesterase-5, angiotensin-I-converting enzyme, tyrosinase, arginase, acetylcholinesterase, butyrylcholinesterase, monoamine oxidase, tyrosinase, and tyrosinase. It also increased the activity of Na^+/K^+ -ATPase [51].

Research conducted on diabetic mice revealed that administering 2% w/v *C. chayamansa* extract for four weeks resulted in hypoglycaemic effects by reducing absorption of glucose [52].

HEPATOPROTECTIVE ACTIVITY

In experimental animals, the leaf extract of *C. aconitifolius* showed protection against hepatotoxicity and chemotoxicity caused by carbon tetrachloride (CCl4) [53]. In addition, the CCl4 significantly raised serum transaminases (ALT, AST, and phosphatase; ALP), which in turn raised serum blood urea nitrogen (BUN) and creatinine in comparison to normal mice. Pre-exposure to the leaf extract of *C. aconitifolius* significantly lessened the impact of CCl_4 on blood parameters and improved the levels of ALT, AST, and ALP, the liver damage enzymes [53].

Mice with liver damage induced by an antitubercular drug (RIF/INH/PZA) were used to assess the hepatoprotective activity at 39 days. Compared to a positive control (Silymarin), the extract at 200 and 400 mg/kg caused animals with liver damage to gain more body weight; additionally, mice treated with the extract at 200 mg/kg and Silymarin showed a slight steatosis; and finally, the serum levels of creatinine and urea were lower than those of untreated mice with hepatotoxicity and those of animals treated with Sil [54].

ANTI-MICROBIAL ACTIVITY

A variety of bacteria were found to be inhibited in growth by the *C. aconitifolius* extract, including Escherichia coli, Psuedomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, P. fluorescence, Klebsiella, Salmonella enterica, Gallinarum, Candida albicans, Bacillus subtilis, Salmonella typhi and Streptococcus pyogenes. Factors such as microbe type, contact time, and concentration all affected the *C. aconitifolius* extract's inhibition zone.

Aspergillus niger and *Aspergillus* tamari, which were used as test fungus [55], showed that *Cnidoscolus aconitifolius* potential antifungal properties had inhibited their mycelial growth. Both moretenol and moretenyl acetate exhibited strong antimycobacterial and antiprotozoal properties in *C. chayamansa*; additionally, kaempferol-3,7-dimethyl ether and 5-hydroxy-7-3′,4′-trimethoxyflavanone were found to have a significant antiprotozoal effect.

ANTI-CANCER ACTIVITY

Cancer is treated with *C. aconitifolius* in ethno medicine. Breast cancer (MCF-7) and lung cancer (NCI-H460) can be treated with methanol extract of the leaves, stems, and bark of CA roots. In vitro tests using leaf extract at a concentration of 100 g/mL demonstrated impressive growth inhibition against lung and breast cancer. Cardiac glycosides, phenolic compounds, terpenes, and saponins are all present in the *C. aconitifolius* extract [39].

CONCLUSION

The plant Cnidoscolus spp. has significant medicinal qualities in addition to being a highly nutritious one. Promising sources of bioactive molecules from various leaf extracts of Cnidoscolus species, such as terpenoids, alkaloids, tannins, flavonoids, and phenolic compounds exhibits biological effects like antimicrobial, antidiabetic, hepatoprotective, antioxidant, antihypercholesterolemia, and anti-inflammatory properties. The macronutrients like proteins, fats, carbohydrates, vitamins, and minerals found in Cnidoscolus spp. leaves give them the potential to be developed into an inexpensive and readily accessible food source. The goal to be accomplished in the upcoming years should be to carry out clinical trials for identified bioactive compounds and to recover potential biological activities in a safe, economical, and highly desired manner.

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Chapter 15

ECO-FRIENDLY FARMING AND SUSTAINABILITY

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ABSTRACT

As the global population continues to rise, the demand for food production puts increasing pressure on agricultural systems. Traditional farming practices often contribute to environmental degradation, soil erosion, water pollution, and the loss of biodiversity. In response to these challenges, there is a growing emphasis on transitioning towards eco-friendly farming methods that prioritize sustainability, conservation, and environmental stewardship. This abstract explores the principles and practices of eco-friendly farming, which encompasses a range of techniques designed to minimize the ecological impact of agriculture while promoting long-term resilience and productivity. These methods include organic farming, agro-ecology, precision farming, integrated pest management, and the use of renewable energy sources. Organic farming emphasizes the elimination of synthetic pesticides and fertilizers, relying instead on natural alternatives, crop rotation, and cover cropping to maintain soil health and fertility. Agro-ecology integrates ecological principles into agricultural systems, fostering biodiversity, enhancing ecosystem services, and promoting efficient resource use. Precision farming utilizes technology such as sensors, drones, and data analytics to optimize resource allocation, reduce waste, and enhance overall productivity. Integrated pest management seeks to control pests through a combination of biological, cultural, and mechanical approaches, reducing the reliance on chemical pesticides. Additionally, the adoption of renewable energy sources, such as solar and wind power, contributes to the overall sustainability of farming operations. This abstract also highlights the economic, social, and environmental benefits associated with eco-friendly farming practices. These benefits include improved soil health, water conservation, reduced greenhouse gas emissions, enhanced biodiversity, and healthier ecosystems. Furthermore, the adoption of such practices can lead to increased resilience to climate change, improved livelihoods for farmers, and the production of healthier and more nutritious food for consumers. The transition to eco-friendly farming represents a crucial step towards achieving a sustainable and resilient agricultural system. As the world grapples with the challenges of feeding a growing population while addressing environmental concerns, embracing eco-friendly farming practices offers a pathway to a greener and more sustainable future.

KEYWORDS: Environmental Degradation, Biodiversity, Sustainability, Agroecology, Renewable Energy

INTRODUCTION

Eco-friendly farming, also known as sustainable agriculture or organic farming, is an approach to agriculture that aims to cultivate crops and raise livestock in an environmentally responsible and resource-efficient manner. This method of farming seeks to minimize the negative impact on the environment, promote biodiversity, and prioritize the well-being of both the ecosystem and the community.

PRINCIPLES OF ECO-FRIENDLY FARMING

Organic Practices:

Eco-friendly farming often involves the use of organic practices, which avoid synthetic pesticides, herbicides, and fertilizers. Instead, farmers rely on natural alternatives and traditional farming methods to maintain soil fertility and crop health.

Soil Conservation:

Sustainable farming emphasizes soil conservation to prevent erosion, improve soil structure, and promote long-term fertility. Techniques such as cover cropping, crop rotation, and minimal tillage are employed to enhance soil health and reduce the need for chemical inputs.

Water Conservation:

Eco-friendly farming aims to use water efficiently by implementing irrigation systems that minimize waste and by choosing crops that are well-suited to the local climate. Water-saving technologies and practices help reduce the environmental impact of agricultural water use.

Biodiversity Preservation:

Maintaining biodiversity is a key aspect of eco-friendly farming. This involves cultivating a variety of crops, preserving natural habitats on the farm, and avoiding monoculture, which can contribute to the spread of pests and diseases.

Reduced Chemical Inputs:

Sustainable farming seeks to minimize the use of synthetic chemicals, such as pesticides and fertilizers, to reduce the environmental impact and potential harm to ecosystems. Integrated Pest Management (IPM) strategies are often employed to control pests with minimal reliance on chemical interventions.

Figure 1: Eco Friendly farming by using oragnic manure (Source- Google)

Energy Efficiency:

Eco-friendly farms strive to be energy-efficient by adopting renewable energy sources, optimizing machinery usage, and employing energy-conserving technologies. This helps reduce the carbon footprint associated with agricultural practices.

Animal Welfare:

For farms that raise livestock, an eco-friendly approach includes providing animals with humane living conditions, access to natural habitats, and avoiding the use of growth hormones and antibiotics whenever possible.

Community Engagement:

Sustainable farming practices often involve engaging with local communities. This can include selling products locally, supporting farmers' markets, and collaborating with nearby businesses to create a more resilient and interconnected food system.

Eco-friendly farming represents a holistic and environmentally conscious approach to agriculture, addressing the interconnected challenges of food production, environmental sustainability, and community well-being. As concerns about climate change and environmental degradation grow, the adoption of eco-friendly farming practices becomes increasingly important for the long-term health of our planet.

Natural farming, also known as Eco- farming or do-nothing farming, is an agricultural philosophy and method that emphasizes working in harmony with nature to achieve sustainable and regenerative farming practices. Developed by Japanese farmer and philosopher Masanobu Fukuoka, natural farming diverges from conventional agricultural approaches by promoting minimal intervention, reducing external inputs, and allowing natural processes to govern the farming ecosystem. Here are key principles and practices associated with natural farming:

No-till Agriculture:

Natural farming discourages traditional plowing and tilling of the soil. Fukuoka argued that disturbing the soil structure through tillage disrupts natural ecosystems, leading to erosion and loss of soil fertility. Instead, farmers practicing natural farming advocate for minimal disturbance and encourage the growth of cover crops to protect the soil.

Seed Balls or Seed Bombs:

Fukuoka introduced the concept of seed balls or seed bombs, which are small balls of clay containing seeds. These seed balls are scattered on the fields, allowing plants to grow naturally without the need for planting rows or using heavy machinery. This method supports the spontaneous growth of a diverse range of plants.

Mulching:

Mulching is a crucial component of natural farming. Farmers cover the soil with a layer of organic materials like straw, leaves, or cover crops. This helps retain soil moisture, suppress weeds, and enhance soil fertility. The mulch also creates a favorable environment for beneficial microorganisms.

Crop Diversity:

Natural farming encourages the cultivation of a variety of crops rather than relying on monoculture. Diverse crops help create a more resilient and balanced ecosystem, reducing the risk of pests and diseases while promoting biodiversity.

No Chemical Fertilizers or Pesticides:

Natural farming avoids the use of synthetic fertilizers, pesticides, and herbicides. Instead, it relies on organic matter, cover crops, and the natural processes of decomposition to enrich the soil and control pests.

Observation and Adaptation:

Natural farmers emphasize careful observation of natural processes. This includes understanding the cycles of plants, weather patterns, and the behavior of insects. By observing and adapting to the natural environment, farmers can work in harmony with nature rather than against it.

Livestock Integration:

Some natural farming systems integrate livestock into the agricultural ecosystem. Animals contribute to nutrient cycling, help control pests, and enhance soil fertility through manure. The integration of animals is often done in a way that mimics natural grazing patterns.

Philosophical Approach:

Natural farming extends beyond specific techniques; it encompasses a philosophical approach to farming that emphasizes working with the inherent wisdom of natural systems. Fukuoka's "do-nothing" farming philosophy encourages a more hands-off approach, allowing nature to take its course.

Natural farming is regarded as a holistic and sustainable approach that promotes harmony between agriculture and the natural environment. While its principles have gained attention and followers worldwide, the degree of adoption may vary based on local conditions and agricultural traditions.

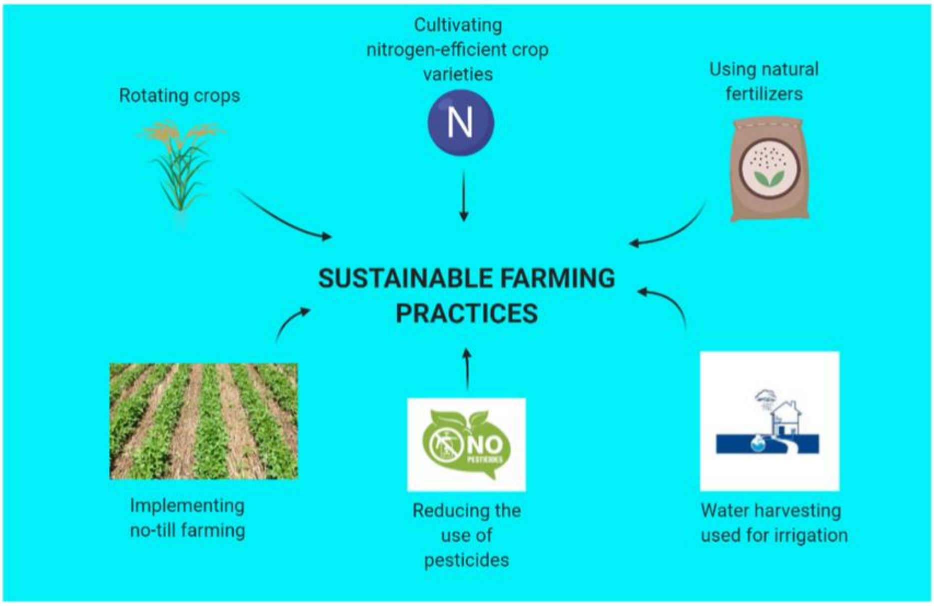

Figure 2: Sustainable Farming Practices (Source- Google)

The goal of eco-friendly agriculture is to reduce the adverse effects of farming on the environment while enhancing long-term sustainability and the welfare of farmers, regions, and clients.

For the sake of human well-being, the safety of food, the environment, long-term food security, soil health preservation, farmer empowerment through stable economic conditions, water resource conservation, biodiversity preservation, and climate change mitigation, eco-friendly or sustainable farming is crucial.

In order to successfully adopt sustainable and environmentally friendly farming practices, we must prioritise training, encourage environmentally friendly agriculture, invest in development and research, subsidise green technologies, and offer farmers and cultivators scientific support to boost agricultural profitability and productivity.

ECO-FRIENDLY APPROACHES FOR SUSTAINABLE AGRICULTURE

The world's most significant industry is agriculture. Agriculture is the practice of cultivating plants and rearing tamed animals in order to provide food, feed, fibre, and other desired items. All of nature's free gifts—land, light, air, temperature, precipitation, water, humidity, etc.—are merged into one main unit that is essential to human survival, making it a productive unit in the truest sense. Prolonged and excessive use of chemicals in crop cultivation has contaminated ground water and the environment, posing health risks to humans. Currently, the debate centres on whether to stick with intensive technologies that rely heavily on chemical inputs or return to more environmentally friendly farming methods like organic farming in order to support the farming community's socioeconomic growth and sustainably increase productivity. Within this framework, biological insecticides are regarded as a selective, inexpensive, renewable, biodegradable, and environmentally safe alternative for use in organic farming systems. Ecological pesticides, often known as green pesticides, are thought to be less harmful to the environment and animals' health. Pesticides are assessed in agrology for their lowest possible average environmental impact. Antibiotics, antibacterial, antiviral, antifungal, germicidal, antitrotozaols, and antiparasites are examples of biocides. Usually, pesticides were sold as dusts and sprays. Biological pesticides are a common type of ecological pesticide. Environmentally friendly agricultural technologies for food safety appropriate technologies that do not harm the environment would play important roles in guaranteeing food security, enhancing human health, and preserving and repairing the environment to protect future generations' well-being. Actually, rather than focusing only on short-term financial rewards, all development initiatives and activities should fall under clearly established ecological regulations. For long-term food security, sustainable agricultural systems need to be ethically and ecologically sound, fair in delivering social justice, and considerate of the needs of future generations and other species.

TYPES OF ORGANIC FARMING

Agroforestry-

Agro-forestry is the raising of trees and agriculture crops on the same land inclusive of the waste patches. It modifies the simultaneous production of food, fodder, fuel, timber, and fruit by combining forestry and agriculture.

Organic farming-

The use of artificial materials, such as pesticides, artificial fertilisers or medications, and genetically engineered organisms, is prohibited in organic farming.

Crop rotation –

Planting different crops in succession on the same piece of land is known as crop rotation, and its goals include enhancing soil health, maximising nutrient content, and reducing insect and weed pressure.

Cover crops –

In agriculture, *cover crops* are plants that are planted to cover the soil rather than for the purpose of being harvested.

Permaculture-

Permaculture is the concept of utilizing land, resources, people and the environment in a manner that doesn't produce any waste – and encourages the use of closed loop systems seen in nature. Closed loop systems, when speaking about the environment, refer to the ideal where nothing should be wasted.

Polycultures-

Polyculture is the growing of multiple crops together in the same place at the same time. It has traditionally been the most prevalent form of agriculture.

Biodynamic agriculture –

Biodynamic farms aspire to generate their own fertility through composting, integrating animals, cover cropping, and crop rotation. Through composting, plant matter, soil, and animal manures are brought into harmony and transformed into a powerful source of fertility and vigour for the farm body.

Hydroponics-

Growing plants hydroponically involves utilising a water-based nutrition solution in place of soil, together with an aggregate substrate or growing media like perlite, vermiculite, or coconut coir. Commercial enterprises, hobbyists, and small farmers all use hydroponic production systems.

Renewable energy –

Renewable energy is energy that comes from a source that won't run out. In addition to being self-replenishing and natural, they typically leave little to no carbon imprint. Examples of sustainable energy sources are hydroelectric power, which includes tidal energy, solar power, wind power, and bioenergy, which is fuel made from burning organic matter.

Soil enrichment –

As a fertiliser catalyst, soil enrichment increases the efficiency of fertiliser by preparing the soil to hold onto more nutrients and promote healthier grass.

Mulching-

Mulching is the process of covering the ground's exposed surface with a layer of external material; the substance that is employed is referred to as "mulch." Mulching is typically used for growing trees for fruit, vegetables, flowers, nursery saplings, and other commercially significant crops.

Urban agriculture –

City farms/*Urban farms* are agricultural plots in urban areas, that have people working with animals and plants to produce food.

Integrated pest management –

Integrated Pest Management (IPM) is a practical and eco-friendly method of controlling pests through a blend of common sense techniques.

Water conservation –

The technique of conserving water involves using it wisely in order to cut down on wasteful water use.

Biotechnology –

Biotechnology is the branch of technology that develops or produces new goods by using biological systems, living organisms, or components of them.

Composting –

By means of controlled aerobic decomposition (requiring oxygen), organic materials can be naturally broken down into a rich in nutrients, biologically-stable mulch or soil amendment through the process of composting.

Drip irrigation –

Drip irrigation, also known as trickle irrigation, uses a system of small-diameter plastic pipes with emitters or drippers as their outlets to drip water onto the soil at extremely low rates (2–20 liters/hour).

Integrated farming system –

This is a mix of farm enterprises such as crop, livestock, aquaculture, poultry, sericulture and agro-forestry to achieve economic and sustained agricultural production through efficient utilization of resources.

Ecological farming-

By preserving the soil, water, and climate, ecological farming guarantees good farming practices and nutritious food for the present and future.

Biological pest control –

Utilising living things to reduce pest numbers and lessen their potential harm is known as biological control. Insect natural enemies are crucial in controlling the populations of possible pests.

METHODS OF ORGANIC FARMING

Agriculture is a profession that has always been practiced, regardless of a person's level of advancement, because it meets among the most fundamental necessities of the populace: food. In order to meet the rapidly rising need for food brought on by the sharp rise in population, technology is being introduced to this sector of the economy to boost agricultural productivity. To harvest and grow the highest-quality crops possible, numerous innovative techniques and strategies are being presented in this industry. Organic farming can be defined as an agricultural practice that employs the following methods:

Crop Rotation: A method of growing different crops in the same space in accordance with the seasons one by one.

Green Manure: Alludes to withered plants that are removed and buried in the ground to serve as a source of nutrients that improve the organic matter.

Biological Pest Control: An approach to pest management that uses live things instead of or in conjunction with chemicals.

Compost: This regenerated organic debris, which is extremely rich in nutrients, is used as fertiliser to farmers.

Crop Diversity: Crop diversity or crop *biodiversity is the variety and variability of crops, plants used in agriculture.*

Soil Management: One of the most important resources that farmers and ranchers have is their soil, and soil management is the collection of tactics they employ to safeguard it.

Weed Management: The management of weeds involves their prevention, eradication, and control through controlled application, limitation of invasion, growth suppression, stoppage of seed development, and total elimination.

Livestock: Domesticated animals raised in agricultural settings for work and the production of a variety of consumable goods, including meat, eggs, milk, fur, leather, and wool, are known as livestock.

STANDARDS OF ORGANIC FARMING

Standards of organic farming are a set of guidelines that define the principles, practices, and procedures of organic agriculture. These standards are designed to ensure that organic farming methods are environmentally sustainable, socially responsible, and economically viable. Here are the general principles and standards of organic farming:

Soil health:

The ability of soil to continue functioning as a vital living ecosystem that supports plants and animals is known as soil health. Organic farming aims to build healthy soil that supports a diverse community of microorganisms and promotes plant growth. This is achieved through techniques like cover crops and crops rotation and the use of natural fertilizers like compost.

Biodiversity:

Organic farming emphasizes the importance of biodiversity, including the conservation of native species and the use of diverse crop rotations to lower the likelihood of pests and disease.

No synthetic chemicals:

The use of synthetic fertilizers, pesticides, herbicides, and genetically modified organisms (GMOs) is prohibited in organic farming. Instead, natural inputs such as compost, green manure, and natural pest control methods are used.

Animal welfare:

Organic farming standards include requirements for the humane treatment of animals, including access to pasture and outdoor space, and prohibition of growth hormones and antibiotics in animal feed.

Traceability and labelling:

Organic farming standards require proper documentation and traceability of organic products from the farm to the point of sale. Products that meet organic standards can be labelled as "organic" and may use a certification mark to signify compliance with organic standards.

Environmental protection:

Organic farming promotes the use of sustainable practices to protect the environment, including reducing greenhouse gas emissions, conserving water resources, and promoting renewable energy.

Social responsibility:

Organic farming standards promote fair labour practices, including ensuring fair wages, safe working conditions, and the right to organize.

Water conservation:

Organic farming prioritizes the conservation of water resources through practices such as efficient irrigation and the use of drought-resistant crops.

Certification:

Organic farming standards are typically enforced through third-party certification programs that verify compliance with established organic farming guidelines.

No genetically modified organisms (GMOs):

Organic farming prohibits the use of genetically modified organisms in crop or livestock production.

These standards are designed to promote environmentally sustainable, socially responsible, and economically viable practices in agriculture. An agricultural method known as "organic farming" makes use of biological pesticides and fertilisers derived from plant or animal waste. The practice of organic farming was really developed as a response to the harm that synthetic fertilisers and chemical pesticides were causing to the environment. Stated differently, organic farming represents a novel approach to farming or agriculture that restores, preserves, and enhances the natural equilibrium.

ADVANTAGES OF ORGANIC FARMING

Economical: Crop planting in organic farming does not involve the use of costly pesticides, fertilisers, or HYV seeds. Consequently, there are no additional costs.

Good return on Investment: By using locally available and less expensive inputs, a farmer can increase return on investment.

High demand: India and other countries have a significant demand for organic products, which increases export revenue.

Nutritional: Organic products are healthier, tastier, and more nutrient-dense than items made with chemicals and fertilisers.

Environment-friendly: Growing organic food does not damage the environment because it uses no chemicals or fertilisers.

DISADVANTAGES OF ORGANIC FARMING

Incompetent: The main problem with organic farming is that there is insufficient infrastructure and product promotion.

Less production: In the early years, the amount of products produced by organic farming is lower than that of chemical products. Thus, it is challenging for farmers to support large-scale production.

Shorter shelf life: Compared to chemical products, organic products are more prone to faults and have a shorter shelf life.

Limited production: In organic farming, there are fewer possibilities and fewer off-season crops.

CONCLUSION

Eco-friendly farming represents a transformative and sustainable strategy to agricultural farming that emphasizes on environmental responsibility, social well-being, and long-term resilience. The principles and practices associated with eco-friendly farming aim to address the challenges posed by conventional farming methods, which often contribute to environmental degradation, loss of biodiversity, and reliance on synthetic inputs. Here are some key points to summarize the importance and impact of eco-friendly farming on Environmental Stewardship, Sustainable Resource Management, Climate Resilience, Biodiversity Conservation, Healthy human and Animal, Local Community Engagement, Economic Viability, Global Impact etc.

In essence, eco-friendly farming is not just a set of techniques; it is a holistic and mindful approach to agriculture that recognizes the interconnectedness of the environment, society, and the food we produce. As the world faces ongoing challenges related to climate change, biodiversity loss, and food security, the principles of eco-friendly farming become increasingly vital for creating a resilient and sustainable future.

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Chapter 16

ADVANCEMENTS IN ANTIBIOTICS, MICROBIAL METABOLITES AND SECONDARY METABOLITES: A COMPREHENSIVE REVIEW

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ABSTRACT

This paper provides a comprehensive review of advancements in antibiotics, microbial metabolites, and secondary metabolites. It covers the historical significance of antibiotic discoveries, the rise of antibiotic resistance, and the potential of secondary metabolites as a source of bioactive compounds with antimicrobial properties. The testing methods for antimicrobial activity of secondary metabolites are also discussed, highlighting their crucial role in identifying novel antimicrobial agents.

KEYWORDS: Microbial Metabolites, Secondary Metabolites, Antibiotics, Antibiotic Resistance, Enzyme Inhibition, Bioactive Compounds, Antimicrobial Activity, Minimum Inhibitory Concentration (MIC)

INTRODUCTION

Bacteria, the smallest and oldest living organisms, have evolved a variety of metabolic pathways to thrive in diverse ecological niches. Secondary metabolites are microbial and plant compounds that are not required for the organisms that produce them to grow and reproduce. Each secondary metabolite is produced by a small number of species and is expressed by a set of redundant genes. These chemicals are formed at the last stage of the exponential growth phase and are heavily impacted by the growth circumstances, particularly the composition of the culture medium. They play pivotal roles in bacterial survival and interactions with their environment. Their study has significant implications in fields like medicine, agriculture, and industry, as they contain bioactive molecules with potent antimicrobial, anticancer, and immunomodulatory properties. They are also used in agriculture as natural pesticides and growth promoters, and in biotechnology for enzymes and biofuels (Martín *et al.,* 2005).

The main breakthrough inventions from the microbial metabolites is antibiotics. Antibiotics were created and discovered, revolutionising medicine, and fundamentally changing the trajectory of human history. These amazing substances have given humanity the power to fight off and eradicate infectious diseases that were previously fatal and widespread. Understanding the development of antibiotics provides insight into the successes and setbacks of medical science. It also highlights the creativity and tenacity of the pioneering researchers and scientists.

To comprehend the complex and fascinating history of antibiotics, it is crucial to acknowledge the pioneers and visionaries who laid the groundwork for these life-saving medications. Foremost among them is Sir Alexander Fleming, whose serendipitous discovery of penicillin in 1928 initiated a transformative era in medicine. Fleming's observation of the bactericidal properties of *Penicillium* mold paved the way for the development of the first antibiotic, heralding a new era of disease control and patient care.

From Fleming's breakthrough, the field of antibiotics rapidly expanded as scientists and researchers across the globe sought to harness the power of these compounds. The subsequent decades witnessed an influx of scientific investigations, clinical trials, and a flurry of breakthroughs that led to the discovery and development of a multitude of antibiotics targeting various pathogens.

From the point of view of human clinical medicine, the invention of the prodrug form of the sulfa drug prontosil, a metabolic precursor to an inhibitor of an enzyme necessary for bacterial DNA manufacture, in 1935 marks the beginning of the antibiotic era(Trefouel *et al.,* 1935; Scholar and Pratt, 2000).

With penicillin as the trailblazer, the field of antibiotics witnessed a rapid expansion in subsequent decades. The discovery of streptomycin by Selman Waksman and Albert Schatz in 1943 marked the first breakthrough in the development of antibiotics beyond penicillin. Their research paper, "Streptomycin, a Substance Exhibiting Antibiotic Activity Against Gram-Positive and Gram-Negative Bacteria" (1944), published in the Proceedings of the Society for Experimental Biology and Medicine, opened up new avenues for combating infectious diseases.

The post-World War II era witnessed an exponential increase in antibiotic discoveries and developments. Tetracycline, erythromycin, and chloramphenicol emerged as potent antibiotics in the 1940s and 1950s, followed by a wave of further discoveries in subsequent years. Key contributions include the elucidation of the structure of vancomycin by scientists Robert B. Woodward and William N. Lipscomb in 1963, which led to a deeper understanding of antibiotic mechanisms.

As the field advanced, the literature expanded to encompass the historical, social, and ethical dimensions of antibiotics. Scott H. Podolsky's book "The Antibiotic Era: Reform, Resistance, and the Pursuit of a Rational Therapeutics" (2015) offers a comprehensive exploration of the complex interplay between antibiotics, medical practice, and societal changes.

The history of antibiotics is not devoid of challenges and concerns. One significant issue is the emergence of antibiotic resistance, whereby bacteria evolve mechanisms to render antibiotics ineffective. The understanding of this phenomenon and its potential consequences has been extensively documented. Notable works on antibiotic resistance include "The Antibiotic Paradox: How the Misuse of Antibiotics Destroys Their Curative Powers" by Stuart B. Levy (2002) and "Antibiotic Resistance: Understanding and Responding to an Emerging Crisis" edited by Karl S. Drlica and David S. Perlin (2011).

The rise of antibiotic resistance has emerged as a significant global health concern, challenging the efficacy of antibiotics and complicating the treatment of infectious diseases. The phenomenon of antibiotic resistance began to surface shortly after the introduction of antibiotics. In 1940, just a few years after the discovery of penicillin, Sir Alexander Fleming warned about the potential for bacteria to develop resistance to this life-saving drug. Fleming's prescient observations were later confirmed as resistant strains of bacteria emerged in the clinical setting (Fleming, 1945).

One notable example of early antibiotic resistance was the development of penicillin-resistant Staphylococcus aureus, commonly known as MRSA (methicillin-resistant Staphylococcus aureus). MRSA first appeared in the 1960s and rapidly spread, becoming a major public health concern. The emergence of MRSA demonstrated the adaptability and resilience of bacteria in the face of antibiotic treatment.

The historical trajectory of antibiotic resistance continued to evolve over subsequent decades. In the 1970s, multiple drug-resistant strains of bacteria, such as multidrug-resistant tuberculosis (MDR-TB), emerged, posing significant challenges for treatment and control. The proliferation of resistance genes among bacterial populations was fueled by factors such as overuse and misuse of antibiotics, inadequate infection control practices, and the ability of bacteria to transfer resistance genes to other bacteria.

The scientific community recognized the gravity of the situation and endeavored to understand the underlying mechanisms of antibiotic resistance. The landmark discoveries of resistance genes and genetic elements shed light on how bacteria acquire and transmit resistance. In 1961, Joshua Lederberg and Edward L. Tatum elucidated the process of bacterial conjugation, the transfer of genetic material, including resistance genes, between bacteria.

As the understanding of antibiotic resistance deepened, research focused on identifying and characterizing specific resistance mechanisms. The discovery of beta-lactamase enzymes, which can inactivate penicillin and related antibiotics, played a pivotal role in the emergence of resistance among bacteria. The groundbreaking work by Abraham and Chain, "An Enzyme from Bacteria Able to Destroy Penicillin" (1940), published in the British Journal of Experimental Pathology, provided the first evidence of beta-lactamase-mediated resistance (Abraham & Chain, 1940).

The history of antibiotic resistance is replete with warnings and calls to action. In 1992, the World Health Organization (WHO) launched the Global Strategy for Containment of Antimicrobial Resistance, recognizing the need for a coordinated, global response to combat the spread of resistance. This strategy aimed to raise awareness, promote rational antibiotic use, strengthen surveillance systems, and foster research and development of new antibiotics (World Health Organization, 1997).

Antibiotic resistance in bacteria is a growing global concern that hampers the effectiveness of antibiotics in treating infectious diseases. The misuse and overuse of antibiotics have contributed to the development of resistant strains, posing challenges to healthcare. However, with advancements in research, antimicrobial stewardship, and the exploration of new therapeutic options, there is hope for addressing antibiotic resistance and ensuring effective treatment options for the future.

TESTING OF MICROBIAL METABOLITES FOR BIOACTIVE ACTIVITY.

Microbial metabolites are valuable sources of bioactive compounds with diverse pharmacological activities. Testing microbial metabolites for their bioactive potential in categories such as antibiotics, antitumor agents, aflatoxins, and alkaloids is essential for identifying novel therapeutic compounds.

Testing Microbial Metabolites for Antibiotic Activity: The evaluation of microbial metabolites for antibiotic activity involves employing various antimicrobial assays. Methods such as agar diffusion assays (e.g., Kirby-Bauer disk diffusion method) and broth dilution assays are commonly used to determine the inhibitory effects of metabolites against specific bacteria or fungi. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) assays are performed to quantify the potency of the metabolites. Well-known antibiotics, such as penicillin and streptomycin, are included as positive controls for comparison.

Testing Microbial Metabolites as Antitumor Agents: The bioactivity of microbial metabolites as potential antitumor agents is assessed using various in vitro and in vivo models. Cell viability assays, such as MTT or ATP-based assays, measure the effect of metabolites on cancer cell growth and survival. Apoptosis assays, cell cycle analysis, and angiogenesis inhibition assays provide insights into the mechanisms underlying the antitumor activity. Animal models, such as xenograft or orthotopic models, are employed to evaluate the efficacy of metabolites in inhibiting tumor growth and metastasis.

Testing Microbial Metabolites for Alkaloid Characterization: Microbial metabolites containing alkaloids can be characterized using techniques such as liquid chromatography-mass spectrometry (LC-MS) or gas chromatography-mass spectrometry (GC-MS). These methods provide detailed information about the chemical structure, composition, and identification of alkaloids produced by microorganisms. Additionally, bioassays, such as receptor-binding assays or enzyme inhibition assays, can be employed to evaluate the biological activities and potential therapeutic applications of alkaloids.

Testing Microbial Metabolites for Aflatoxin Detection: Aflatoxins are highly toxic secondary metabolites produced by certain fungi. Microbial metabolites with potential bioactivity against aflatoxins can be screened using techniques such as thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). These methods help identify metabolites that can inhibit the synthesis or growth of aflatoxin-producing fungi. Additionally, bioassays, such as enzyme-linked immunosorbent assays (ELISAs), can detect and quantify aflatoxin levels in samples treated with the metabolites.

TESTING FOR ANTIMICROBIAL ACTIVITY OF A SECONDARY METABOLITES.

Secondary metabolites produced by microorganisms are a rich source of bioactive compounds with potential antimicrobial properties. The testing of these secondary metabolites for antimicrobial activity plays a crucial role in identifying novel antimicrobial agents. There are some approaches and methods like determination of minimum inhibitory concentration (MIC), the agar diffusion method, different methods like membrane permeability assays to asses to assess disruption of microbial membranes, enzymatic inhibition assays to determine the inhibition of specific enzymes, cell viability assays to examine the effect on cellular viability.

The agar diffusion method, such as the Kirby-Bauer disk diffusion assay, is widely used. In this assay, paper disks impregnated with the metabolite extract are placed on an agar plate inoculated with the target microorganism, and the zone of inhibition is measured. Broth dilution assays, such as the microdilution method, can also be employed to determine the antimicrobial activity by evaluating the growth inhibition in liquid cultures (Bauer *et al.,* 1966)

Determination of Minimum Inhibitory Concentration (MIC):

The determination of minimum inhibitory concentration (MIC) provides quantitative data on the antimicrobial potency of secondary metabolites. MIC is the lowest concentration of the metabolite that inhibits visible microbial growth. The broth microdilution method, where serial dilutions of the metabolite are prepared in a microplate, is a commonly used technique. The MIC is determined as the lowest concentration without visible growth. Positive and negative controls are necessary for validating the accuracy of MIC results (Mohan *et al.,* 2020)

Evaluation of Mechanism of Action:

Understanding the mechanism of action of secondary metabolites is essential for elucidating their antimicrobial activity. Various techniques can be employed to evaluate the mode of action. These include membrane permeability assays to assess disruption of microbial membranes, enzymatic inhibition assays to determine the inhibition of specific enzymes, and cell viability assays to examine the effect on cellular viability. DNA binding assays, protein synthesis inhibition assays, and electron microscopy can also provide insights into the mechanism of action.

TESTING FOR ENZYME INHIBITION OF SECONDARY METABOLITES.

Applications of Enzyme Inhibition Assays with Secondary Metabolites: Enzyme inhibition assays using secondary metabolites have various applications in drug discovery, including the identification of lead compounds, evaluation of natural product extracts, and determination of mechanism of action. These assays are particularly valuable in screening libraries of secondary metabolites to identify potential enzyme inhibitors for therapeutic purposes.

The formula for calculating enzyme inhibition is as follows:

Enzyme Inhibition (%) = [(Control Activity - Test Activity) / Control Activity] × 100

Where, Control Activity refers to the measured enzyme activity in the absence of any inhibitor (control sample).

Test Activity refers to the measured enzyme activity in the presence of an inhibitor (test sample).

By using this formula, the percentage of enzyme inhibition can be calculated by comparing the enzyme activity between the control and test samples. The resulting percentage represents the degree of inhibition exhibited by the inhibitor on the enzyme's activity (Berg, 2018)

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Chapter 17

PTERIDOPHYTES: UNVEILING THE MEDICINAL POTENTIAL AND THERAPEUTIC APPLICATIONS IN TREATING VARIOUS ILLNESSES

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ABSTRACT

Pteridophytes are a diverse group of vascular plants that reproduce and disperse through spores. This plant division includes ferns, horsetails, and whisk ferns, among others. Pteridophytes have a significant place in the evolutionary history of plants, representing a transition from non-vascular to vascular plants. One of the key characteristics of pteridophytes is the presence of vascular tissues, including xylem and phloem, which enable them to transport water, nutrients, and sugars throughout the plant. Unlike non-vascular plants, pteridophytes exhibit a more complex structure with true roots, stems, and leaves.

Pteridophytes have been important throughout human history, with some species serving as ornamental plants and others having traditional uses in medicine and agriculture. Additionally, their fossil record provides valuable insights into the evolution of land plants. Despite their ecological and evolutionary significance, pteridophytes face challenges, including habitat loss, climate change, and invasive species. Conservation efforts are crucial to preserving the diversity and ecological roles of these ancient plants. In summary, pteridophytes represent a diverse and fascinating group of plants with a rich evolutionary history, ecological importance, and relevance to human culture and ecosystems. It's important to handle pteridophytes responsibly, especially in natural ecosystems, as some species may be invasive or threatened. Additionally, if you're using pteridophytes for food or medicinal purposes, it's essential to ensure proper identification and knowledge of any potential toxicity.

While pteridophytes may not have the same economic impact as some other plant groups, they play important ecological roles and offer various potential applications in fields such as horticulture, ecology, medicine, and research. As our understanding of these plants continues to grow, their potential applications may expand.

KEYWORDS: Pteridophytes, Horticulture, Ornamental Plants, Potential Toxicity.

INTRODUCTION

Medicinal plants have been a crucial part of traditional medicine systems for centuries, providing humanity with natural remedies to treat various ailments. These plants, often endowed with therapeutic properties, have played a significant role in the development of pharmaceuticals and continue to be a source of inspiration for modern medicine. since the earliest times, and people from all continents have practiced the use of botanicals for silver ages. In spite of the significant growth in synthetic organic chemistry in the 20th century, a quarter of approved drugs in industrialized countries are obtained directly or indirectly from plants. (Baskaran *et al.,* 2018). The pteridophytes are non-flowering, vascular, and spore-bearing plants. Pteridophytes are ferns; historically, several groups of plants were considered fern allies. They form a prominent part of the earth's vegetation as they provide evidence of vascular system evolution and reveal the emergence of seed habitat in the plants. Thus, the pteridophyte group forms a connecting link between non-vascular lower-group plants and the higher-group seed-bearing plants. Though they were the predominant part of the earth's vegetation 250 million years ago, seed-bearing plants replaced them to a greater extent. The pteridophytes grow abundantly in moist tropical and temperate forests, rocky faces, shady tree branches, wetlands (which include marshes, swamps, bogs, ferns, and similar areas lying between the land and water interface), mangroves, and water bodies. Interestingly, they exist in different eco-geographically endangered regions, from sea level to the highest mountains. (Malati and Narasimha Rao, 2020) with natural remedies to treat various ailments.

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India is profusely rich in the history of medicinal plants, and its 75% folk population is still using herbal preparations in the form of powder, extracts, and decoctions because these are easily available in nature and the natives have stronger faith in traditional knowledge. Pteridophytes (fern and fern allies), by virtue of possessing great variety and fascinating foliage, have drawn the attention and admiration of horticulturists and plant breeders for centuries. (Leena Parihar *et al.,* 2010)

In tropical countries, ferns are widely used as food and fodder. The dried fern biomass is sprayed in the cattle shed as supplementary fodder, to protect the animals from extreme cold, and as an absorbent of urinal excreta. (Priti Giri *et al.,* 2021) Pteridophytes plant extract had secondary metabolites, such as phenolic components, that enhanced biological activity. Plant secondary metabolites are known to possess antimicrobial and antioxidant properties, and some of them are categorized as generally recognized safe substances. With the growing interest in these compounds, phytocompounds from therapeutic plants with some biomedical activities are in great demand. (Baskaran *et al.,* 2018)

PTERIDOPHYTES OF TAMIL NADU

In Tamil Nadu, in various hills, more than 42 species of pteridophytes are identified in terrestrial, aquatic, and epiphytic forms. Psilotum nudum, Huperzia species, Actinopteris radiata, etc. are the important identified pieces. The distribution of most of the species was reported to be rare and limited. At the study location, the genus Polypodium was reported to be present in higher numbers. These pteridophytes face threats such as habitat destruction, deforestation, and climate change. Conservation efforts are essential to protecting these plants and their habitats. Understanding the distribution and ecology of pteridophytes in Tamil Nadu is crucial to implementing effective conservation strategies.

Actiniopteris radiata (Sw.) Link (Actiniopteridaceae) "Fan fern": these are highly decorative small ferns with their palm-like attractive fronds. It is best suited for rockeries and can also be used as a pot plant. (Sonia Abraham *et al.*, 2012) Actiniopteris radiata (Sw.) Link, belonging to the Actiniopteridaceae family, is an herb with great medicinal value. According to Ayurvedic texts, Mayurashikhaa (A. radiata) is used as an astringent, anti-inflammatory, tonic for the genitourinary tract, alleviates vitilated blood, is indicated in cough, bronchitis, asthma, diarrhea, dysentery, dysuria, and is used internally as well as externally for infected wounds, ulcers, and erysipelas (Khare, 2004). The leaves and stem of the herb have been reported to possess rutin, hentriacontane, hentriacontinol, and β-sitosterol palmitate, as well as antibiotic properties. The fronds juice of this herb was also reported for its anti-diabetic effect. A. radiata has been reported to possess anti-helminthic and styptic properties. (M. Manjunath *et al.*, 2011)

Drynaria quercifolia (L.) J. Sm. (Polypodiaceae) "Oak leaf fern" This can be grown in gardens on the ground or as epiphytes. This is an outstanding plant to grow as a pot plant indoors. (Sonia Abraham *et al.*, 2012) Drynaria quercifolia can aid in the healing and strengthening of damaged bones. It promotes bone density, protects against osteoporosis, and aids in the recovery of fractured bones and ligaments. Drynaria tonic is good for the liver and kidneys. They can aid in the treatment of bleeding gums and toothaches, and your teeth will be strengthened if you consume them on a regular basis. Plants from the Drynaria genus can be used topically as a hair tonic to promote hair development and improve the condition of the hair. Drynaria as a whole plant can be used to treat tuberculosis, rheumatoid fever, dyspepsia, and cough. The pounded fronds were applied to the affected areas of inflammation. On the forehead, macerated rhizome paste was applied for the relief of headaches. (Kanimozhi N.V., *et al.*)

Lygodiumflexuosum (L.) Sw. (Schizaeaceae), "Climbing Fern"

Lygodiumflexuosum, a climber, is a pteridophyte belonging to the family Schizaeaceae. It is commonly known as 'Bhutraj' or 'Maiden hair creeper', which is an important medicinal plant. In these plant parts, roots are used in external applications for rheumatism, sprains, scabies, and eczema, and to cut wounds; leaves are used to treat boils. The whole plant has an ethnomedicinal value as it is used in the treatment of jaundice (Biswadeep Das *et al.*).

Asplenium nidus L. (Aspleniaceae)

Nidusnot is not only extensively used to treat elephantiasis and reduce fever but also showed remarkable antiviral activity toward Herpes simplexvirus Type 1. A. nidus has been used locally in folk medicine for asthma, sores, weakness, and halitosis (Rini Jarial *et al.*).

Selaginella

The cosmopolitan genus Selaginella, possessing many species, is conventionally utilized for food, medicine, handicrafts, and also as ornaments. Selaginella plants are usually used to cure many illnesses, which include fever, jaundice, hepatic disorders, cirrhosis, diarrhea, cholecystitis, sore throats, and coughs of the lungs. Additionally, it promotes blood circulation, helps in the removal of blood stasis, controls external bleeding after injury, etc. The cosmopolitan genus Selaginella, possessing many species, is conventionally utilized for food, medicine, handicrafts, and also as ornaments. Many different species of the genus Selaginella are exploited for various ethnomedicinal purposes around the globe, mainly to cure many illnesses and stop external bleeding after trauma and separation of the umbilical cord. Though a high content of various phytochemicals has been isolated from Selaginella species, flavonoids have been recognized as the most active component in the genus. Crude extract and different bioactive compounds of this plant have revealed various in vitro bioactivities, for example, antimicrobial, antiviral, anti-diabetic, anti-mutagenic, anti-inflammatory, anti-nociceptive, anti-spasmodic, anticancer, and anti-Alzheimer. (Mohd Adnan, *et al.,* 2021)

Ceratopteristhalictroides

Ceratopteristhalictroides occurs in semi-shaded localities mostly rooted in mud, occasionally free-floating, and common in paddy fields and ponds. The fronds of C. thalictroides are used as a vegetable. The fronds of C. thalictroides are used as poultices for skin diseases. The uncurled fronds are eaten as a salad or as a substitute for asparagus. The tribal people use the plant as a poultice for skin problems. The whole plant parts are ground into paste and mixed with turmeric. The mixture is applied to the affected areas to treat skin diseases and wounds. In Madagascar, C. thalictroides leaves are eaten as salad or cooked as vegetables, whereas in Swaziland, leaves are eaten as leafy vegetables (Smitha and Vadivel 2019).

Anemiawightiana

Anemiawightiana (Family: Anemiaceae) was used in the treatment of tuberculosis, and it also exhibits strong antimicrobial activity. Species of this genus contain several essential oils and terpenes (Sujatha V. *et al.,* 2021).

CONCLUSION

Inconclusion, the exploration of the medicinal potential of pteridophytes has revealed promising avenues for their therapeutic applications in treating various illnesses. The diverse range of bioactive compounds present in these ancient plants showcases their significance in traditional medicine and opens new possibilities for modern pharmaceutical research.

Through comprehensive studies, it has become evident that pteridophytes harbor compounds with anti-inflammatory, antimicrobial, antioxidant, and other beneficial properties. These compounds have demonstrated effectiveness in addressing a spectrum of health issues, from infectious diseases to chronic conditions. Moreover, the sustainable utilization of pteridophytes for medicinal purposes holds promise for both conservation efforts and healthcare advancements. The integration of traditional knowledge with contemporary scientific methods will facilitate the identification and isolation of specific compounds, paving the way for the development of novel pharmaceuticals. As we navigate the complexities of modern healthcare, acknowledging the medicinal potential of pteridophytes contributes to the growing repertoire of natural sources for drug discovery. This research not only emphasizes the importance of preserving biodiversity but also highlights the potential of these ancient plants to provide solutions to the health challenges of our time.

In the future, continued exploration and understanding of pteridophytes' medicinal properties will likely lead to the development of new, effective treatments. This field of study stands at the intersection of traditional wisdom and cutting-edge research, offering a bridge between the past and the future of medicine. As we move forward, the collaboration between ethnobotanists, pharmacologists, and traditional healers will play a crucial role in unlocking the full therapeutic potential of pteridophytes for the benefit of global health.

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Chapter
18

MICROALGAE-BASED BIOGENERATIVE LIFE SUPPORT SYSTEM: A CELL WITH GREAT APPLICATION IN SPACE

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ABSTRACT

Humans have been living and working in space for 22 years, thanks to the establishment of the International Space Station. The National Aeronautics and Space Administration (NASA) has committed to returning humans to the Moon within the next two years and to placing the first humans on Mars by 2033. The first human presence on Mars will be the most technically difficult venture in the long history of exploration beyond our planet. The long-distance between Mars and Earth, harsh surface conditions such as low atmospheric pressure and severe temperatures, and the requirement for basic resources such as water all pose formidable challenges to this attempt. Microalgae are regarded as innovative sources of appropriate nutrition to overcome food scarcity and ensure nutritional food supply for the sustainable living of a rising population. Currently, dried biomass, carotenoids, phycocyanin, phycoerythrin, omega fatty acids, and enzymes have been used as food additives, coloring agents, and supplements. This article explores the potential for the creation of various synthetic and microbiological food products for long-duration crewed missions, focusing on the technological aspects of food product synthesis rather than the product development aspects. The bioregenerative life support system (BLSS) is essential in long-duration manned missions to space. Microalgae-based BLSS was proposed decades ago, but its deployment in space missions was hampered by several technological difficulties. However, much work has been given to this topic in recent years, and significant progress has been made in the investigation of microalgae-based BLSS. As a result, the primary goal of this work is to summarise previous advances and identify the issues confronting microalgae-based BLSS technology.

KEYWORDS: Atmospheric Pressure, Nutrition, Scarcity, Sustainable, Phycocyanin, Biomass.

INTRODUCTION

NASA intends to send humans to Mars by the 2030s [8]. The pursuit of this aim will necessitate the development of technologies capable of supporting long-duration human missions beyond the Lower Earth Orbit (LEO). Since nutrition and a healthy diet are fundamental to all aspects of health and performance. The sufficiency of the food system has been important to the success of human exploration throughout the history [50, 56]. For long-duration space missions, it is the single most expensive expendable resource. The International Space Station (ISS) experience indicates that the "carry it with you" approach to life-support supplies continues to be too expensive to launch. Over the last century, the field of human nutrition has grown significantly, and we have established the conditions for a safe, healthy, and acceptable food system. As we approach the era of deep space exploration, it becomes obvious that we will use this knowledge in future exploratory food systems. However, resource limits and shelf-life need aboard spacecraft can conflict with what appear to be basic and required food system solutions (e.g., cold storage) on Earth, making food system provisioning more difficult as missions are longer and further away from Earth. Life support systems (LSSs), which are one of the essential components in manned spacecraft determining the distance and duration of space exploration missions, assure human survival in space [28,51]. In a traditional life support system (TLSS), packed food is transported in the spacecraft, and human waste is retrieved for further treatment when the spacecraft returns to Earth, significantly increasing the logistics cost. The oxygen required by an astronaut is often supplied by alkali-metal peroxide or compressed oxygen gas, which are both which are non-recyclable in spacecraft. Due to spacecraft volume limitations, the quantities of these essential supplies are limited to support long-duration space missions. As a result of the aforementioned issues, considerable efforts are being made to develop more sustainable LSSs to further extend the duration of space research missions. As a result, there is interest in emerging technologies that simultaneously reduce cost, risk (by redundancy in process or output), and raise the probability of mission success. The National Aeronautics and Space Administration (NASA) now seeks promising new technologies for

- Producing fuel for the primary spaceship and secondary personal conveyances [23,32,33,38];
- Manufacturing components in space (e.g. By 3d printing) [21, 30];
- Replacing cargoes of pre-packaged food [26, 46];
- Establishing redundant life support systems for gas purification, water treatment, etc.[29,42,52];
- Treating diseases and enhancing the shelf life of therapies and pharmaceuticals [34,36,37,47,48];

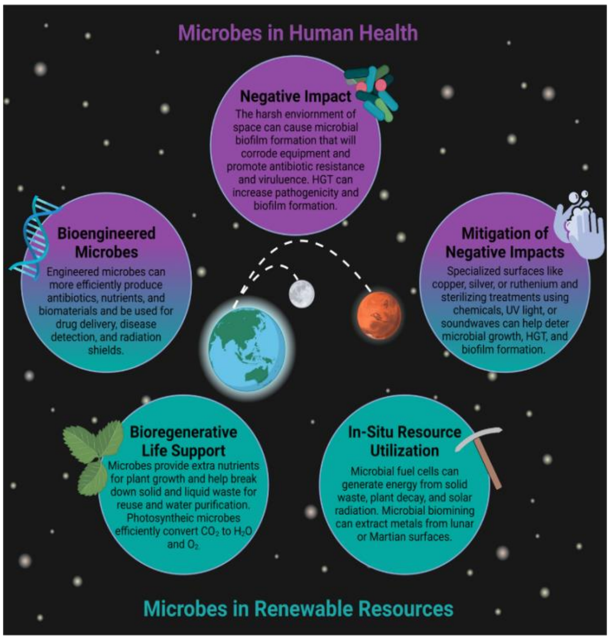

Figure 1: An overview of the microbiological impact on long-duration space missions [1]

MICROALGAE BASED BIOGENERATIVE LIFE SUPPORT SYSTEM

The majority of Earth's ecosystems are sustained by photoautotrophs, which use light energy to fix carbon dioxide into organic macromolecules and split water to release O_2. Terrestrial and aquatic plants, macroalgae (seaweeds), microalgae (microscopic species of eukaryotic algae), and photosynthetic bacteria are examples of oxygenic photoautotrophs. Although plants are a vital source of nutrition and oxygen, cyanobacteria and microalgae provide numerous advantages that make them attractive as a future commercial technology on Earth and for space travel. This includes faster growth rates, fewer nutritional requirements, the ability to grow on non-arable land, and the production of several valuable byproducts [5,22]. In practice, microalgae-based BLSS requires growing condition management, dead cell treatment, algal seed preservation, and bacterial contamination control. The biogenerative life support system (BLSS) is a man-made ecosystem that uses photosynthetic organisms for oxygen generation, food supply, and even waste treatment, recognizing the need for resource recycling in spacecraft and maintaining a safe habitat for astronauts [18,39,44]. The Environmental Control and Life Support System (ECLSS) generates breathing oxygen by water electrolysis, as well as a Sabatier reactor which employs catalytic CO_2 conversion and extra CO_2 scrubbing from the atmosphere. The majority of the water recovered from urine is recycled as drinking water via vacuum distillation, whereas solid human waste and other garbage are placed into resupply spacecraft and burned upon re-entry into Earth's atmosphere. Such life support systems are referred to as open since they do not completely recycle matter, causing the loss of resources as waste and a demand for resupply from Earth [57]. Compared to a TLSS, a BLSS that relies on resource recycling is more sustainable, extending the duration of the space mission and reducing logistics costs. Researchers from the United States, the Soviet Union, China, European Union countries, Japan, and Russia have made significant progress in plant species selection, space mutagenesis and genetic stability, system design, system operation testing, and numerous other fields since the 1950s. However, due to the instability and immaturity of the technology, as well as the low efficiency of bio-system performance, BLSS has been rarely used in manned spacecraft till now. As major powers in aviation research, such as the United States, China, the European Union, and Russia, proposed ambitious plans for long-duration manned space exploration missions, such as a permanently inhabited space station on the Moon or Mars, BLSS is gaining prominence due to its critical roles in space missions [15].

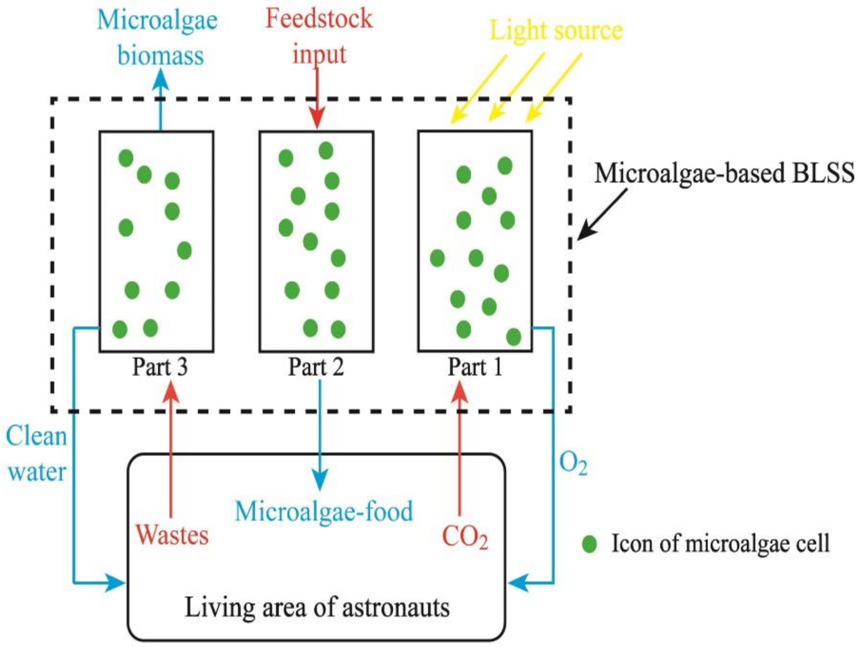

Figure 2: Principles of microalgae-based bioregenerative life support system (Blss)[13].

MICROALGAE AS A FOOD IN SPACE

Protein, glucose, fat, dietary fiber, vitamins, and mineral salts are all essential nutrients for humans and are abundant in algal species. Furthermore, microalgae contain powerful antioxidants such as astaxanthin and carotene, which can protect the human body against radiation-induced oxidation and aging [31]. Algae that have been successfully commercialized in the food sector to provide value-added goods are *Chlorella* sp., *Scenedesmus* sp., *Dunaliella salina*, and *Haematococcus pluvialis*, which can be used for food supply in spacecraft. Microalgae are an important food source for astronauts due to their high nutritional value and productivity. Spirulina and Chlorella vulgaris have a long history as food and food ingredients [19] and are appealing candidates for food cultivation aboard space missions. Edible microalgae biomass may also be easily cultivated with high volume efficiency [15]. Furthermore, based on their substrate and nutrient requirements, microalgal systems can be added to existing ECLSS. This can be performed by diverting CO_2 from cabin air or a portion of it (as substrate), as well as urine and flush water (as nutrition supplies), into a microalgal reactor, which is illuminated by sunshine or artificial LED lights. More importantly, the addition of the microalgal system might not only provide a source of food supplements but also greatly reduce the amount of vitally needed C and H (in the form of CH_4) that is currently vented (wasted) into space. As a result, the integration promotes the development of a near-ideal (closed-looped), self-sustaining life-support system capable of exploring isolated areas with limited resources (to generate life-support substances). Another critical feature of the microalgal system in the ECLSS is its ability to potentially exploit the vast CO_2 resource in Mars' atmosphere, which could be useful for the planet's intended colonization (Mars' atmosphere is over 95% CO_2).

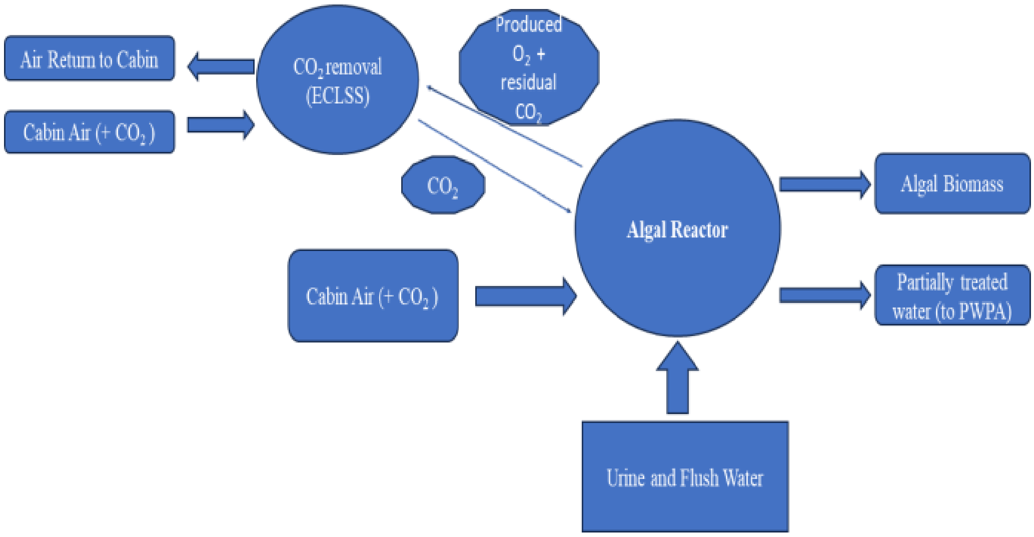

Figure 3: Integration of microalgal system into eclss to include food production (Microalgal Biomass) [2]

A MICROALGAL CELL WITH GREAT POTENTIAL FOR SPACE APPLICATION

Chlorella, Limnospira, and Chlamydomonas are the most well-known and studied microalgae in space travel. Limnospira is a new genus into which commercial Spirulina strains (formerly known as Arthrospira platensis) have been reclassified as Limnospira fusiformis [12]. Chlorella, Limnospira, and Chlamydomonas are ideal candidates for Mars-specific biological life support systems (BLSS) due to their ease of cultivation. As indicated in Table 1, all three genera have been evaluated in various life support systems, mostly for food and air revitalization. Anabaena and Nostoc have the potential for biomining and nitrogen fixation. Euglena gracilis is a well-known model organism in space exploration [24]. It is a new food source with commercially relevant products [11], and it can endure extreme temperatures and desiccation. As shown in Table 1, Euglena is commonly flown in space missions and has successfully given enough oxygen for fish in a bioregenerative life support system [24].

Table 1: Microalgae applications in space

Microalgae	Types of Microalgae	Most Relevant Features	References
Anabaena/ Nostoc	Filamentous cyanobacterium	Extremophile; genetic technologies are available; edible species; can exploit very high amounts of carbon dioxide; biomining potential; nitrogen fixation capability	[20,35,41,43]
Limnospira fusiformis (Spirulina)	Filamentous cyanobacterium	Edible with high nutritional and nutraceutical value	[19,27]
Synechocystis/ Synechococcus	Unicellular cyanobacterium	Well-studied metabolism; a wide range of genetic tools (e.g., increase biopolymer content); survival in extremely high atmospheric carbon dioxide concentrations	[17, 20]
Chlorella vulgaris	Green microalgae	Edible; air revitalization; well-characterized; most extensively investigated in spaceflight conditions	[15, 19,45]
Chlamydomonas reinhardtii	Green microalgae	A wonderful array of genetic tools is accessible (for example, for pharmaceutical manufacture)	[14,16]
Chroococcidiopsis	Unicellular cyanobacterium	Extremophile (desiccation and radiation tolerance)	[35,53]
Euglena gracilis	Green microalgae	Extremophile; edible with great nutritional characteristics; air revitalization; model organism in gravitational studies	[11]

Among the many microalgae species, *Chlorella vulgaris* has been the focus of the majority of investigations involving space missions, including an ISS experiment that began in 2019 [4]. These cells have the ability to be cultured in all known PBR types due to their tiny cell size and spherical shape. Its biomass is a nutritious dietary supplement that is high in protein (B 50%) and contains all important amino acids. It also contains a lot of unsaturated fats, carotenoids, dietary fibres, vitamins, minerals, and other bioactive compounds [6]. It can grow heterotrophic, photoautotrophic, or mixotrophic depending on the carbon supply (e.g., organic glucose or inorganic CO_2) [40]. As a result, in addition to other well-studied microalgal taxa such as Arthrospira sp. and Chlamydomonas sp., Chlorella sp. is also highly suitable for use as model organisms for space applications due to its cultural versatility and outstanding robustness. In order to be used in space, microalgae must have the ability to regenerate in the presence of cosmic radiation. Rea *et al.,* (2008) demonstrated that Chlorella sp. could tolerate continuous ionising irradiation while retaining more than 90% of their initial photosynthetic capability in numerous radiation trials [49]. Spirulina and Chlorella vulgaris are the best-studied microalgal species in terms of a healthy diet[3,7].

Dunaliella species are another edible eukaryotic alga with space crop potential. The majority of research on this genus has concentrated on beta carotene production, which can account for 14% of dry biomass [9]. Again, due to the lack of a solid cell wall, Dunaliella species such as D. salina have an advantage over C. vulgaris and have been demonstrated to be highly digestible in rats [53]. Analysis of D. salina biomass shows it to have an equivalent protein content (~57%) to C. vulgaris and Spirulina [9]. Dunaliella are not currently regarded as a "mainstream" microalgal food, despite being sold as dietary supplements; however, a recent request for additional nutritional research on this genus may change this [10].

Lastly, two species of filamentous cyanobacteria in the order Nostocales that are already available as dietary supplements, Aphanizomenon flos-aquae (AFA) and Nostoc, may prove to be promising as space crops in the future. Although some strains from these species are toxic, edible Nostoc species have been reported as being highly [25,55], although a nutritional profile of AFA is not available in the scientific literature. AFA is harvested and sold as a supplement from lake Klamath in Oregon, US, but it has not yet been developed for mass cultivation in commercial ponds or PBRs [54]. More research has been conducted on cultivating edible Nostoc species, specifically N. commune, N. sphaeroides, and N. flagelliforme, although none of these are currently grown at the mass scale required to make them profitable – largely due to their slow growth rate [25]. But Nostoc's energy-intensive ability to fix nitrogen—a valuable trait for regenerative life support on Mars—is most likely the cause of its sluggish growth rate.

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Chapter
19

AN ASSESSMENT ON THE ANGIOSPERMIC DIVERSITY OF THE *KOLONG* RIVERINE ZONE OF NAGAON MUNICIPALITY AREA, ASSAM

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ABSTRACT

The present article focuses on the angiospermic plant diversity of the *Kolong* riverine zone of Nagaon municipality area, Assam. From the analysis of the data a total of 60 angiospermic species belonging to 29 families has been recorded. Among the recorded species, 41 are dicot and 20 are monocots. *Pluchea wallichiana* DC. (Asteraceae) and *Rumex dentatus* L. (Polygonaceae), *Dendrobium aphyllum* (Roxb.) C.E.C. Fisch. (Orchidaceae), *Papilonanthe teres* (Roxb.) Schltr. (Orchidaceae), and *Chrysopogon aciculatus* (Retz.) Trin. (Poaceae) are assessed as threatened (Th). For monocot families, Poaceae (7 No's) appeared to be most dominated followed by Cyperaceae (6 No's), whilst in dicots, Asteraceae (8 No's) were found to be dominated followed by Lamiaceae (8 No's). In the statistical representation Poaceae possess highest in degree (126˚) among monocot families, and Asteraceae represents highest among the dicot (70.25˚). The study also revealed a decline in the variety of significant plants that add to the socioeconomic fabric of the vicinity through various anthropogenic actions resulting environmental deterioration or elevated stresses on the riverine vegetation.

KEYWORDS: *Kolong* River, Angiospermic Diversity, Anthropogenic Threats.

INTRODUCTION

Comprising about 250,000 species in 350 families, angiosperms serve the most varied group in the plant kingdom. Certain families viz., Asteraceae, Orchidaceae and Poaceae have much larger numbers of species than others (Stebbins, 1981). These are special type of seed plants that are distinguished by certain traits in common. Carpels encircling the ovules, pollen grains germinating on the stigma, sieve tubes with companion cells, double fertilization leading to triploid endosperm, and drastically diminished male and female gametophytes are some of these significant characteristics.The category of flowering plants is also distinguished by the presence of stem vessels and seeds that nestle into the carpels (Eames, 1961). The angiospermicflower typically hermaphrodite in structure with carpels surrounded by stamens. Monophyletic plants are typically perceived as angiosperms (Dahlgren, 1983; Doyle & Donoghue, 1993; Faegri, 1980; Takhtajan, 1969).

Assam's eclectic topography, ideal climate, and enviable geographical setting led to the nation's top-notch biodiversity. The vegetation of Assam is predominantly tropical, encompassing evergreen, semi-evergreen, grassland, deciduous woodland, and riverbankwoods. Francis Buchanan Hamilton (1762–1829) undertook the survey on lower Assam's vegetation for the first time in 1808 (Bhuyan & Bahadur, 1940). Griffith (1836) had prior explored the Sadiya region in upper Assam. However, the 'Flora of British India' (Hooker 1875–1897) and 'Flora of Assam' (Kanjilal *et al.* 1934–1940; Bor 1940) were utilized as the primary guides for the systematic plant collecting and floristic investigations. About 3895 species of angiospermic taxa are now known to exist in the state of Assam (Chowdhury, 2005). The plains of Assam are mostly draped in trees, such as *Shorea assamica*, *S. macrocarpus*, *S. tubinatus*, *Keyea assamica*, *Dalbergia sissoo*, *Palaquium polyanthum* etc. These are the most commercial source that serve for crafting furniture, plywood, pulpwood, machinery for farming, timber, alongside NTFP's. Leaves of *Litsea monopetala*, *L. cubeba* get utilized for rearing silkworms. Approx. 293 species of orchids recorded to flourish in Assam, accounted for 24.42% of Indian species and 44.39% of species found in the Northeast India.

The Nagaon district lies in the middle Assam blessed with several hills and hillocks and a number of rivers. The*Kolong* is the one of major rivers in the district harbors a good number of aquatic and semi aquatic plants. Some sporadic floristic study on the district indicates a great deal of diversified flora (Khan *et al.*, 2012) (Bora *et al.*, 2015), yet no comprehensive work on floristic account of the riverine zone have been made except about few particular aquatic plants. During the period of 1960-1972, several major floods occurred in the district causing a serious threat to the district. Decision was executedto block the mouth of the river. Since the time of blockage, *Kolong* become degraded and stagnant in major parts of the year. However, during the monsoon period rain fed water fills the river bed, as the river become flowing in nature and inspires the abundance of diversified floral elements in the riverine zone. Several of them are often used by the local inhabitants as medicine or other purposes.

METHODOLOGY

STUDY AREA, DEMOGRAPHY

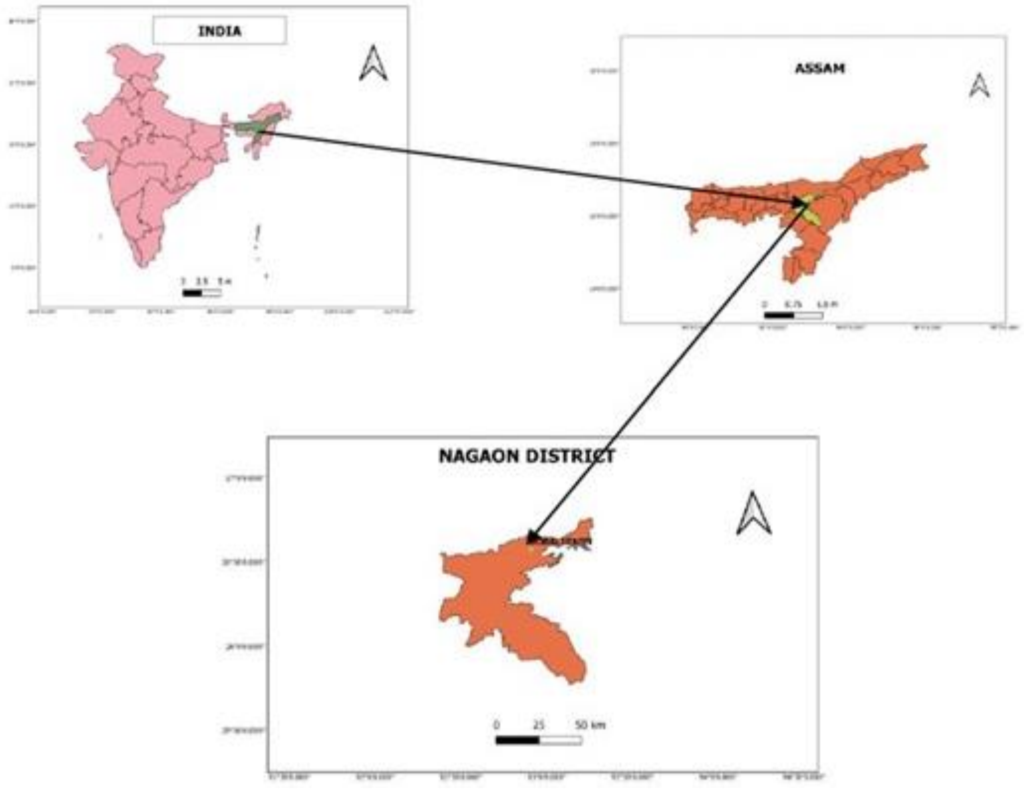

Figure 1: Map of the study area (*Source- Internet*)

The *Kolong* river is an anabranch of the Brahmaputra, which diverts from the river in the Hatimura region of the Nagaon district (latitude of 25˚47◎ N-26˚ 42′ N and longitude of 92˚25´-93˚18´ E) lies in the middle assam region. At present Nagaon municipality board consist of wards with a population of 1,17,722.

Figure 1: Map of the study area (*Source- Internet*)

SURVEY, SAMPLE COLLECTION AND ANALYSIS

Extensively planned survey spread over 3 months from September-December,2023 was carried out and the abasement of theangiospermic elements have been collected from the *kolong* riverine zonebearing their reproductive parts. Taxonomic Keys were produced for the same and validated by consulting with the available national and international floras; subsidiary information including, Red list category (IUCN database). Specimens were preserved following standard herbarium techniques (Jain & Rao, 1977) and deposited in the herbarium of the Department of Botany, Nowgong College (A), Nagaon.

STATISTICAL ANALYSIS

Pie representation of the reported specimens (Table 1-2) was prepared and the statistical presentation (In degree) of the same was performed by the following formula-

$$\frac{\text{Number of species of the family} \times 360^\circ}{\text{Total no of species}}$$

RESULTS AND DISCUSSION

The current study assembled data on 60 angiospermic species, comprising their scientific name, IUCN sorting, family name, and vernacular name (whenever available). Out of the sixty species that have been identified, 41 are dicot (Table 1) and 19 are monocots (Table 2). 2 dicot species, *Pluchea wallichiana* DC. (Asteraceae) and *Rumex dentatus* L. (Polygonaceae) are ranked as threatened, whilst 3 monocot species viz., *Dendrobium aphyllum* (Roxb.) C.E.C. Fisch. (Orchidaceae), *Papilonanthe teres* (Roxb.) Schltr. (Orchidaceae), and *Chrysopogon aciculatus* (Retz.) Trin. (Poaceae) are assessed as threatened. For monocot families, Poaceae (7 No's) appeared to be most dominated followed by Cyperaceae (6 No's), whilst in dicots, Asteraceae (8 No's) were found to be dominated followed by Lamiaceae (8 No's). In the statistical representation of the pie diagram (Figure 2-3) Poaceae possess highest in degree (109.57˚) among dicot families, and Asteraceae represents highest among the monocots (70.25˚).

Analyses combining the different locations of the study area, it became apparent that an array of anthropogenic factors, especially gentrification and the construction of housing units, roadways, industrial growth, and forest felling, gradually diminish the diversity of plant species in the riverine zone. Furthermore, a decline in the variety of significant plants that add to the socioeconomic fabric of the vicinity could arise through environmental deterioration or elevated stresses on the vegetation. It is high time to take necessary action to sustainably manage these wild and semi-domesticated elements. The repercussions of genome size (*gs*) on plant growth and viability have garnered significant curiosity in recent times, since data suggests that *gs* influences the physical position and growth layout of plants. Studies demonstrating the synergies with *gs*, photosynthesis, water-use efficiency, and environmental factors (CO_2, water, and nutrients) on riverine growth should be addressed.

Table 1: Reported dicot species alongside families, vernacular names and IUCN status

Sl. No.	Botanical name	Family	Vern. Name	IUCN Status
1	*Acmella paniculata* (Wall. ex DC.) R.K. Jansen	Asteraceae	Huhoni-bon	LC
2	*Rorippa montana* (Wall. Ex Hook.f. & Thomson)	Brassicaceae	--	NE
3	*Argemone maxicana* L.	Papaveraceae	Siyal-kata	NE
4	*Sida acuta* burm. f.	Malvaceae	Boriala	NE
5	*Abutilon hirtum*(Lam.) Sweet	Malvaceae	--	NE
6	*Crotalaria pallida pallida*	Fabaceae	--	NE
7	*Cannabis sativa* Linn.	Cannabinaceae	Bhang	LC
8	*Oxalis corniculate* L.	Oxalidaceae	Tengechi tenga	LC
9	*Vernonia cinerea* Less.	Asteraceae	Lohpohi	--
10	*Pluchea wallichiana* DC.	Asteraceae	--	Th
12	*Centratherum puncatatum* Cass.	Asteraceae	--	NE
13	*Xanthium strumarium* L.	Asteraceae	Ogora-kata	NE
14	*Leucas plukenetii* (Roth) Spreng.	Lamiaceae	Durun	--
15	*Cleome rutidosperma* DC.	Cleomaceae	--	NE
16	*Ocimum gratissimum* L.	Lamiaceae	Gandha-tulasi	V
17	*Ocimum sanctum* L.	Lamiaceae	Tulasi	LC
18	*Leonurus sibircus* L.	Lamiaceae	Ronga Durun	NE
19	*Heliotropicum indicum* L.	Boraginaceae	Biyoni-sabota-Hati-huria	LC
20	*Solanum nigrum* L.	Solanaceae	Los kochi	NE
21	*Nicotiana plumbaginifolia* Viv.	Solanaceae	--	NE
22	*Lantana camara* L.	Verbenaceae	Gu-phul	LC
23	*Lippia javanica* (Brum.f.) Spreng.	Verbenaceae	Pahu-kuta	LC
24	*Andrographis paniculata* (Burm.fil.) Nees	Acanthaceae	Kalmegh	LC
25	*Ruellia tuberosa* L.	Acanthaceae	Chatpati	LC
26	*Scoparia dulsis* l.	Scrophulariaceae	--	--
27	*Rumex dentatus* L.	Polygonaceae	Jangali chuka	Th
28	*Euphorbia hirta* L.	Euphorbiaceae	Gakhiroti-bon	NE
29	*Amaranthus spinosus* L.	Amaranthaceae	Hati-Khutura	NE
30	*Amaranthus viridis* L.	Amaranthaceae	Khutoriya	LC
31	*Alternanthera sessilis* (L.) R.Br. ex DC.	Amaranthaceae	Mati-kanduri	LC
32	*Chenopodium album* L.	Amaranthaceae	Jilmil-sak	LC
33	*Ageratum conyzoides* L.	Asteraceae	Gendhali-bon	LC
34	*Chromolaena odorata* (L.) R.M.King & H.Rob.	Asteraceae	Jarmani bon	NEx
35	*Eclipta prostrata* (L.) L.	Asteraceae	Kehraj	NE

36	*Ipomoea batatas* (L.) Lam.	Convolvulaceae	Mitha-alu	LC
37	*Lucus aspera* (Wild.) Link	Lamiaceae	Durun	NE
38	*Mimosa pudica* L.	Mimosaceae	Nilaji-bon	LC
39	*Morus indica* L.	Moraceae	Nuni	NE
40	*Stellaria media* (L.) Vill.	Caryophyllaceae	Morolia	NE
41	*Scoparia dulcis* L.	Plantaginaceae	Bon chini	LC

NB: IUCN category/ LC-*Least concern*, EN-*Endangered*, NE-*Not Evaluated*, R-*Rare*, DD-*Data Deficient*, NEx-*Not Extinct*, Th-*Threatened*, V-*Vulnerable*

Table 2: Reported monocot species alongside families, vernacular names and IUCN status.

Sl. No.	Botanical name	Family	Vern. Name	IUCN Status
1	*Amorphophallus paeoniifolius* (Dennst.) Nicolson	Araceae	Ol-kochu	LC
2	*Dendrobium aphyllum* (Roxb.) C.E.C.Fisch.	Orchidaceae	Haliki-thutia-phul	Th
3	*Papilonanthe teres* (Roxb.) Schltr.	Orchidaceae	Bhatou phul	Th
4	*Rhynchostylis retusa* (L.) Blume	Orchidaceae	Kopou phul	NE
5	*Dendrobium aphyllum*	Orchidaceae	--	--
6	*Papilonanthe teres*roxb.	Orchidaceae	--	--
7	*Cyperus rotundus*l.	Cyperaceae	Keya-bon	LC
8	*Cyperus pangorei*	Cyperaceae	--	
9	*Eleusine indica* (L.) Gaertn	Poaceae	Binoi-bon/ Binoi	NE
10	*Poa annua* L.	Poaceae	--	LC
11	*Cynadon dactylon* (L.) Pers	Poaceae	Dubori-bon/Dubori	NE
12	*Piptetharum miliaceum* (L). Coss.	Poaceae	--	--
13	*Chrysopogon aciculatus* (Retz.) Trin.	Poaceae	Bon-guti	Th
14	*Echinochloa crus-galli* (L.) P.Beauv.	Poaceae	Binoi-bon/ Binoi	NE
15	*Eleusine indica* (L.) Gaertn.	Poaceae	Bobosa-bon	LC
16	*Kyllinga brevifolia* Rottb.	Cyperaceae	--	--
17	*Cyperus rotundus* L.	Cyperaceae	Keya bon	LC
18	*Cyperus pangorei* Rottb.	Cyperaceae	--	LC
19	*Kyllinga bulbosa* P.Beauv.	Cyperaceae	--	LC
20	*Commelina diffusa Burm.f.*	Commelinaceae	Kona-simalu	LC

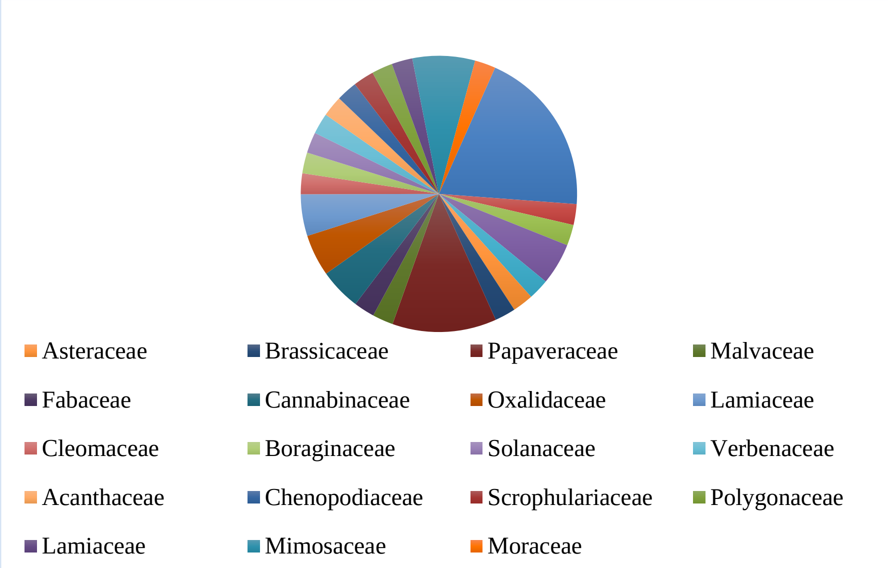

Figure 2: Pie diagram of the family wise diversity of dicots

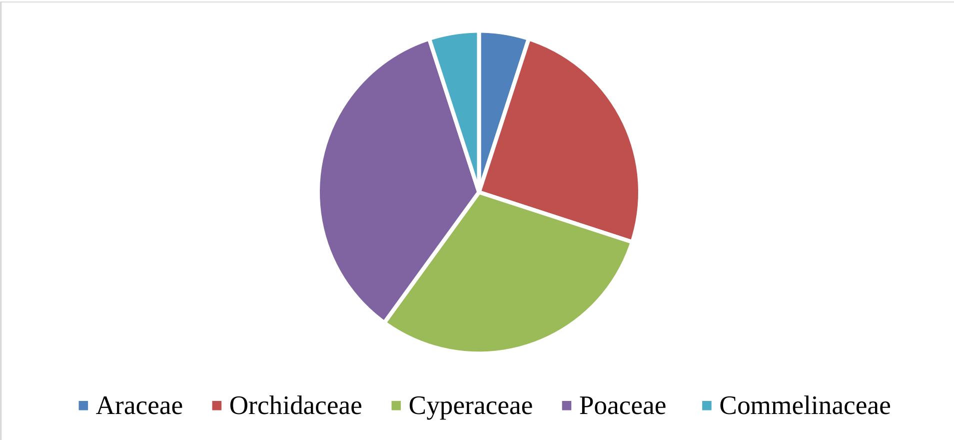

Figure 3: Pie diagram of the family wise diversity of monocots

Table 3: Calculation of the pie diagram of monocots

Family	No of species	Presentation (In degree)
Araceae	1	$1 \div 20 \times 360^\circ = 18^\circ$
Orchidaceae	5	$5 \div 20 \times 360^\circ = 90^\circ$
Cyperaceae	6	$6 \div 20 \times 360^\circ = 108^\circ$
Poaceae	7	$7 \div 20 \times 360^\circ = 126^\circ$
Commelinaceae	1	$1 \div 20 \times 360^\circ = 18^\circ$

Table 4: Calculation of the pie diagram of dicots

Family	No of species	Presentation (In degree)
Asteraceae	8	8÷41×360°= 70.25°
Brassicaceae	1	1÷41×360°= 8.79°
Papaveraceae	1	1÷41×360°= 8.79°
Malvaceae	2	2÷41×360°= 11.57°
Fabaceae	1	1÷41×360°= 8.79°
Cannabinaceae	1	1÷41×360°= 8.79°
Oxalidaceae	1	1÷41×360°= 8.79°
Lamiaceae	5	5÷41×360°= 43.91°
Cleomaceae	1	1÷41×360°= 8.79°
Boraginaceae	1	1÷41×360°= 8.79°
Solanaceae	2	2÷41×360°= 11.57°
Verbenaceae	2	2÷41×360°= 11.57°
Acanthaceae	2	2÷41×360°= 11.57°
Chenopodiaceae	1	1÷41×360°= 8.79°
Scrophulariaceae	1	1÷41×360°= 8.79°
Polygonaceae	1	1÷41×360°= 8.79°
Lamiaceae	1	1÷41×360°= 8.79°
Mimosaceae	1	1÷41×360°= 8.79°
Moraceae	1	1÷41×360°= 8.79°
Caryophyllaceae	1	1÷41×360°= 8.79°
Plantaginaceae	1	1÷41×360°= 8.79°
Euphorbiaceae	1	1÷41×360°= 8.79°
Amaranthaceae	3	3÷41×360°= 26.35°
Ipomeaceae	1	1÷41×360°= 8.79°

a

Figure 4:4(A) The habitat; 4(B) *Oxalis corymbosa* DC.; 4(C)*Cynadon dactylon* (L.) Pers; 4(D)*Oxalis corniculate* L.; 4(E)*Ricinus communis*;4(F)*Kyllinga brevifolia* Rottb.; 4(G)*Solanum nigrum* L.; 4(H)*Ocimum* sp; 4(I)*Euphorbia hirta* L.; 4(J)*Amaranthus viridis* L.

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