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“BIOBRIDGES: Connecting Technology with the Future of Life" explores the intersection of biotechnology and technology, focusing on how advancements in both fields can collaboratively shape the future of life on Earth. The book delves into the various ways in which innovative technological solutions are being applied to solve critical biological challenges and enhance human well-being, environmental sustainability, and global health.

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Biobridges: Connecting Technology with the Future of Life

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BIOBRIDGES

Connecting Technology with the Future of Life

Dr. Bidisha Ghosh

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Biobridges: Connecting Technology with the Future of Life

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Chapter - 1

Reduction of Bacterial Diversity in Environment Due to Indiscriminate use of Antibiotics

Sayan Mondal, Arunava Maity, Subhasis Sarkar, Suranjana Sarkar and Bidisha Ghosh

Abstract

The overuse of antibiotics has had a negative effect on the environment and significantly decreased the diversity of bacteria. A startling reduction in species richness and evenness is revealed by this study, which offers a thorough analysis of the impacts of antibiotic contamination on soil and water ecosystems. Antibiotics have been used extensively in human health, agriculture, and aquaculture, which has led to their discharge into the environment, where they can linger and build up. It has been demonstrated that high levels of antibiotic contamination upset the microbial community's delicate balance, jeopardizing ecological services like nitrogen cycling, decomposition, and promoting plant growth. The ecosystem may suffer greatly if beneficial bacteria, such as those that fix nitrogen and sequester carbon, disappear. Beneficial bacteria that fix nitrogen and sequester carbon are examples of those whose absence can have a profound impact on the resilience and overall function of ecosystems. Furthermore, antibiotic-resistant bacteria now pose a serious threat to human health due to the selection pressure that antibiotics have exerted. The abuse and misuse of antibiotics has been connected to the spread of antibiotic resistance genes in the environment, underscoring the importance of using and managing these drugs responsibly. Our findings highlight the significance of addressing antibiotic misuse's environmental effects in order to preserve ecosystem health and human welfare. The study's findings emphasize the necessity of sustainable practices, including better waste management, a decrease in the usage of antibiotics, and the creation of substitute therapies.

Keywords: Antibiotic contamination, antibiotic pollution, antibiotic resistance, bacterial diversity, ecosystem

Introduction

Since the start of the industrial era, anthropogenic activities have contributed significantly to an increase in environmental contamination [1].

Urban garbage, both liquid and solid, isa global pollution management concern [2, 3, 4]. The biodiversity of microbial ecosystem is altered by high concentration of chemicals, micropollutants, and antibiotics that are discharged into the environment through anthropogenic waste. Bacterial populations are stressed by xenobiotics produced from pollution in the surrounding environment. Bacterial populations naturally respond to stress by attempting to adapt by acquiring unique genetic features or resistance genes that enable them to survive and grow in the stressed environment, or by trying to resist the stressors. Pollution has been demonstrated to increase the selection of bacterial pathogens in the environment, while simultaneously decreasing the biodiversity of both terrestrial and aquatic microbial ecosystems [1, 2]. Although bacterial pathogens are able to complete a large portion of their life cycle outside of their host, once they are introduced, they can pose a considerable threat to public health since they can infect humans, animals, and plants and cause serious illness [5, 6, 7].

One of the most significant therapeutic developments in medical history is the development of antibacterial medications [8]. But over the past 20 years, there has been a significant rise in bacterial resistance [9], and multidrug-resistant bacteria are becoming more widespread and posing a major threat to public health [10, 11, 12, 15]. Notably, antibiotic resistance is not exclusive to the field of human medicine [13]. Human populations, food, farm animals, food products generated from livestock, animal waste, sewage, water, and the general environment can all develop and propagate antibiotic resistance [10, 12, 15].

Bacteria belonging to different phyla carry antibiotic resistance genes (ARGs) in a variety of environmental niches [16, 17, 18]. The Current Selective Pressure of clinical antibiotic use does not precede the natural phenomena of antibiotic resistance. Consequently, it has been suggested that every niche that is home to microorganisms can serve as a reservoir for ARGs, a concept known as resistome [19]. Approximately 30,000 years ago, Beringian permafrost sediment samples were found to contain ancient DNA, which included antibiotic resistance genes encoding mechanisms of resistance against clinically significant antibiotics like β-lactam antibiotics, tetracycline, and vancomycin [16, 17]. Antibiotics' function in nature is still poorly understood. Only a small portion of the bioactive chemicals that bacteria normally create are antibiotics. Only a small portion of the bioactive chemicals that bacteria naturally create in a variety of habitats are antibiotics ('The parvome') [28]. They may be produced by bacterial strains to inhibit the growth of a neigh boring bacterial strain in particular niche [22, 23], and they

are hypothesized to play a significant role in bacterial-cell interaction and communication. Interestingly, the amounts of antibiotics found in nature are much lower and not equivalent to those found in pharmaceutical formulations used in food production (aquacultural and agricultural) and clinical settings [20, 22, 25].

The global selective pressure for antibiotic-resistant bacteria and antibiotic-resistant genes (ARGs) has increased due to the use of antimicrobial agents (biocides and antibiotics) in clinical practice, veterinary medicine, agriculture, and aquaculture [25, 26]. When bacterial populations are exposed to high, medium, or low concentrations of antibiotics and other xenobiotic chemicals found in different environmental matrices, ARGs are selected and co-selected [26, 27, 28, 29].

Impact on global scenario

As infections become more difficult to treat with existing antibiotics, a global scenario of antibiotic pollution show that the environment is widely contaminated with antibiotic residues, mostly from human and agricultural use. This leads to the emergence and spread of bacteria that are resistant to antibiotics, which poses a serious threat to public health. This problem is especially problematic in areas with inadequate waste management systems, which further contaminates the environment.

Between 2000 and 2015, India's antibiotic usage increased by 103%, which is comparable to other low- and middle-income countries due to the prevalent practice of giving antibiotics without a proper prescription [27]. In 2004, the most often used medications in Delhi, India, were fluoroquinolones, cefuroxime, and cefixime, a combination of cefixime and clavulanic acid [20]. Cephalosporins, broad-spectrum penicillin, fluoroquinolones, tetracyclines, and trimethoprim are the five most often used antibiotics in India. It has also been observed that between 2000 and 2015, the average dosage of antibiotics consumed by per 1000 Indian citizens grew by 63%. Similarly, the rate of intake of antibiotics per 1000 persons increased by 39%, and their use increased by 65% globally [26, 27]. To encourage sensible antibiotic policies and stop the abuse of antibiotics, the World Health Organization (WHO) and the top medical, pharmaceutical, and research associations in India created the Chennai Declaration in 2012 [27].

One of the main markets for veterinary antibiotics worldwide is China. International efforts to stop the emergence of antibiotic resistance are hampered by a lack of awareness of emissions and environmental contamination. In order to forecast the total 2010-2020 emissions of 80

veterinary antibiotics, ranging from 23,110 to 40,850 tonnes/year, after 36-50% antibiotic elimination by manure treatment, this groundbreaking study integrated empirical data and mathematical methodologies. After rising by 10% between 2010 and 2015, emissions decreased by 43% in the years that followed. Even though 85% of emissions are released into the soil, under steady-state conditions, roughly 56%, 23%, and 18% of environmental residue ended up being dispersed over freshwaters, seawaters, and soils. 2020 saw 657 (319-1470) tons of freshwater from inland sources enter the ocean. The calculated median concentration of antibiotics in freshwaters was 4.7 × 103 ng/L, while in soils, it was 2.9 ng/g with the two main ingredients being tetracyclines and sulfonamides. 44 veterinary medicines were found to be at high risk of developing resistance in freshwater environments. Of these, seven were shown to be at high risk in more than 10% of freshwater locations in China. The majority of antibiotics in the tetracycline category showed heightened hazards; however, the largest risk of any single chemical was shown by sulfa methylthiazole. Overall, the susceptibility is highest in the Haihe River Basin. The results provide important backing for China's efforts to reduce veterinary antibiotic contamination [28].

Pollution sources include waste from industry, cities, medicine, agriculture and aquaculture, as well as their common denominator: (Waste)-Water

Wastewater (WW) releases a large amount of industrial, medical, and urban waste into the environment [1]. WW is discharged into the environment either treated, not sufficiently treated, or untreated, with the latter having the greatest negative effects on the receiving environment, depending on the infrastructure that is available and national restrictions [10]. WW is discharged into the environment either treated, not sufficiently treated, or untreated, with the latter having the greatest negative effects on the receiving environment, depending on the infrastructure that is available international restrictions [29]. Following WW's passage through wastewater treatment plants (WWTPs), the cleaned water is either returned to nearby surface waters or used Aasa crop irrigation source. After Undergoing Certain Treatments, WWTP sludge is occasionally utilized as fertilizer in agriculture [31, 32, 33, 34]. It has been suggested that WW and WW treatment plants act as hotspots for the selection and environmental spread of ARGs and bacterial diseases [35, 36, 37, 38]. Antibiotics, other xenobiotic compounds, pathogenic bacteria, and ARGs actually enter the environment most frequently through sewage out falls [39, 40, 41], agricultural fertilizers, and natural fertilizers like pig slurry and cow dung [29, 42, 43], which contain chemicals that select against

resistance, such as metals, biocides, and antimicrobials. Globally, food production sites in both terrestrial and aquatic environments serve as hotspots and sites of selection for antibiotic-resistant bacteria (ARBs) and ARGs due to the use of antibiotics and other antimicrobial agents in varying concentrations during food production [38, 44, 45].

In low-income nations without special WWT laws, the pharmaceutical sector contributes significantly to high levels of resistance-selecting chemical the receiving environment [46, 47]. Non-antibiotic pharmaceuticals like ibuprofen, found in high concentrations in urban WW systems, have been linked to the selection and transfer of ARGs [48, 49, 50]. According to the 2020 UN World Water Development Report, 80% of WW globally is released into the environment without treatment, with only 8% treated on lower income countries, 30% in lower-middle income countries, and 70% in high-income countries [51]. Fecal pollution significantly increases the amount of ARGs and ARBs in the environment [40]. Improving WW treatment options is crucial for reducing chemical and pharmaceutical pollution on a global scale [24, 41,48].

Sources	Types of Pollutant's (Antibiotic Compound)	Reference
Lake water	Cefradine 1st Cefuroxime 2nd Ceftriaxone 3rd Cefepime 4th	[52]
Ultrapure water	Amoxicillin, Ampicillin, Penicillin V, Piperacillin	[52, 55]
River water, Surface water	Tetracycline, Oxytetracycline, Chlortetracycline	[52]
Surface water, STP effluent's River water	Sulfamethoxazole	[52]
Surface water, River water	Sulfamethazine	[52]
River water, Surface water, deionized water, kaolinite suspension river water	Enrofloxacin, Ciprofloxaein, Ofloxacin, Norfloxacin	[52, 45]
Sea water, Wastewater, River water	Erythromycin Roxithromycin Clarithromycin Azithromycin	[52]

The Effects of Antibiotic Contamination Include Poor Microbial Diversity, Water Waste, Ecological Impact, Increased Risk of Infection, and Human Health

Every year, hundreds of thousands of people die as a result of antibiotic resistance in microorganisms. The most critical issue is the ever-increasing number of microorganisms resistant to frequently used antibiotics, even last resort medication like vancomycin [53]. This widespread pollution of

antibiotics in the environment exacerbates the establishment and spread of ARB, as these bacteria are frequently exposed to low levels of antibiotics, allowing them to adapt, live, and evolve. Mutation generated by low antibiotic dosage appear to be the most important cause of antibiotic resistance. Antibiotics can cause specific mutations in bacteria, even if they are not fatal. These mutations can accumulate over time, creating antibiotic-resistant bacterial species that are difficult to treat. For example, sublethal doses of bactericidal drugs such as norfloxacin, kanamycin, and ampicillin can induce mutagenesis in *E. coli*. Sublethal antibiotic dosage increased the generation of reactive oxygen species (ROS), resulting in the creation of mutations in E. coli that provided resistance to antibiotic therapy. Similarly, exposure to sub-MIC (1/4) ciprofloxacin resulted in the rapid generation of ciprofloxacin resistance mutants with 100 times higher minimum inhibitory concentrations (MICs) than the initial population [54].

ARBs and ARGs are transported from resistance evolution hotspot to pristine habitats using a variety of vectors. Vectors can be used solely for transporters are reservoir for ARBs and ARGs to survive, multiply, and evolve. While ARGs are likely to be carried between environments within ARBs, most studies do not look into this directly, instead assessing various putative vectors for the presence of a panel of resistance genes. As a result, the genetic context of the majority of ARGs in the environment remains unclear [55].

Surface waters collect WWTP and pharmaceutical effluent, as well as runoff from manure-fertilized field sand AFOs, and hence serve as primary hub for the transportation and diffusion of ARBs. ARG profiles discovered in rivers were heavily influenced by the pollution source (e.g., WWTP or AFO), indicating that ARBs are transferred rather than selected there. In addition to the existence of ARGs in rivers, substantial amounts of gene transfer elements to aid SGT were also found, showing the possibility for ARGs to modify their genetic background during transfer [41, 56, 55].

Antibiotics have also been found to affect higher species by changing the microbial communities associated with animal hosts. Animal microbiomes serve a variety of functions for their hosts, including effective food metabolism and bone building. As a result, disrupting a host's microbiome, known as dysbiosis, can have serious health effects such as developmental abnormalities, allergies, metabolic illnesses, and increased vulnerability to pathogens [1, 20, 55].

Antibiotics affect higher organisms in addition to microbial populations. Standardized clinical trials and pharmacology reports have established the side effects of several antibiotics in human medicine. However, some contend that the environmental levels of antibiotic compounds are incredibly low and that human health risks are negligible. Low levels of environmental antibiotics can build up in human populations from prolonged exposure to food, drink, or consumer goods, with unidentified health consequences. For instance, drinking water that has been chlorinated has been shown to include quinolones and macrolides. Streams and rivers all across the world contain triclosan, an antibacterial substance used in consumer products like soap and clothing. Additionally, it has been detected in non-users' human serum, urine, and breast milk, which may result in muscle weakness and reproductive problems. According to estimates, 75% of Americans have come into contact with triclosan or other antibiotics through consumer products.

While toxicity thresholds for people are frequently established, there is relatively little knowledge about harmful concentrations in wild animals, particularly smaller and more vulnerable creatures. Low quantities of antibiotics like streptomycin and erythromycin can affect the survival and behavior of macro invertebrates like Daphnia magna and Artemis in lab settings. Further research indicated that antibiotic toxicity can be increased after exposure to UV radiation, as is the case with most species in the wild, underlining the challenge of determining the risk associated with antibiotic pollution in natural habitats through lab experiments [1, 55, 28].

Bacteria and fungi that live in soil and water are not only the foundation of the world’s most diverse and densely populated ecosystems, but they also play critical roles in important ecological functions such as nutrient cycling, decomposition, and primary productivity in a variety of environments. Antibiotic pollution-induced selective pressures can have an impact on the general makeup of the microbial community by diminishing taxonomic diversity or altering it. In general, antibiotic exposure leads to an increase in antibiotic exposure leads to an increase in than Gram-positive bacteria. The latter are more susceptible to antibiotics and disinfectants due to the lack of an outer cell membrane. Antibiotic exposure may therefore result in the loss of essential microbial taxa that play critical ecological roles [55].

For instance, it has been demonstrated that antibiotic contamination reduces aquatic environments' overall microbial diversity, including species that are essential for primary productivity and carbon cycling. Antibiotics in soil have also been how to alter the structure of microbial communities,

leading to a reduction in microbial respiration, nitrification, and denitrification as well as a loss of biomass. Antibiotics can also affect the activity of bacterial enzymes, including phosphatases, ureases, and dehydrogenases, which are important markers of soil activity. Lastly, the number of parasites and pathogens in soil and water might rise as a result of antibiotics disrupting microbial populations. For instance, it has been shown that antibiotic contamination in aquatic environments increases the prevalence of dangerous Cyanobacteria species, which leads to freshwater eutrophication [50, 54, 55].

Treatment Procedures of Antibiotic Pollutants Water

Both primary (mechanical treatment, including as filtration and sedimentation) and secondary (biological processes to remove organic contaminants via aerobic or anaerobic systems) treatment procedures are commonly used in conventional WWTPs. Traditional activated sludge is the most widely used biological method. Membrane bioreactors (MBRs) are less common, probably because of the high energy consumption and operational costs involved in maintaining sustainable filtering conditions. The MBR method combines biological degradation through active sludge with modern membrane filtering technology, utilizing both aerobic and anaerobic processes.

The main benefit of MBR is the high quality and suitability for reuse of the treated water. By eliminating a wide spectrum of bacteria by size exclusion, membrane bioreactors, which employ micro-or ultra-filtration membranes with a diameter ranging from 0.04 to 0.4 µm, greatly improve the microbiological purity of the effluent that is produced. A Swiss hospital's influent removed 51% of ciprofloxacin, 47% of norfloxacin, less than 60% of erythromycin, 7% of sulfamethoxazole, and 96% of trimethoprim, according to a pilot-scale MBR research. An anaerobic membrane bioreactor (AnMBR) successfully decreased the amounts of trimethoprim and sulfamethoxazole in wastewater by 94.2 ± 5.5% and 67.8 ± 13.9%, respectively, in a study by Xiao *et al.* Additionally, high ampicillin eradication rate of 94.4% was achieved with MBR therapy. In Municipal wastewater treatment plant, 82% of ampicillin was nominated by the activated sludge process whereas 91% was destroyed by the disinfection process. The superior effluent produced by MBR sewage treatment sets it apart from traditional activated sludge systems. Trailer parks, motels, and ski resorts all use MBR, a portable technique with several advantages. Instead of being released into the environment, MBR effluent could be utilized for technical purposes like irrigation, snowmaking on ski slopes, or other

industrial processes involving non-potable water. Given the current worldwide water problem and water management regulations, reusing water has many advantages.

As a result, less water is used, and new contaminants like antibiotics that are released into the environment can be eliminated or minimized. More recent practices combine MBR with cutting-edge water treatment techniques such reverse osmosis, UV irradiation, post-ozonation, and activated carbon. According to Xiao *et al*., the elimination of sulfamethoxazole from the MBR bioreactor rose from 67.8 ± 13.9% to 95.5 ± 4.6% when 1 g/L of powdered activated carbon was added. Ala cable used activated carbon made from pumpkin seed shells to remove ciprofloxacin from aqueous settings with over 99% efficacy. In activated carbon adsorption systems, Vander Waals interactions are the main mechanism for removing organic chemicals, including antibiotics. Because of their high specific surface area, porosity, and reaction activity, carbon-based materials-such as graphene, carbon nanotubes, and activated carbon-make good adsorbents for antibiotics that pollute water. Both the properties of the chemical (such as size, charge, and hydrophobicity) and the adsorbent (such as surface polarity and porosity) affect how successfully different compounds are removed. Liu et al. looked into treating surface water tainted with antibiotics and pharmaceutical personal care products using a membrane bioreactor with ultraviolet/chlorine (MBR-UV/Cl2).

The single MBR system was obviously unable to remove sulfamethoxazole in the study that was provided. However, 95.6% of the sulfamethoxazole in the contaminated surface water is effectively removed by treating the MBR permeate with UV irradiation and chlorination (at a chlorine concentration of 3 mg/L). The effectiveness of antibiotic elimination was greatly increased by the UV/CI2 method. Similar outcomes with sulfamethoxazole elimination (100 µg/L) were reported by Tambosi *et al*. The treatment of wastewater from a German municipal treatment facility was the main focus of the investigation. Advanced methods like H2O2/UV, H2O2/Fe2+ (Fenton), H_2O_2/Fe2+/UV (photo-Fenton), UVr adiation, and ozone were used to treat the permeate throughout the MBR process. The findings shown that after 30 minutes of treatment, sulfamethoxazole was removed from the MBR process at a rate of 64%. Nevertheless, the sulfamethoxazole was totally removed from the MBR penetrates by ozonation, UV light, and sophisticated oxidation processes (H_2O_2/UV, H_2O_2/Fe2+/UV). The sensitivity of sulfamethoxazole to every UV treatment process was quite high. Nevertheless, the Fenton process was completely

unsuccessful in getting rid of this molecule (elimination rate: 0%). The best method for totally getting rid of trimethoprim and sulfamethoxazole from the MBR permeate is ozone treatment. Additionally, tetracycline antibiotics are effective eliminated from synthetic wastewater by the use of anoxic/aerobic-MBR treatment. Tetracycline, chlortetracycline, and oxytetracycline had elimination efficiencies of 93.6%, 82.9%, and 88.6%, respectively, following 60 days of solid retention. One of The Most Prevalent antibiotics discovered in wastewater is tetracycline. In an integrated pilot-scale membrane bioreactor combined with reverse osmosis (MBR-RO) for the treatment of municipal wastewater, Dolar *et al*. demonstrated promising outcomes. The MBR therapy eliminated 87% of erythromycin and 75% of azithromycin from macrolide medication. Ofloxacin removal wax ineffective in this trial, however MBR therapy largely eliminated sulfamethoxazole (69%) in this investigation. For every drug under investigation, the RO membrane (pore size range < 0.001µm) exhibit antibiotic clearance rate of >99%. Dolar *et al*. found that antibiotic removal performance was much greater in MBR paired with the RO system, efficiently removing low-molecular-weight pharmaceutical substances. Metal-organic frameworks (MOFs), which are multi-dimensional materials linked together by bonds between metal atoms and organic ligands, have recently been proven to be efficient in cleaning antibiotic-contaminated wastewater. MOFs have favorable qualities such as large surface area and pore volume, hierarchical structures, biocompatibility, non-toxicity, regeneration capabilities, and programmable pore size and functional groups, making them appropriate for wastewater treatment procedures MOFs can keep their structures intact in aqueous environments. Using MOFs in WWTPs can considerably improve treatment efficiency. MOFs can be used in wastewater treatment for adsorption, filtration, and degradation, including the catalytic breakdown of antibiotics by immobilized enzymes. Zhou *et al*. studied the detection and removal of 0.1 mM tetracycline solution in water using a luminous MOF. Zhou et al. used a luminous MOF to detect and remove a 0.1 mM tetracycline solution in water, and 56% of the antibiotic was eliminated after 30 minutes. Dong et al. demonstrated the photocatalytic degradation of oxytetracycline using a stable 8-connected Cd(II) MOF as a photocatalyst. Metal-organic frameworks are thought to be useful materials for adsorbing and removing emerging contaminants from wastewater, such as antibiotics.

Zeolites are beneficial minerals that can be used to remediate sewage that contains antibiotics. Zeolites are sorption materials that can hold onto antibiotic residues in wastewater treatment system if they are appropriately engineered and functionalized. One advantageous property of zeolites that

improves antibiotic adsorption in aqueous solutions is their hydrophobicity. Sulfonamide antibiotics were successfully removed from water by high silica-zeolites (>90%). Antibiotics are also extracted from water using both natural and synthetic minerals. They provide special features such a high specific surface area, affordability, accessibility, and superior removal effectiveness. Using the natural colemanite mineral (a mesoporous substance) as an adsorbent, four widely used fluoroquinolones were eliminated from wastewater and surface water samples. The following antibiotic was discovered using batch adsorption trials. Ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin were all removed at 81.9%, 78.4%, 80.3%, and 79.7% of their initial levels, respectively, according to batch adsorption studies.

According to a number of studies, MBR systems that use ultrafiltration are only partially successful in eliminating antimicrobial substances from wastewater. Advanced oxidation processes (AOPs), UV radiation, activated carbon, and adsorption techniques (MOFs, zeolites, and natural materials) in conjunction with MBR treatment have the potential to become successful technologies for treating surface water and wastewater contaminated with antibiotics. Wastewater that has been cleansed by the MBR process can be recycled into artificial snow or discharged into delicate waterways. It is suitable for usage in spaces with limited space and can be utilized for municipal sewage treatment because of its small size. It eliminates the disadvantages of traditional activated sludge procedures, specifically the necessity for a lot of area for secondary clarifiers and the generation of excess sludge. The employment of sophisticated wastewater treatment techniques offers excellent opportunities for the cost-effective reuse of wastewater free of micropollutants like antibiotics. The quantity of antibiotics discharged into the environment after treatment is decreased when wastewater's antibiotic content is decreased. This lessens the possibility of drug resistance in environment microbes and other detrimental effects on the environment.

Conclusion

It has been determined that antibiotics are emerging micropollutants that affect microbial communities. Antibiotics and drug resistance factors are mostly transported between environmental compartments via water. Antibiotics enter the aquatic environment more quickly than they are eliminated. Long-term exposure to sub-inhibitory antibiotic doses (ng/L-µg/L) in water is the primary cause of bacterial genome changes that lead to drug resistance and the horizontal gene transfer (HGT) of drug resistance

genes. Antibiotics, which serves as signaling molecules and alter the ecological activity and makeup of bacterial communities, are the ecological force driving bacterial evolution. This lowers the biodiversity of bacteria, which is essential for ecosystems' biological processes to operate properly.

Drug-resistant mutations can arise from the alteration of bacterial populations and the impact of antibiotics with low concentrations and bioavailability of transcriptional control. Antibiotics are one example of how microorganisms adjust to new environmental factors. This is especially true in sensitive areas, such a pristine alpine ecosystems, where human activities pertaining to agriculture, tourism, and animal husbandry may have a substantial impact on rivers.

The majority of research findings that have been published so far have concentrated on metropolitan areas that have been impacted by antibiotic pollution and the changes that have occurred as a result. The effects of antibiotic pollution in pristine alpine environments, which are especially vulnerable to change and under a great deal of anthropogenic strain because of popular mountain tourism, among other factors, have not, however, been well studied. Heavy tourist traffic overloads wastewater treatment systems with effluent carrying micropollutants, causing antibiotic poisoning of the mountain ecosystem. After being administered, antibiotics are only partially digested in the bodies of humans and animals, leading to parents for delivery to wastewater treatment facilities and unchanged excretion at levels between 5 to 85%. These micropollutants cannot be sufficiently removed by conventional wastewater treatment facilities, which use primary and secondary wastewater treatment techniques. Surface waters containing treated sewage also contain antibiotics. Surface water in mountainous regions is utilized for tourist recreation, crop and green area irrigation, and snowmaking on ski slopes. Significant or complete removal of Anti-microbial chemicals from wastewater is made possible by additional wastewater treatment using contemporary technologies (MBR, UV radiation, activated carbon, membrane methods, oxidation processes, and MOFs). The possibility of implementing advanced wastewater treatment methods reduces the quantity of antibiotics discharged into the environment, offers a cost-effective way to use water resources, and allows wastewater to be safely reused for technological purposes.

The upper river course of protected high- mountain national parks have already been shown to be contaminated by antibiotics. Antibiotic pollution can also come from mountain lodges without sewage system. The continued growth of mountain tourism and protecting the pristine mountain ecosystem

are incompatible. The greatest risks to public health are the emergence of medication resistance and the potential spread of ARGs from environmental trains to clinical strains. Since alterations in microbial genomes will be influenced by the environment's consistent supply of subinhibitory antibiotic doses, there is possibility that environmental and clinical drug resistance will be connected. Changes in the composition and diversity of microbial populations, which control key ecological processes in the ecosystem, give rise to environmental issues. Monitoring antibiotic contamination in surface waters is therefore essential. Surface stream contamination by antibiotics is particularly harmful in alpine settings.

This is because, in every country, mountain water resources serve as a source of potable water and rea vital natural resource that is primarily located in pristine, protected areas where they supply rivers. Because protected areas are a significant source of biodiversity, they must be maintained. Antibiotics are one micropollutant that is not regularly evaluated.

A substantial knowledge gap is a dearth of knowledge regarding the locations and timing of crucial alterations that impact the development of antibiotic resistance in bacteria as well as modifications to the composition of bacterial populations. Additionally, the knowledge gaps are especially important in the pristine mountain environment, which is the source of drinking water resources. Addressing environmental and public health challenges requires an understanding of how antibiotic contamination affects the environment [52].

Conflict of Interest

The authors have no conflict of interest.

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Chapter - 2
Edible Films from Biodegradable Products in Minimizing Plastic Pollution

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Chapter - 2

Edible Films from Biodegradable Products in Minimizing Plastic Pollution

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Abstract

Food packaging plays an important role in protecting the food from external environment and it also provides information about the manufacturer name and address, preparatory ingredients, expiry date or best before dates. But over the years, these use plastic as packaging material creates a lot of pollution because this petroleum-based plastics are neither biodegradable nor environment friendly and it requires years to decompose. Many microorganisms cannot degrade non-biodegradable substances. To address these issues biodegradable and edible materials are advised to be used because are quickly and easily degraded by the microbes and other environmental factors. Especially edible materials are safe to humans or animals to consume, so they can be used for packaging food while also reduce waste production. Edible films are biobased products that are usually transparent. There are several polysaccharides such as cellulose, starch and animal proteins like milk, egg white, gelatin, whey protein and plant proteins like soy protein, chickpeas which are the renewable sources of edible food films. Edible films provide sustainability as well as antimicrobial, antioxidant and nutritional properties. The antimicrobial property helps to extend the shelf life of food product by incorporating naturally derived active components (essential oils, ash gourd wax, green tea leaf extract) into the biopolymers. These biodegradable products offer biocompatibility and low toxicity in our environment.

Keywords: Plastic pollution, food packaging, biodegradability; edible films; antimicrobial p shelf life, naturally derived active component

1. Introduction

Food packaging is essential to maintain food products' quality, safety, and shelf life (Pei *et al*. 2024). As stated in ASTM E460, low oxygen and moisture permittivity in ambient circumstances, inertness toward food

material, and chemical and mechanical stability in specified environmental conditions are the primary traits of packaging materials (Ashfaq *et al*., 2022). A wide range of materials that includes papers, glass, metals and plastics (such as polyethylene, polypropylene and polyethylene terephthalate) have traditionally been used to packaged food products. The food packaging industry utilizes plastic extensively because of its qualities, which include low production costs, durability, strong mechanical capabilities, and resistance to chemicals and oil. But the primary cause of plastic pollution in the world is food and drink packaging (Gaspar *et al*. 2023). Around 37% of the market for food packaging is made up of petroleum-derived plastics because of its low cost of production, lightweight, ease of transportation, high tensile strength, and form adaptability. However, because plastic garbage is ignited, petroleum-derived plastics are accountable for number of hazards. Burning synthetic plastics contributes to global warming because it releases a significant amount of carbon dioxide. It is therefore essential to design and develop new packaging materials at this time. (Gupta *et al*. 2022). Researchers, government and organizations have recently undertaken initiatives to mitigate the effects of plastic pollution on the environment. However, current research indicates that this industry is moving slowly and unevenly away from packaging composed of petroleum and toward more environmentally friendly options. In actuality, businesses choose to gather and recycle plastic instead of switching to environmentally friendly packaging options. Therefore, the authors believe that in order to hasten the switch from conventional to environmentally friendly packaging solutions, additional funding, actions, pledges, and even incentives must be made available to research institutes and companies (Gaspar *et al*. 2023).

It has been studied to replace conventional packaging techniques with cutting-edge materials like edible films (Gaspar *et al*. 2023). This alternative is sustainable to our environment. A variety of guidelines are included in sustainable packaging, with the goal of minimizing the environmental impact of packaging systems and materials over the course of their lifetimes. This entails cutting down on the use of resources, lowering greenhouse gas emissions, encouraging compost ability and recyclability, and lessening pollution. In addition to addressing environmental issues, sustainable packaging adapts to changing customer tastes of goods that uphold ecological and ethical standards. Additionally, it shows how governments and companies are committed to implementing more ethical practices in response to global environmental challenges (Pei *et al*. 2024).

The origins of edible films made from biodegradable plant and food residues, as well as the use of antibiotics in edible films, are the main topics of this review study. Because of their accessibility, affordability, biodegradability, and ability to form films, polysaccharides like cellulose, starch, chitosan, and alginate were widely used. Furthermore, research was done on the potential applications of proteins including soy protein and wheat gluten in food packaging as well as their ability to produce films. These films demonstrated positive attributes such flexibility, oxygen and moisture resistance, and biocompatibility. (Olawade *et al*. 2024).

2. Edible films from Plant and Animal-based Materials

2.1 Polysaccharides

Numerous polysaccharides in plants, and they have a variety of properties that make them valuable for making plant-based goods and edible films. Alginate, starch, chitosan, and cellulose and its derivatives are some of the most common polysaccharides used in this situation. Plant cell walls are the source of cellulose, the most prevalent biopolymer on the planet. It has outstanding barrier qualities, mechanical strength, and thermal stability.

Nevertheless, its direct application in film creation is restricted by its water solubility. Cellulose derivatives including methylcellulose, carboxymethylcellulose, and hydroxypropyl cellulose are frequently used to get around this. These derivatives maintain some of the favourable characteristics of cellulose but show increased solubility and film-forming ability (Olawade *et al*. 2024).

Another readily available polysaccharide that comes from a variety of plant sources, mainly corn, potatoes, and wheat, is starch. Because of its affordability and natural availability, starch is greatly preferred. it has the potential to form films, is biodegradable, and has high oxygen barrier qualities. Materials based on starch can be altered to improve their mechanical strength, stability, and resistance to water by using techniques including plasticization, cross-linking, or combining with other polymers (Olawade *et al*. 2024).

Chitin, is present in the cell walls of fungus and the exoskeletons of crustaceans, is the source of chitosan. It is biodegradable, has strong film-forming ability, and antimicrobial qualities. Because chitosan-based films can prolong the shelf life of perishable goods by preventing microbial growth and preserving product quality, they have shown promise in a variety of applications, including food packaging (Olawade *et al*. 2024).

Because of its capacity to form films, alginate-which is generated from brown seaweeds-has been the focus of extensive research. Alginate-based films are extremely biodegradable, have good water vapour barrier properties, and can be engineered to have controlled release characteristics. These films are used in wound dressings, food packaging, and pharmaceutical delivery systems. In are cent work, for example, citric acid is utilised to crosslink composite films composed of chitosan and soybean protein isolate to produce an edible bioplastic film with increased hardness and strength. Promising outcomes included a 5-day delay in fruit ripening, a 75% edible rate for cherries and strawberries 5-7 days after storage, and the prevention of heat decomposition by chemical inhibition and antibacterial qualities (Olawade *et al*. 2024).

Food firms have been using plant gums for a long time. They are all polysaccharides with different functions that are produced by different plants. Plants can produce vegetable gums through the natural exudation processor removing tissue from different parts of the plant. Chemically speaking, these are polysaccharides, consisting of glycosidic bonds joining several monosaccharide units. Many exudate gums, including karaya and acacia, and seed/mucilaginous gums, like locust bean and guar, are widely recognized for their commercial use. Arabic gum extracted from Acacia species is the most widely used polysaccharide in industry due to its exceptional emulsification, encapsulation, and film-forming abilities. Arabic gum is utilized in the food, cosmetics, and pharmaceutical industries It is made up of galactose, rhamnose, arabinose, and glucuronic acid (Song *et al*. 2021).

2.2 Proteins

As potential replacements for plant-based materials and edible films, plant-based proteins have attracted attention. Casein, wheat gluten, soy protein, and other plant-based proteins are often researched in this regard.

Made from soybeans, soy protein isolates and concentrates have a number of beneficial properties that make them suitable for use in film making. The soy protein-based films good mechanical strength, flexibility, and oxygen barrier properties. They can also be modified by mixing with other polymers, adding additives, or cross-linking to enhance their properties and expand their range of applications.

A byproduct of processing wheat, wheat gluten has drawn a lot of interest as a sustainable film-forming substance. It has outstanding a barrier qualities, biodegradability, and film-forming ability. Applications for wheat

gluten films in food packaging have been investigated; these films provide a biodegradable and renewable substitute for plastic derived from petroleum.

Casein is milk protein with remarkable mechanical properties, a water vapor barrier, and the ability to produce translucent films. Casein-based films are widely used in food packaging due to their high oxygen barrier, biodegradability, inability to stop moisture transfer. Additionally, by including nanoparticles or antimicrobial agents, casein films can be modified to enhance their functionality. (Olawade *et al*. 2024).

Collagen can be found in fish or animal skin and bones. For many years, meat products have used collagen edible films to keep moisture in and give them a uniform look. In animals, 20-25% of their total body mass is made up of collagen and gelatin. Its structure is comprised of three cross-linked α-chains, whereas gelatin-a denatured collagen derivative made up of several polypeptides and proteins-is made up of only three. The amino acids methionine, hydroxyproline/proline, and glycine makeup the majority of collagen. Collagen is hydrolyzed to form gelatin. Gelatin is produced by pre-treating collagen, which is mostly found in animal skins, muscles, bones, and connective tissues, with an acid/alkaline solution and then heating it to 40 °C. Pure and dry gelatin has mild yellow tint and is transparent, brittle, tasteless, and odourless. It also resembles glass. Typically, the gelatin solution is dissolved in hot water, the gelatin-derived edible film is then poured onto a plate, and it is dried in an oven. (Song *et al*. 2021).

2.3 Lipids

Casein is milk protein with remark able mechanical properties, a water vapor barrier, and the ability to produce translucent films. Casein-based films are widely used in food packaging due to their high oxygen barrier, biodegradability, inability to stop moisture transfer. Additionally, by including nanoparticles or antimicrobial agents, casein films can be modified to enhance their functionality (Song *et al*. 2021).

The potential of natural waxes derived from plants, such as candelilla, carnauba, and beeswax, for the creation of edible films has been investigated. These boxes have advantageous qualities include the ability to form films, being hydrophobic, and repel water vapor (Olawade *et al*. 2024).

It has been discovered that essential oil shave strong antibacterial and antioxidant properties. Secondary metabolites called essential oils are created by aromatic plants and may have therapeutic, fragrance, and antioxidant effects. and antioxidant effects, terpenes, and other fragrant chemicals, they have a significant antimicrobial impact. Their chemical

makeup and amount of individual compounds determine their antimicrobial action, and the growth stage of spoilage microorganisms determines how effective they are (Song *et al*. 2021).

Plant cells produce compounds called resins in response to damage or infection in trees and shrubs. If not, certain insect species may generate them; *Laccifer lacca*, for example, produces shellac resin. Most resins are physically solid or semisolid and transparent with tones of golden brown.

Table 1: Sources of Edible Films

Polysaccharides	Proteins	Lipids
• Starch • Chitin • Cellulose • Alginate • Gum	• Soyprotein • Wheatgluten • Milk protein • Collagen • Gelatin	• Essentialoils • Waxes • Resins

3. Edible Films from Food Residues

Particular food residues and by-products that could be used as components in edible and biodegradable films for the preservation of fruits, vegetables, meat, fish, and derivative products have been the subject of numerous research recently. Different plant parts-skin, peel, seed, pomace, husk, and straw, among others-are often thought of as residues because they are high in polymers like proteins and polysaccharides, which are used as the primary raw materials to make edible films.

Pumpkin wastes have been used by authors to extract pectin and protein, which they subsequently used as materials to build films. Are cent study also looked into the possibility of using pomegranate peel and mung bean protein from the food industry to create edible films for food product packaging. Peel potatoes are used to make edible films, and when curcumin was added, the amount of lipid oxidation in the fresh pork during storage was significantly reduced. Chitosan films infused with banana peel extracts for use as an apple covering also showed antioxidant activity. Recent research has looked into using fibres from mushroom byproducts to make edible films. The authors have previously looked into the potential of a number of food residue, including various tomato wastes (rotten, green, and tomato branches). They discovered that these residues are rich in flavouring compounds, antioxidant activity, and phenolic compounds, making them potential sources to include in edible films or coatings (Gaspar *et al*. 2023).

4. Challenges for Industrial Scale

There is virtually no disposal problem because edible packaging can be consumed with the food or drink it contains. Human-consumable materials that have the capacity to create a continuous and coherent network make up edible packaging. Even if it is not eaten, it decomposes faster than synthetic and biodegradable packaging materials. Landfill space may be greatly reduced as a result. Since the materials used for packaging are safe for human consumption, the transfer of packing molecules into food does not cause any health issues. It is safe to conclude that because of these advantages, edible packaging has become very popular as a substitute for synthetic and biodegradable plastic packaging in food packaging applications. Lipids, proteins, and polysaccharides are the traditional polymers used to make edible films. The polysaccharide-based edible films are excellent gas barriers despite having poor mechanical strength and negligible water vapour resistance. The protein films are mechanically strong, however they are not very resistant to water vapour. Lipids, on the other hand, can't be used to make edible films or construct self-supporting structures, although they do show good resistance to water vapour. In order to make edible composite films, lipids are used in applications involving edible coatings or as an ingredient in additives with protein and/or polysaccharide films. (Jeevahan *et al*. 2020).

A good edible film should meet a number of requirements, including having excellent sensory qualities, high barrier qualities, high mechanical strength, high microbiological stability, no toxins, health safety, ease of production, non-polluting, and affordability. Production of edible films is still done in laboratories. For edible films to be commercially successful, a number of obstacles still need to be solved. Edible films have a number of drawbacks over synthetic plastics, including low mechanical strength (particularly poor elongation), poor gas and liquid resistance, inadequate evaluation of edibleness and biodegradability, challenges with processing scale-up, etc. The research community put great importance on the commercial side of edible films. The scaling up and commercialization aspects of this article are-film making and drying methods; lack of knowledge; and consumer acceptance. Each section covers research opportunities, challenges, and advancements in the field to ensure the commercial success of edible films in food packaging applications (Jeevahan *et al.*, 2020).

Film Making Drying Methods

Thecastingtechniqueistypicallyappliedtotheproductionoffilmsinlaboratoryscale. A substantial amount of water in cast films needs to evaporate, which is thought to be a highly energy-intensive process. There is no need for an evaporation stage with dry procedures. As a result, drying techniques may reduce the amount of time needed for drying. However, the properties of these films are greatly influenced by several process variables, such a barrel temperature, die pressure, screw speed, energy input, moisture content, and die diameter. Post-extrusion methods such as thermo-compression, injection molding, and film blowing are also required to create specific file properties. Further research is required to completely understand the mechanism of film creation and optimize the process parameters in order to create edible films on an industrial scale. The current laboratory-scale film-making methods' incapacity to create films larger than 25 cm, their long drying times (2-4 days), and their imprecise thickness control make them suitable for scaling up to industrial production. To generate edible films on a big scale, continuous filmmaking with short production times and high production rates needs to be established. (Jeevahan *et al*. 2020).

Lack of Knowledge

The structure of edible polymers, their orientation, the amount of bases and acids present, the degree of cohesion and adhesion, the crystallinity index, temperature and pressure, free volume, additives, film thickness, and other factors can all affect the properties of edible films. The effects of a few of these elements have been studied by very few researchers, and the effects of the majority of these factors are still unknown. The ability of edible films to withstand carbon dioxide, oxygen, and water vapor is often used to assess their barrier qualities. However, there has been relatively little research on the permeability of food ingredients, such as tastes and oil permeability, despite the fact that these permeabilities are crucial for food packaging applications (Jeevahan *et al*. 2020).

Acceptance by Consumers

Only when they perceive edible films to be safe do consumers accept them. Only a small number of studies were able to provide conclusive proof of the biodegradability and edibility of edible film, despite researchers' claims that the film can be consumed with the food they contain. Recent studies have shown that the film-forming process, the film-forming materials, the type of plasticiser, additives, structural features, composition, chain length, molecular arrangement, and the crystallinity index can all have

a significant impact on digestibility and digestion rates. The acceptance of edible films may be hampered by lack of understanding and fear. in order to draw the attention of customers, marketing techniques including running awareness campaigns, offering discounts, making enticing offers, and running commercials could be useful (Jeevahan *et al*. 2020).

5. Conclusion and Future Perspectives

There is an immediate need for the alternation of petroleum-based plastic packaging due to increasing global plastic pollution. Edible films, from polysaccharides and proteins and food residues, are biodegradable and eco-friendly to nature. This can lessen the amount of plastic that ends up in the ground. Many benefits, including biodegradability, antioxidant, and antibacterial qualities, being investigated in relation to edible films; however, in order for edible films to be accepted as food packaging, a few issues must be resolved soon. Key conclusions about the challenges described above are listed below:

- The current laboratory-scale production of edible films is not suitable for scaling up to an industrial scale due to a number of problems, such as the inability to produce continuous films, the time needed for drying, the inaccuracy of controlling thickness, the high energy consumption and the high cost. To increase production, these problems need to be addressed in future study.
- A few of the quality of the films were the subject of the majority of investigation, with many additional aspects going unexplored. In the future, edible film will have multiple uses and work with contemporary packaging technology. On the other hand, a thorough investigation of the fundamentals of the disregarded elements and film qualities is necessary.
- Consumer adoption of edible films may be impacted by a number of factors, including a lack of proof about edibility and biodegradability, organoleptic elements, alack of legal considerations, lack of marketing, public awareness, cultural concerns, and a fear of toxicological and health repercussions. To increase the commercialisation success, these factors should be taken into account in future edible film research. (Jeevahan *et al*. 2020).

Conflict of Interest

The authors have no conflict of interest.

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Chapter - 3
Phytoremediation: Harnessing Nature's Power for Heavy Metal Decontamination in the Anthropocene

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Chapter - 3

Phytoremediation: Harnessing Nature's Power for Heavy Metal Decontamination in the Anthropocene

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Abstract

Heavy metal (HM) contamination of water resources, driven by industrial, agricultural, and mining activities, poses significant environmental and health risks. As HMs accumulate in the food chain, effective remediation strategies are crucial. Phytoremediation, an eco-friendly and cost-effective approach, utilize hyperaccumulator plants to extract, detoxify, or stabilize HMs in contaminated environments. This method encompasses five main techniques: Phyto stabilization, phytodegradation, rhizofiltration, phytoextraction, and phytovolatilization. Each targets specific aspects of HM contamination, from immobilization to extraction and volatilization. While conventional phytoremediation faces limitations in large-scale applications, biotechnological advancements aim to enhance plant efficiency in HM decontamination. Research focuses on identifying and developing high-efficiency metal hyperaccumulators for both phytomining and phytoremediation purposes. These efforts seek to overcome current constraints and expand the technology's applicability. This review comprehensively examines various strategies and biotechnological approaches for removing HM contaminants from the environment. It highlights recent developments in phytoremediation techniques and their applications in treating diverse toxic pollutants. Additionally, the view explore the origins and impact of HM contamination, as well as the factors influencing phytoremediation effectiveness, providing a holistic overview of this promising environmental restoration technology.

Keywords: Nanotechnology, sustainability, waste management, heavy metals, phytoremediation, phytoextraction and genetic engineering

1. Introduction

Heavy metal (HM) pollution of soil has become a major global environmental concern due to industrialization's rapid growth and

agricultural practices. In nature, HMs are highly persistent and have a half-life of more than twenty years. As a result, they are regarded as ubiquitous contaminants. Elements having densities more than 5 g/cm3 are listed as heavy metals. Partitioned into two groups, essential and non-essential are HMs. These basic HMs, which are regarded as micronutrients but are poisonous when ingested in excessive quantities, include a variety of elements such as manganese (Mn), zinc (Zn), cobalt (Co), nickel (Ni), copper (Cu), iron (Fe), chromium (Cr). Incredibly deadly to living things are the non-essential heavy metals (HMs) cadmium (Cd), mercury (Hg), and lead (Pb).

Although HMs are naturally present in rocks, human activity has led to an increase in their concentration. Sources of heavy metals (HM) in soil include overuse of sewage sludge, agrochemicals, wastewater, biosolids, and manure. Heavy metals (HMs) build up in the soil and seriously harm plants, animals, and people's health. Literature indicates that contaminated soil with heavy metals (HM) has impacted about 10 million individuals globally. As a result, HMs accumulate in crops through the soil-root contact and represent a major risk. The two mostimportantnaturalresourcefoundationsforthelong-termsustainabilityofagricultureand the existence of civilizations are water and soil. Furthermore, soil and water contamination is a result of both natural and human activity.

In addition to producing garbage, these activities endanger the environment, natural habitats, and public health. The organic parts of the trash decompose naturally, but because of their persistent persistence in the environment, metalloids and heavy metals provide a new problem. There must be certain concentration range for certain of these HAs since they have beneficial effects in biological systems. Fe, Co, Mn, Mo, and Zn are necessary for many physiological and metabolic processes in humans, however excesses of these elements can be harmful. Curtain heavy metals, including Hg, Pu, Cd, and Pb, do not benefit living things. Heavy metal pollution in the soil is a global problem.

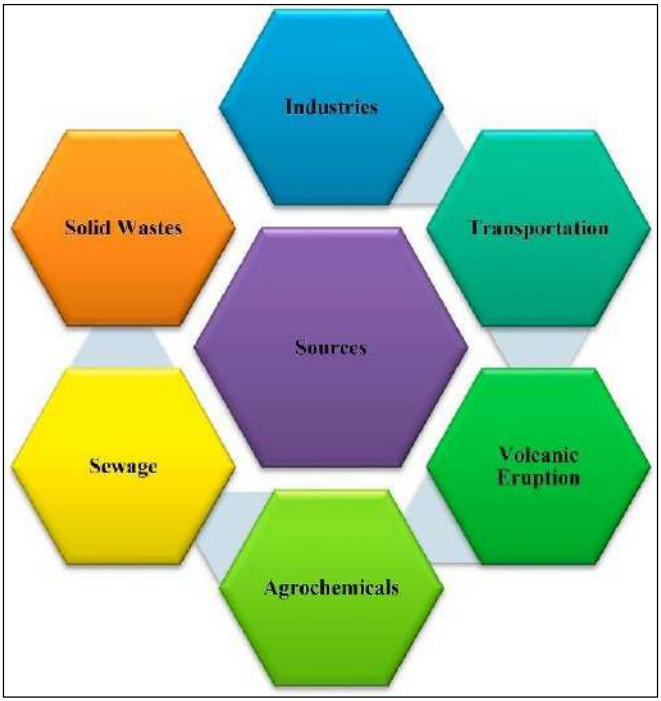
2. Heavy Metal Contamination in the Anthropocene

Thetoxicityofheavymetalpoisoningtolivingthingshasdrawnconcernfroma llaroundthe world. Typically, HMs are categorized as higher elemental density metals or metalloids.

Based on factors like density, weight, o'atomic number, these are separated. Alietal. proposed new standard that specifies that HE should have an atomic density of >41g/cm3, using as examples Zn, Pb, Hg, Cu, Cr, Co,

Cd and other metals. Terrestrial and aquatic ecosystems are heavily impacted by even minute concentration of heavy metals (HMs) in the system. As air emissions are known to be the main cause of pollution in soil and water, this could be the result of diffuse influences. These are the most hazardous and dangerous water pollutants for both natural systems and human health.

In contrast to incomplete fossil burning, mineral extraction, landfilling, metal refining, the production of electronic goods, dyes, agricultural chemicals, military operations, and vehicle emissions are examples of anthropogenic activities. Examples of natural sources include volcanoes, soil erosion, and rock disintegration. Sewage, dyes, alloys, mine effluent, and other sources of hazardous metals (HMs) include Pb, Cu, Hg, Cr, As, Cd. As opposed to natural sources, anthropogenic practices contribute more to environmental contamination. As a result of their high level of reactivity, many HMs have detrimental effects on the environment and people. Agrochemical operations, mining, smelting, and the addition of Cd, As, Ni, U, Pb, Zn, and Hg metals can result in increased levels of hazardous heavy metals (HMs) in the environment. Fig. 1 list the main anthropogenic behaviors that can cause these levels.



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3. Phytoremediation Techniques

3.1 Phytoextraction

The uptake and transport of poisons by factory roots into shoots is nominated as phytoextraction, occasionally known as phytoaccumulation, photo absorption or phytosequestration. In order to remove oil poison, the roots and shoots are gathered. (Chandra and Kumar 2017) demonstrated that, twelve suitable native shops growing on sludge observed their capability to accumulate Fe. *A. spinosus* L. was discovered to be Zn and Mn shoot accumulator. Except for *A. spinosus* L. and Ricinus communis, all shops were set up to be splint accumulators for Fe, Mn and Zn. Lajayer *et al*., 2019 have demonstrated the promising eventuality of cosmetic shops for phytoextraction of HMs from weakened surroundings. When compared to traditional remittal ways, this strategy appears to be more ecologically friendly. Ugya *et al*. (2019) looked at the phytoextraction capacity of certain vegetables as well as the possible health pitfalls related to eating vegetables that are grown in agrarian soils rinsed with refinery wastewater. For Cd and Hg, the results revealed a strong accumulation pattern in the vegetable. Makarova *et al*. (Makarova *et al*., 2021) studied the possibility to enhance the phytoextraction process by Trifolium repens of Cd, Ni and Cu. The results showed the effectiveness of the potassium swab of K2HEDP, factory growth controllers and iron chelate for the phytoextraction of Cd.

3.2 Phytostabilization

Phytostabilization uses the essence-tolerant shops for HM immobilization in polluted soils thereby, dwindling their bioavailability in the terrain (Yan *et al*., 2020). As a result, they are unfit to resettle to groundwater or join the food cycle (Ali *et al*., 2013). (Luo *et al*., 2019) delved the felicity of using four wooden shops in combination with organic emendation stop hy to stabilize zinc smelting sediment. The results showed that planting foliage directly in zinc smelting waste sediment increased nutrient accumulation and dropped heavy essence bioavailability (Cu, Zn and Cd). (Saran *et al*., 2020) performed a phytostabilization demonstration to determine the capacity of growing *Helianthus petiolaris* (an sweet factory) in HMC soils. In terms of phytostabilization, *H. petiolaris* could develop in soils with over to 500 mg/Kg Pb2 and 50 mg/Kg Cd2, accumulating> 3 times the soil Cd content in the upstanding corridor and translocating large quantities of Pb to the upstanding corridor. (Galal *et al*., 2017) delved the capability of Vossia cuspidate (imperative macrophytes) as a phytoremediator to absorb HM from polluted water sources in another

analysis. The results revealed substantial seasonal variation in the attention of the delved HMs in the colorful factory apkins of *V. cuspidate*, with HMs accumulating in advanced attention in below- ground sections than in above-ground shoots.

3.3 Rhizofiltration

Rhizofiltration is a fashion in which factory roots are employed to exclude adulterants from wastewater. Root exudates can absorb HMs, therefore altering the pH of rhizosphere (Yan *et al*., 2020). A study (Benavides *et al*., 2018) revealed that Zea mays has advanced eventuality for uptake and bioaccumulation of Hg. In a sewage treatment factory of KwaZulu-Natal (a fiefdom of South Africa), a rhizofiltration device was planted with two species (*Phragmites australis* and *Kyllinga nemoralis*), was tested for its effectiveness in rooting HMs from external wastewater. HMs were deposited in the planted and reference corridor of the rhizofilter in varying attention. Cd situations were also setup to have risen by 33 and 21 on of Nemoralis root system, indicating that the system is effective at rooting HMs from wastewater (Odinga *et al*., 2019).

3.4 Phytovolatilization

Shops are used to convert different adulterants into lower dangerous volatiles, which are also released into the atmosphere through the foliar system. This conception maybe used to remove organic adulterants as well as heavy essence including Se, Hg and As (Yan *et al*., 2020). According to previous exploration, *Astragalus racemosus* may convert Se into dimethyl diselenide by phytovolatilization, whereas arabidopsis thaliana can convert Hg 2 to Hg 0, hence boosting Hg volatility (Awa and Hadibarata, 2020). also, Sakakibara *et al*. (2010) observed the capability of Pteris vittate to metabolize arsenic into unpredictable arsenic forms. In phytovolatilization, harvesting or disposing of HM adulterants are collected from the soil and spread as gassy composites (Yan *et al*., 2020).

4. Conclusion

Heavy Essence (HMs) are one of the serious troubles to soil, water and humans. The use of low-cost and environmentally-safe strategies seems to be a implicit approach to remediate these adulterants. As a result, phytoremediation is among the most popular and effective factory-grounded approaches for removing pollutants from defiled areas while causing minimum damage to water bodies or soil structure. The need to consolidate and accelerate phytoremediation surface from exploration and operations not only because of the benefits it provides as an ecological process but also

because of the downsides associated with the long time it takes to remove HMs as well as pitfalls HM toxin pose to plant integrity, heavy essence. The exploration examine the origin of HM pollution, HM impurity of soil and water, and their dangerous goods on mortal health in this terrain. The full study assists us in determining how to choose fashion for a certain essence-bearing effluent grounded on time consumption and effectiveness, as well as relating generally employed shops for phytoremediation. also, some of the possibilities and strategies for enhancing phytoremediation viz., different phytoremediation ways, biotechnological approaches, shops used in colorful approaches, and variables impacting phytoremediation have been bandied therefore creating perspectives for the development of new strategies. In nutshell, phytoremediation may be employed as an provident, effective, environmentally and eco-friendly system related to the operation of essence-accumulating shop Satna indispensable system for HM remittal. new factory species suitable for rooting heavy essence from weakened soils must be introduced to ameliorate the process. Essence bioavailability- adding strategies, similar as using bio-augmented acidified ordure, can be used to lower the pH of weakened soil and thereby increase heavy essence vacuity to shops. likewise, inheritable engineering and molecular wisdom exploration can make significant and innovative benefaction to adding the impact of phytoremediation salutary fashion for perfecting adding soil quality, as well as a system of recovering critical essence from heavily defined areas. Chelating agents and microbes can be employed in confluence with this fashion to boost HM bioavailability, allowing for lesser heavy essence accumulation in shops and soil remittal. Further, further exploration is demanded to more understand the impacts of different types of catalysts on phytoremediation effectiveness in order to increase the practicability of phytoremediation for environmental restoration.

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Chapter - 4
Aturmeric Extract-Study of its Possible Anti-Tumor Properties

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Chapter - 4

Aturmeric Extract-Study of its Possible Anti-Tumor Properties

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Abstract

One of the world's leading causes of death, cancer continues to take many lives despite growing awareness and the development of multi targeted therapeutic options. Chemotherapy is a therapeutic approach for treatment of cancer. But significant adverse effects from chemotherapy can lower quality of human body. So, we should found an alternative safe way to get rid from this deadly diseases. One species that may have anticancer properties is turmeric, which contains curcumin. Curcumin exerts its chemo preventive effects by promoting apoptosis, inhibiting survival signals, scavenging reactive oxidative species (ROS), and reducing the inflammatory cancer microenvironment, thereby modulating multiple molecular pathways involved in prolonged carcinogenesis. Curcumin's low toxicity, reasonable price, and convenient availability make it an excellent chemo preventive agent. Here, we go over curcumin's molecular targets, modes of action, and prospects for cancer prevention.

Keywords: Chemotherapy, anti-cancer, ROS, molecular targets, turmeric, therapy

Introduction

Currently, cancer is one of the primary causes of mortality among individuals. The existing therapeutic modalities for cancer treatment encompass chemotherapy, surgery, phototherapy, and radiotherapy (Barati *et al*. 2018). But significant adverse effects from chemotherapy can lower quality of life and include nausea, vomiting, diarrhea, exhaustion, and hair loss. So we should find an alternative safe way to get rid from cancer. One species that may have anticancer properties is turmeric. The active ingredient in turmeric, known as curcumin (diferuloylmethane), a polyphenol derived from the rhizome. Curcumin (75-77%), demethoxycurcumin (17-18%), and

bis-demethoxycurcumin (3%-5%) are the three primary curcuminoids found in curcumin extracts and commercial products. Turmeric gets its yellow hue from phenolic pigments found in this diarylheptanoid, which belongs to the curcuminoid group (Boyanova *et al*. 2024). A new antioxidant peptide called turmerin has been discovered in turmeric, and it is water-soluble. Turmeric essential oil have been shown to have inhibitory effects on methicillin-resistant bacteria, including *E. coli*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Additionally effective against MDR isolates of *A. baumannii*, *K. pneumoniae*, and *Proteus mirabilis* were ethanolic turmeric extracts. It is used to treat lung cancer, breast cancer, gastric cancer, leukemia, prostate cancer, according to (Kostelecka *et al*. 2024). They are also used as food preservative and in cooking (Boyanova *et al*. 2024). Many animal studies have demonstrated the significant roles of curcuminplaysinpreventinginitialcarcinogenesisinavarietyoforgansthatservea smetastatic sites, including the gastrointestinal track and mammary glands (Davoodvandi *et al*. 2021). This paper will summarize the current research on curcumin's effectiveness in treating various types of cancer.

Discussion

There is several health benefits associated with turmeric and curcumin, the main element. These include hepatoprotective, anti-inflammatory, antioxidant, and antibacterial characteristics. Research has shown that circum in inhibits the growth of cancer by interacting with cancer cells in a number of ways. Curcumin's immunomodulatory ability, however, also affects cellular constituents including dendritic cells, macrophages, and T and B Lymphocytes addition to molecular targets. Curcumin induces cell cycle arrest and death in gastric cancer cells through a myriad of mechanisms that differ across cell types and medication. Curcumin inhibits tum our progression and metastasis by blocking various signal transduction pathways in cancer cells, including p53, Ras, Wnt-β, MAPKs, ERK, PI3K, and Akt. Of the numerous therapeutically important molecules that curcumin can inhibit, some include EGFR, IKK, cyclinD1, β-catenin, tumor necrosis factor α(TNF-α), and antiapoptotic genes such as Bcl-2 and Bcl-X (Barati *et al*. 2018).

Effects of curcumin on cancer cell	Induction	Epigenetic alteration	micro RNAs
		Cell cycle inhibition	p21, p27
		Apoptosis	bcl-2, bax
		Activation of tumor suppression gene	p53, PTEN
	Inhibition	Cell cycle promotion	Cyclin D1, Cyclin D3, CDK2
		Metastasis and angiogenesis promotion	MMP2, MMP-9, VEGF
		Transcription	NF-κB, β-catenin, p65, notch-1, mTOR

Fig 1: The impact of curcumin on cancer cells, summarized. An important set of abbreviations include: cyclin-dependent kinase 2(CDK2), matrix metallopeptidase 2(MMP-2), matrix metallopeptidase 9 (MMP-9), vascular endothelial growth factor (VEGF), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kb), phosphatase and tensin homolog (PTEN), B-cell lymphoma-2 (bcl-2), and bax (Bcl-2-associated X protein)

Lung Cancer

Lung cancer is the third most prevalent cancer in women, whereas it ranks among the most common cancers in men. Lung cancer is often managed with surgical intervention, succeeded by chemotherapy, immunotherapy and radiation therapy. Curcumin causes apoptosis in human lung cancer cells by number of different ways, such as preventing cell cycle progression at the growth/mitotic (G2/M) phase of A549 cells and synergistically inducing cell death and apoptosis with (Mehta *et al*. 2014). Additionally, curcumin produces ROS, which triggers the signaling path way for DNA damage.

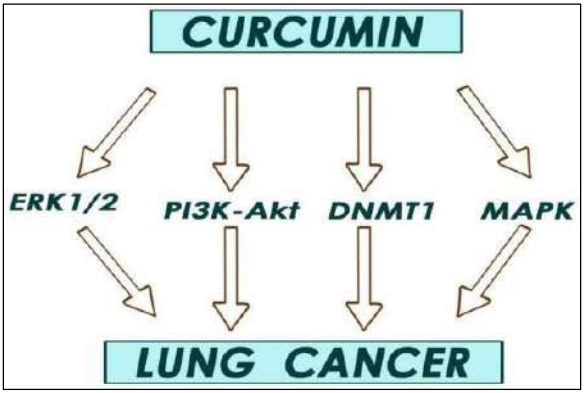

Fig 2: The involved signaling pathways in antitumor activity of curcumin

Curcumin directly targets and diminishes the expression of the ERK 1/2 pathway by 75%. A recent study shown that curcumin and paris saponin 2 (PS2) collectively caused apoptosis in lung cancer cells via blocking the PI3K-Akt pathway. Conversely, another study discovered that a new

curcumin derivative, T59, stimulated the PI3K/Akt pathway and triggered apoptosis in lung cancer cells (Zoi *et al*. 2021). In the treatment of lung cancer, curcumin has a favorable effect on TCF21. By downregulating DNMT1 (75% drop in expression), curcumin (5 and 10μM) for 48 hours increases TCF21 expression (up to fourfold increase) and inhibits lung cancer cell motility, proliferation, and colony formation (G.-Q. Wu *et al*. 2016). A major contributing factor to the development of lung cancer is tobacco smoking. Research indicates that tobacco smoking (TS) is linked to over 90% of occurrences of lung cancer. Nicotine smoke induces an epithelial-mesenchymal transition (EMT), which is mediated by the mitogen-activated protein kinase (MAPK) pathway (Liang *et al*. 2015).

The impact of curcumin on the TAp63α transcription factor. Curcumin was found to enhance TAp63α expression, hence exerting a beneficial effect on smoking-induced carcinogenesis. This factor is suppressed by TS-induced lung cancer epithelial-mesenchymal transition (Zoi *et al*. 2021). Curcumin possesses the capability to regulate the production of miRNA, a minor subset of human RNA that encompasses non-coding sequences associated with several diseases, including cancer. Curcumin therapy was shown to diminish the expression of miRNA-186 in lung cancer cells (Zhang *et al*. 2010).

Breast cancer

Breast cancer is presently the most prevalent malignant tum our among adult females. It is the leading cause of cancer-related mortality among women globally. NF-κB is crucial for the proliferation, invasion, and metastasis of breast cancer cell lines. Curcumin was found to impede the nuclear translocation of NF-κB and to reduce p100 and p52 levels in MCF-7 and MDA-MB- 453 breast cancer cells, respectively. Curcumin induces apoptosis in MCF-7 breast cancer cells via regulating the spermidine/spermine N1-acetyl transferase (SSAT) gene, which is intricately associated with the NF-κB-dependent signaling system. Clinical evidence suggests that inhibiting the Akt/mTOR-dependent pathway is a promising therapeutic strategy, as it is a primary signaling mechanism in the growth of breast cancer cells. Curcumin was demonstrated to suppress the phosphorylation of Akt, mTOR, and their downstream proteins, resulting in cell cycle arrest in various breast cancer cell lines, including T47D and MCF7 (Zoi *et al*. 2021). Curcumin can disrupt the EGFR family of receptor tyrosine kinase, whose signaling pathways implicated in the proliferation, adhesion, migration, and differentiation of cancer cells.

Regulating EGFR is prudent strategy for cancer therapy. Curcumin inhibited the proliferation and growth of breast cancer cells by diminishing EGFR signaling and reducing the levels of EGFR and Akt.

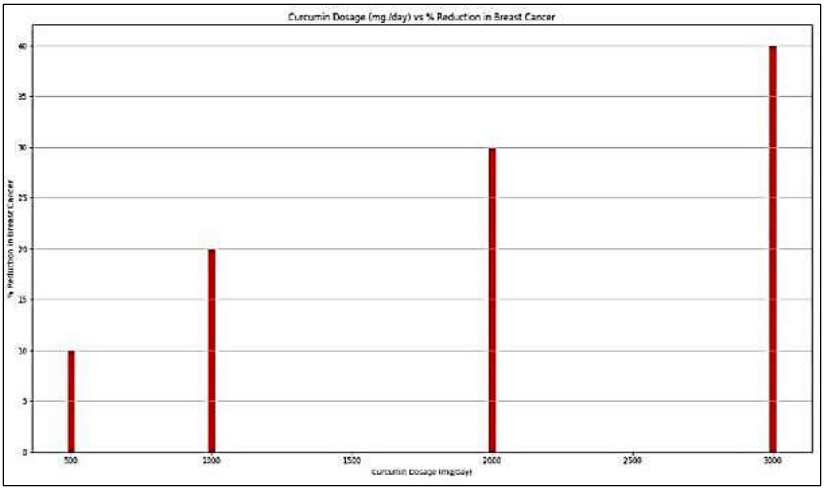

Fig 3: Curcumin dosage (mg/day) and reduction in breast can coincidence

It has recently been discovered that curcumin has the ability to alter the expression of miRNAs in breast cancer cells. MiRNAs are 18-22 nucleotide non-coding sequences that are implicated in a number of illnesses, including cancer. Tumor-suppressive (miR-15a, miR-16, miR-34a, miR-146b-5p, and miR-181b) and oncogenic (miR-19a and miR-19b) miRNA expression in breast cancer cells were both impacted by curcumin (Giordano 2019). Consequently, Apoptosis induction and the inhibition of tumorigenesis and metastasis were noted.

Gastric Cancer

Gastric cancer ranks as the third leading cause of cancer- related mortality globally. Curcumin's ability to inhibit many signal transduction pathways, such as p53, Ras, Wnt-β, extracellular signal-regulated kinases (ERK), PI3K, MAPKs, and protein kinase B (Akt) in gastric cancer cells, indicates its potential anticancer efficacy (Kasi *et al*. 2016). Curcumin therapy markedly decreased the expression of MMP-2, MT1-MMP, integrin receptors, and focal adhesion kinase (FAK) in laryngeal squamous carcinoma cells. This led to significant reduction in the invasive properties of the tumor cells. It is essential to note the expression levels of integrin receptors, MMP-2, and MT1-MMP (Mitra *et al*. 2006). The tumour suppressor gene p53 is essential in neoplastic cells. Upregulating p53

expression can induce apoptosis and limit cancer cell proliferation (Liu *et al*., 2019). Curcumin may induce autophagy and apoptosis in gastric cancer cells by inhibiting the PI3K pathway and activating the p53 signalling pathway (Fu *et al*., 2018). Furthermore, curcumin can inhibit MKN 45 cell migration and invasion, elevate the expression of E-cadherin and Bax, reduce the expression of N-cadherin, Snail1, Wnt3a, p-β-catenin, p- LRP6, and Bcl-2, and augment the activity of Caspase-3, Caspase-8, and Caspase-9. It may also induce apoptosis. Curcumin can inhibit H19 expression in gastric cancer cells, indicating that H19 is crucial for the curcumin-mediated reduction of gastric cancer cell proliferation and apoptosis. Curcumin has demonstrated the ability to upregulate mi R-34a expression and inhibit the proliferation of gastric cancer cells in SGC-7901 cells. Moreover, curcumin therapy can reduce the levels of Bcl-2, CDK4, and cyclin D1 in gastric cancer tissue (Zhang *et al*. 2022).

The expression of phosphatase and ten sin homolog (PTEN) elevated, whereas the level of MicroRNA-21 (miR-21) diminished in human gastric cancer Mgastric cancer-803 cells with increasing doses of curcumin. The results indicate that curcumin exerts a negative regulatory influence on the miR-21/PTEN/Akt pathway (Qiang *et al*. 2019).

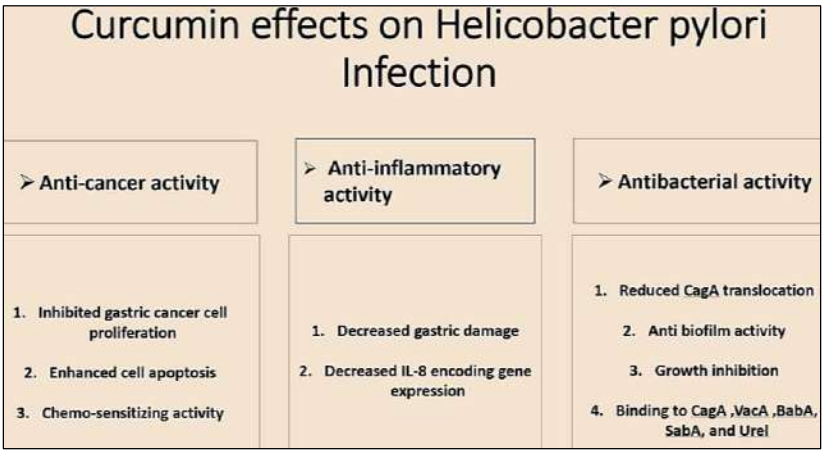

Fig 4: Gastric cancer mainly caused by a bacteria *Helicobacter pylori*

Leukemia

Globally, leukemias constitute 8% of all cancer cases. It constitutes 30% of all pediatric cancer cases and is the most common type. Four principal types of leukemia have been recognized: acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL),

and chronic myeloid leukemia (CML). Leukemia is a cancer of the bone marrow or blood characterized by excessive proliferation of blood cells. Curcumin has been found to decrease TNF-α-induced nuclear translocation and NF-κBDNA binding inhibiting the phosphorylation and degradation of IκBα in human myeloid ML-1a cells. Moreover, curcumin induced apoptosis in B-cell chronic lymphocytic leukemia (CLL-B) via downregulating STAT3, AKT, NF-κB, and X-linked inhibitor of apoptosis protein (XIAP) (Giordano 2019). The anticancer properties of curcumin were evidenced in chronic myelogenous leukemia (CML) cells via the overexpression of PTEN, a target of miR-21, a microRNA that is overexpressed in several cancers and represents one of the mutated or silenced tumor suppressor in human malignancies. Curcumin inhibited leukemic cell growth both in vitro and in vivo by triggering miR-21-mediated modulation of the PTEN/AKT pathway (Taverna 2015). The chemical mechanism by which curcumin acts on human M5 leukemia cells (SHI-1cellline) demonstrate that this compound, due to its anticancer qualities, can inhibit the MAPK and matrix metalloproteinase (MMP)-dependent signaling pathways (Zhu *et al*. 2019).

Table 1: Due to its anticancer qualities of curcumin in cancer prevention

Mechanism	Description
Anti-proliferative	Inhibits Cancer Cell Growth
Pro-apoptotic	Induce Cancer Cell Death
Anti-angiogenic	Inhibits tumor blood vessel formation
Immunomodulatory	Enhances Immune Response

Prostate Cancer

Currently, in the Western world, prostate cancer is the second leading cause of cancer-related mortality in males. Androgen Receptor (AR)-dependent signaling significantly contributes to the initiation and progression of the disease. Aberrant NF-κ an activity has been detected in human prostate cancer cells and xenografts. Curcumind is rupts multiple molecular signaling pathways, including NF-κB, mitogen-activated protein kinase (MAPK), and epidermal growth factor receptor (EGFR) pathways (Zoi *et al*. 2021). Curcumin suppresses C-X-C motif chemokine ligand 1 (CXCL-1) and CXCL-2 in androgen-independent prostate cancer (AIPC) cells via targeting the NF-κB pathway. Curcumin therapy yields a 60-80% reduction in the proliferation of the PC3-insensitive prostate cancer cell line. Curcumin is notably significant inhibiting the progression of cancer in individuals with castration-resistant prostate cancer (CRPC) who are unresponsive to androgen deprivation therapy. Curcumin has demonstrated the ability to

inhibit the transcription of the AR gene in prostate cancer cells *in vitro*. The downregulation of the AR gene expression in the lymph node cancer of the prostate (LNCaP) cell line is accomplished by suppressing steroidogenic acute regulatory protein, including CYP11A1 and HSD3B2 (Ide *et al*. 2018). Curcumin has been shown to inhibit the phosphorylation of Ser473AKT/PKB and the expression of the p110 and p85 subunits of PI3K. Matrix metalloproteinases (MMPs) are essential for tumour invasion and metastasis; curcumin significantly decreased the activity of MMP-2 and MMP-9, resulting in fewer metastatic nodules in tumor-bearing mice. Subsequent investigation into the mechanism of curcumin's action revealed that the inhibition of IκBα kinase activity, IκBα phosphorylation, IκBα degradation, p65 phosphorylation, p65 nuclear translocation, and p65 acetylation occurred sequentially, leading to the suppression of p65 nuclear translocation (Teiten *et al*. 2009).

Conclusion

Curcumin, a polyphenol derived from the rhizomes of Curcuma longa, is among the most promising bioactive natural chemicals in the treatment of several cancer types. Curcumin influences multiple cells signaling pathways, including growth factors, cytokines, transcription factors, and genes that govern cellular proliferation and apoptosis, significantly affecting the progression of numerous cancer types. This enables curcumin to exhibit anticancer effects.

However, curcumin is not devoid of adverse effects, which may include headache, diarrhoea, nausea, and yellow stools. Moreover, it exhibited restricted bioavailability due to rapid metabolism, systemic excretion, and inadequate absorption, all of which diminish its efficacy in disease treatment.

Future Aspects

Here are some future aspects of curcumin in cancer therapy:

Clinical Trials: More extensive Phase II and III trials to confirm curcumin's efficacy and safety.

Combination Therapies: Investigating curcumin with other anticancer agents.

Nano-technology: Developing targeted curcumin delivery systems.

Personalized medicine: Identifying biomarkers for curcumin responsiveness.

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Chapter - 5
Molecular Pathways and Therapeutic Approaches in Parkinson's Disease

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Chapter - 5

Molecular Pathways and Therapeutic Approaches in Parkinson's Disease

Sohani Das, Tiyasha Saha, Tanisha Bhowmick, Ananyo Mazumdar, Ashis Ghosh, Rupesh Dutta Banik, Debjit De and Priyankar Pal

Abstract

Parkinson's disease (PD) is the second most prevalent age-related complicated, idiopathic neurological condition, following Alzheimer's disease. This condition is distinguished by involuntary shaking, Bradykinesia, muscle rigidity, and reduced function. Furthermore, around 50% of individuals with PD also demonstrate executive dysfunction that is mediated by the frontostriatal system. This dysfunction encompasses deficiencies in attention, mental processing speed, language abilities, working memory, and impulsivity. The development of PD may be influenced by both environmental and genetic variables. Exposure to toxins and gene alterations can initiate the formation of brain lesions. The known processes of PD consist of α-synuclein aggregation, oxidative stress, ferroptosis, mitochondrial failure, neuroinflammation, and gut dysbiosis. The interplay between these molecular processes complicates the development of PD and presents significant obstacles to medication discovery. The pathophysiological processes underlying Parkinson's disease PD are highly intricate, encompassing various pathways including the aggregation of α-synuclein, oxidative stress, neuroinflammation, ferroptosis, mitochondrial failure, and gut dysbiosis. Interactions have a significant role in both the onset and advancement of PD. The primary medication utilised in the treatment plan for PD is L-DOPA, with additional medications such as MAO-B inhibitors, COMT inhibitors, etc. being administered in conjunction with L-DOPA. Furthermore, the current medications frequently lack efficacy in treating patients with advanced PD, thus diminishing the quality of life for these individuals. Hence, there is still a want for novel medications to be developed for therapeutic purposes.

Keywords: Parkinson's disease, α-synuclein, neuroinflammation, ferroptosis, L-DOPA

Introduction

Parkinson's disease (PD) is a prevalent neurodegenerative illness that is mainly defined by a decline in motor function brought on by dysfunction of the dopaminergic nigrostriatal pathway. The primary motor symptoms, bradykinesia, rigidity, and postural instability, are specifically brought on by the death of dopaminergic neurons that project from the substantia nigra pars compacta to the caudate-putamen in the striatum. This loss of dopamine neurotransmission is the result of this neuronal death. Despite the fact that Parkinson's disease (PD) was once thought to be a movement disorder distinct from dementia, it is now recognized that the disease's progression impacts other extra-nigral dopaminergic, cholinergic, and serotoninergic tracts. This can result in non-motor symptoms like anosmia, constipation, and sleep disorders as well as cognitive and psychiatric symptoms like depression and dementia [34, 17]. There is experimental evidence that Parkinson's disease (PD) also affects the prefrontal cortex (PFC), anterior cingulate gyrus, and/or frontostriatal pathways [30]. Despite this, it is unclear exactly how dopaminergic neurons in SNpc are lost. The initiation and progression of Parkinson's disease (PD) may be attributed to a number of factors, including mitochondrial damage, energy failure, oxidative stress, excitotoxicity, protein misfolding and subsequent aggregation, impairment of protein clearance pathways, cell-autonomous mechanisms, and "prion-like protein infection" [26].

One of the most popular theories for Parkinson's disease (PD) involves protein mis folding and the buildup of misfolded proteins in intracellular regions [23, 8]. Lewy bodies (LB), which comprise several misfolded amyloid proteins such as phosphorylated tau (p-tau), amyloid beta protein (Aβ) [8, 18], and alpha-synuclein (SNCA), are the main misfolded amyloid protein inclusion seen in the intracellular spaces of SNpc neurons in Parkinson's disease (PD) [8]. Distinct environmental contaminants are linked to sporadic Parkinson's disease (SPD), which may be partially replicated in experimental animal models of Parkinson's disease (PD), including paraquat and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [21]. It is more challenging to comprehend the pathophysiology of PD since, in contrast to SPD, family instances are uncommon and do not exhibit the typical signs of the disorder [23, 19]. Despite the development of several novel PD medications, none of them have been able to stop the disease's progression. There are currently just a few symptomatic therapies that are suitable for a restricted number of individuals. Furthermore, the main obstacles to using these medications to treat Parkinson's disease are their permeability, short half-lives, and adverse

effects. It's interesting to note that new advancements in gene therapy and stem cell transplantation have garnered significant attention as alternate PD treatment options. For instance, in mice models of Parkinson's disease, genetically altered DA neurons have demonstrated encouraging outcomes [37, 15]. Similarly, scientists are able to fix some of the defective metabolic pathways linked with Parkinson's disease (PD) by employing lentiviral or recombinant adeno associated viral vectors (rAAV) [10]. Moreover, a relatively new advancements in the field of gene editing known as clustered regularly-interspaced short palindromic repeats associated protein 9 (CRISPR-Cas9) has promise for the treatment of Parkinson's disease [35]. The goal of this study is to present fundamental conceptual knowledge on the molecular pathways behind Parkinson's disease (PD), in light of the urgent need for the development of novel, sensible therapeutics. This knowledge might aid in the creation of more potent medications or other therapeutic methods.

Gene Mutations and their Role in Parkinson's Disease

A multitude of recent research shows that 5-10% of late-onset forms of D'are related to genetic factors, including the identification of gene mutations in familial or hereditary forms of the disease [19, 10].

i) Parkin. Parkin is a crucial protein linked to the ubiquitin proteasome system, one of the mechanisms for protein clearance that aids in the breakdown of misfolded proteins within the cell. Asan E3 ubiquitin ligase, parkin may bind covalently to ubiquitin on a variety of substrates that are misfolded proteins in order to facilitate the destruction of such proteins [7]. Parkin can create LB inclusions and has also been co-localized with SNCA. On the other hand, misfolded amyloid protein aggregation inside SNpc can result from parkin mutations. Neurons in the midbrain's locus coeruleus are lost in parkin-deficient animals and individuals with idiopathic Parkinson's disease [21]. Additionally, parkin mutations have the potential to dramatically reduce ubiquitin-ligase enzymatic activity in the SNpc, which can greatly slow down the proteasomal breakdown process in the event of autosomal recessive juvenile Parkinsonism.

Furthermore, parkin has role in controlling the release of dopamine from SNpc [21].

i) DJ-1 (PARK7). DJ-1 is an 189 amino acid dimer that is found in the cytoplasm, nucleus, and mitochondria. It has been associated with

Parkinson's disease (PD) that manifests early [1]. Numerous *in vitro* studies have demonstrated its neuroprotective properties, which include transcriptional regulation, anti-oxidant, chaperone, and protease action, as well as modulation of the activity of particular genes essential to cell survival (PI3 K/Akt pathway) [3]. Mice lacking DJ-1 exhibit impaired locomotion, a reduction in D2 type DA receptor activation, and an increase in MPTP sensitivity [16]. Similarly, point mutations and DJ-1 deletions lead to the development of autosomal recessive Parkinson's disease [21]. Furthermore, DJ-1 has been shown to co-localize with SNCA and p-tau, suggesting that DJ-1 may be important in tauopathies and synucleinopathies [2]. Moreover, DJ-1 may bind to a number of chaperones, such as mitochondrial HSP70/mortalin/Grp75, carboxy-terminus of HSP70-interacting protein (CHIP), and HSP70. This can aid in the breakdown of misfolded SNC [5]. In order to sustain TH levels in DA neurons of SNpc, DJ-1 also regulates the expression of the human TH gene by removing the transcriptional repressor poly-pyrimidine tract binding protein-associated splicing factor (PSF) from the human TH gene promoter [1].

ii) PINK1 (PARK6). PTEN-induced putative kinase-1, or PINK1, is a 63 kDa serine/threonine protein kinase that is found in the mitochondria and guard against mitochondrial damage brought on by stress in neurons [36]. Research conducted in vitro indicates that PINK1 may have a role in cell survival.

Numerous PD families have been found to have the PINK1 gene mutation, which increases cell sensitivity [36, 12]. Moreover, PINK1 gene mutations have been connected to SNpc neuron loss and mitochondrial malfunction, which ultimately results in Parkinson's disease (PD) [36].

i) LRRK2/PARK8 (dardarin). The PARK8 gene encodes the 268kDa multi-domain protein known as leucine-rich repeat kinase 2 (LRRK2). Late-onset Parkinson disease (PD) has been associated with several point mutations in the PARK8 gene. Post-mortem PARK8 point mutations have been found in many PD patients' tissues, along with severe degenerative arthritis (DA) and/or LB aggregation.

Furthermore, p-tau pathology found in PD patients' post-mortem brain may be connected to LRRK2 gene alterations [19].

Environmental Pollutants effect on Parkinson's Disease

Farmers are using a range of pesticides, sometimes indiscriminately, to increase crop yields as a result of recent yield-boosting developments in the fertilizer and agriculture sectors. SPD has developed as a result of exposure to certain environmental pollutants, such as pesticides, herbicides, fungicides, and insecticides, among others [4]. Importantly, exposure to such poisons, either by drinking water or direct touch, puts farmers and residents in rural regions at risk for Parkinson's disease (PD). Additionally, a lot of people get infections, bacterial toxins, or illicit street drugs like synthetic heroin (MPTP, also known as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which can cause SPD [31]. The real poisonous metabolite, MPP+, is created when MPTP is exposed to a cell. This metabolite can enter the cell by DAT, assault DA-neurons in the SNpc, and cause parkinsonism [21]. MPTP is presently commonly utilized to induce severe, persistent parkinsonian symptoms in animal models of Parkinson's disease (PD) because of this capacity [21]. A number of environmental toxins, including paraquat, maneb, zineb, nabam, thiram, ziram, and rotenone, have structural similarities with MPTP and have the potential to cause Parkinsonism in animals [21]. In one of our tests, for instance, mice who received a 5-day injection of MPTP (25 mg/kg BW) revealed an 80% decrease of TH-immunoreactivity in the SNpc 5 weeks later, indicating a significant loss of DA-neurons in that region. Additionally, we saw that TH-positive DA-fibers were more sparse in the striatum. This implies that the DA fibers in the striatum become less numerous as a result of the loss of DA neurons in SNPc [21]. Similarly, giving animals an injection of 6-hydroxydopamine (6-OHDA) into the striatum can cause symptoms similar to Parkinson's disease [21]. In a manner similar to that of MPTP exposure, rotenone is another well-known pesticide ingredient that degenerates DAneuronsin SNP casa result of energy failure [21]. According to a number of studies, the majority of environmental pollutants have the ability to interfere with the mitochondrial electron transport system limit complex-function. This can eventually increase the formation of free radicals and cause oxidative stress [21].

Despite the fact that several researchers have created numerous animal models of Parkinson's disease (PD) using environmental toxins, none of them showed the main symptoms of the disease, such as bradykinesia or resting tremor. Furthermore, they are unable to precisely summarize the processes behind DA neuronal loss. Toxins like these can also result in sudden or acute cell death, in contrast to the gradual neurodegeneration seen

in Parkinson's disease. Furthermore, the majority of treatment that are employed in animal models to prevent the neurodegeneration brought on by these environmental pollutants do not work as well in humans. As such, choosing a toxin to create an animal model of Parkinson's disease is a difficult undertaking [21].

The Molecular Causes of Parkinson's Disease

i) **Clustering of Alpha-Synuclein (SNCA):** The intracellular buildup of LB in DA neurons of the SNpc, which include misfolded aggregates of SNCA and other related proteins, is one of the characteristic diseases of Parkinson's disease (PD) [21]. Interestingly, a number of molecular, genetic, and biochemical studies showed that human post-mortem brain of patients who were neuro pathologically diagnosed as having mixed dementia with Lewy bodies (DLB) and PD with dementia (PDD) frequently showed a mixture of multiple misfolded protein aggregates, such as p-tau, Aβ, and SNCA [32]. Upon examining the brains of many Parkinson's disease (PD) patients, Gomperts *et al*. discovered a combination of amyloid deposition in the brain, which was connected to cognitive decreases independent of dementia, indicating that amyloid plays role in cognitive decline overtime, but not in motor decline [16]. In a similar vein, Hepp et al. discovered that PDD and LBD cognitive deficits are influenced by the level and burden of Aβ pathology [2]. By creating a hole in the membrane, the oligomers, proto-fibrils, and fibrils of SNCA or other misfolded amyloid proteins can kill neurons by excitotoxicity, oxidative stress, neuroinflammation, and energy failure [18, 22]. One instance of this is when mice receive a single intracerebroventricular (i.c.v.) infusion of SNCA oligomers (α-SYOs), which results in the development of both non-motor and late motor symptoms, including impairments in the pole and rotarod tests and decreased TH and DA levels in the caudate putamen [14]. Similar to this, familial Parkinson's disease (PD) is brought on by mutations in the SNCA gene (e.g., A53T, A30P, E46K, and H50Q), which has a strong correlation with dementia and an early start [28]. In animal and cell culture models, overexpression of SNCA resulted in a buildup of SNCA aggregates in the mitochondria, pronounced defects in mitochondrial mobility, and a reduction in the potential of the mitochondrial membrane [21]. The electron transport chain was impaired in the SNCA knockout mice, and they had aberrant lipid patterns in their mitochondria. Additionally, the mice's reduction in

the potential of the mitochondrial membrane toxins decreased [21]. Moreover, brain mitochondria degradation is observed in the A53T transgenic mouse model of Parkinson's disease (PD), along with a notable decrease in complex IV activity and an increase of mitochondria harboring SNCA [21]. There have also been reports of oxidative stress, respiratory chain failure, mitochondrial DNA damage, and SNCA inclusion in human DA-neurons in the PD brain [21].

ii) **Oxidative Stress and Dysfunctional Mitochondria in Parkinson's Disease:** Post-mortem human brain histology shows evidence of mitochondrial malfunction; in particular, oxidative stress is a typical pathological mechanism used in Parkinson's disease [29]. Actually, exposure to the toxin 1-methyl-4-phenyl-1,2,3,4-tertahydropyridine (MPTP) produced irreversible inhibition of complex I in the mitochondria, which resulted in significant Parkinsonian symptoms. This suggests that mitochondrial dysfunction have role in the manifestation of Parkinson's disease [29]. Complex I8kDa subunit has been shown to be 34% reduced in mitochondrial electron transport chain (ETC) components from Parkinson's disease (PD) brain participants, although other complexes have not altered [29]. Crucially, preclinical research has convincingly linked the degeneration of dopaminergic neurons caused by loss of mitochondrial complex I activity in PD models [9]. Since the complex I inhibitors, such as MPTP or rotenone, cause Parkinson's disease (PD) in animal models, they offer a chance to reveal the realities of PD [29]. Reactive oxygen species (ROS) from the mitochondria can damage dopaminergic neurons [13]. The primary locations for the production of reactive oxygen species (ROS) in mammalian cells are the mitochondria. The process begins with an electron leak, mainly at complex I, where partial reduction of molecular oxygen (O2) to superoxide radical ($O2*-$) via a single electron uptake. This, in turn, causes the generation of either hydroxyl radical ($OH*$) through the Fenton reaction catalyzed by iron or peroxynitrite ($ONOO-$). Superoxide dismutase 2 also converts superoxide radical ($O2*-$) to hydrogen peroxide (H_2O_2). Catalase then breaks down H_2O_2 into H_2O and O_2 to keep the body in a homeostasis [29]. According to reports, a build-up of ROS can cause extensive harm to many parts of cells [29]. Furthermore, changes to mitochondrial DNA under ROS stress may result in severe consequences as it has been observed that genetically

eliminating TFAM (transcription factor A, mitochondrial), a significant transcriptional factor, causes dementia in mice [33]. MitoPark is the name of the mouse model that demonstrates cardinal PD characteristics. In Parkinson's disease (PD) brain, dopamine undergoes oxidation to produce dopamine quinones and free radicals. This can occur naturally or by the action of mitochondrial outer membrane associated monoamine oxidases (MAO), such as MAO-A in neurons and MAO-B in glia. Dopamine oxidation products are reactive enough to generate superoxide radicals and have the potential to cyclize to make aminochromes [29]. Neurodegenerative disease- related changes in the fusion/fission mechanism of the mitochondria and its transportation are also the subject of much research. In order to keep the mitochondria pool healthy, mitochondria go through fusion/fission dynamics, and the cell separates the injured population for mitophagy [24]. The PINK1/Parkin pathway is activated during mitophagy by the actions of cytosolic Parkin and the mitochondrial kinase PINK1 [11]. Pathogenesis of Parkinson disease (PD) is partly explained by genetic abnormalities that cause loss of PINK1/Parkin based mitochondria quality regulation [27].

iii) **Parkinson Disease-Related Neuroinflammation:** The neuroinflammatory processes associated with Parkinson's disease (PD) entail a series of events, such as increased cytokine release and microglia activation [21]. For instance, after sub chronic MPTP injection in rats, researchers discovered a significant correlation between proinflammatory cytokines and degradation of DA neurons [25]. Numerous clinical investigations have demonstrated that DA-neurons in the postmortem PD brain and in mice models of PD exhibit multiple increases in the levels of inflammatory enzymes, including cyclo-oxygenase-2 (COX2) [21].

Current Therapeutic Approaches

i) **Levodopa (L-DOPA):** In order to restore DA levels, doctors typically administer DA-drugs to patients with Parkinson's disease. Since dopamine (DA) cannot penetrate the blood-brain barrier on its own, levodopa (L-3, 4-dihydroxyphenylalanine/L-DOPA) and other DA precursors are frequently administered [21]. While L-DOPA can effectively lesson "resting-tremors" and other basic symptoms, it cannot arrest the course of Parkinson's disease (PD) or preserve or replace damaged DA neurons [21]. In addition, it could result in low

blood pressure, nausea, vomiting, restlessness, sleepiness, or an abrupt start to sleep. In addition, doctors frequently prescribe carbidopa, which prolongs the therapeutic impact when administered in conjugation with L-DOPA, because L-DOPA converts quickly to DA, lowering its efficacy when it reaches the target region [6].

ii) **MAO-B Inhibitors:** Reduced levels of DA in Parkinson's disease (PD) maybe brought on by the catalytic enzyme monoamine oxidase-B (MAO-B), which is more prevalent in the diseased brain [21]. Therefore, keeping DA levels in the PD brain stable can be achieved by inhibiting MAO-B [20]. The most widely used and well-tolerated MAO-B inhibitors are rasagiline and selegiline (L-deprenyl), which when used with L-DOPA can prolong the effects of L-DOPA for up to a year or more. Despite a number of documents adverse effects, these medications show promise on restoring cell function and delaying the loss of dopaminergic neurons in Parkinson's disease [21].

iii) **COMT Inhibitors:** The enzyme catechol-O-methyl transferase (COMT) further catalyzes the conversion of dihydroxyphenyl acetate, which is produced when MALA breaks down DA, to homovanillic acid. Since COMT is indirectly responsible for the breakdown of DA, inhibiting COMT may offer an additional means of restoring DA and treating Parkinson's disease (PD) [21]. Entacapone and Tolcapone are two typical COMT inhibitors that extend the effects of LDOPA by inhibiting the breakdown of docosa hexamine (DA). These medications can help lessen the susceptibility of PD patients to LDOPA and have less adverse effects [21].

Conclusions

Parkinson's disease (PD) is a multifaceted neurodegenerative condition marked by a steady loss of dopaminergic neurons, which results in symptoms that are both motor and non-motor. The complex molecular mechanisms and treatment modalities for Parkinson's disease are highlighted in this study Numerous interrelated molecular mechanisms, including as α-synuclein aggregation, oxidative stress, neuroinflammation, ferroptosis, mitochondrial dysfunction, and gut dysbiosis, are involved in the etiology of Parkinson's disease (PD). Since each of these variables has role in the development of the disease and the damage to neurons, a comprehensive strategy may be required for successful therapy. Environmental pollutants

(e.g., pesticides, MPTP) and genetic mutations (e.g., in Parkin, DJ-1, PINK1, and LRRK2) are important factors in the development and progression of Parkinson's disease. Environmental contaminants are linked to infrequent incidences of the disease, whereas genetic abnormalities play a role in family types of the illness. Determining at-risk populations and creating preventive solutions require an understanding of these elements. Existing treatments, primarily centered around L-DOPA and adjunct medications such as MAO-B and COMT inhibitors, offer symptomatic relief but are not curative. These therapies can be effective in managing motor symptoms but often become less effective over time and do not address the underlying disease mechanisms. Preclinical models indicate potential for novel treatment approaches such as CRISPR-Cas9 gene editing, gene therapy, and stem cell transplantation. By addressing the disease's biological or genetic causes, these strategies may provide more long-lasting and potent therapies. Problems with medication administration, limited efficacy in advanced stages of Parkinson's disease, and unfavorable side effects plague the present therapy landscape. Moreover, study animal models frequently fall short of accurately simulate human disease, which makes developing novel treatments more difficult.

Future Scope

Future studies should concentrate on creating tailored treatments that target particular biochemical pathways connected to Parkinson's disease. Developing medications that can efficiently control the aggregation of α-synuclein, lower oxidative stress, and lessen neuroinflammation is part of this.

Treatment regimens that are tailored to each patient's genetic profile and illness subtype may become possible as a result of developments in genomics and biomarker research. Personalized methods may reduce adverse effects and enhance therapeutic results. It is imperative to develop novel drug delivery technologies with improved blood-brain barrier crossing. Methods like intranasal administration or delivery via nanoparticles may improve the effectiveness of medications while lowering systemic side effects. Understanding the nature history of Parkinson's disease (PD) and the efficacy of novel therapies will require long-term studies that monitor the disease's course and response to treatment in a variety of groups. These investigations may also reveal biomarkers for early illness detection and prediction.

Conflict of Interest

There is no conflict of interest related to the study.

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Chapter - 6
Comprehensive Review of the Mechanism of Action, Side Effects and Emerging Alternatives to NSAIDs

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Chapter - 6

Comprehensive Review of the Mechanism of Action, Side Effects and Emerging Alternatives to NSAIDs

Sagnik Sardar, Sanjukta Banerjee and Rupesh Dutta Banik

Abstract

NSAIDs, or non-steroidal anti-inflammatory drugs, are among the most often given medications worldwide. It consists of a diverse range of pharmaceuticals used to treat fever, discomfort, and inflammation symptomatically. Notwithstanding the fact that NSAIDs primarily function by preventing the cyclooxygenase (COX) enzyme from synthesising prostaglandins, clinical and experimental evidence clearly suggests the presence of other mechanisms. The ability of NSAIDs to cross biological membranes and interfere with crucial molecular interactions required for a variety of cellular processes, including cell adhesion, is linked to some of the COX-independent effects; these effects, particularly the ones that interfere with neutrophil L-select in function during the inflammatory response, may be part of the NSAIDs' anti-inflammatory effects in vivo. But NSAID use is not safe since, in comparison to non-NSAID users, it mostly raises the risk of cardiovascular and gastrointestinal (GI) problems. NSAIDs cause topical mucosal irritation and deplete prostaglandins generated from COX-1, which damages both the upper and lower gastrointestinal tract besides it also cause cardiovascular side effects and renal disturbance. In this review article we will discuss about the mechanism of action of NSAID mediated drugs, adverse effects of NSAIDs in GI toxicity, CV and renal disturbances and highlighting the necessity of creating fresh, safer COX inhibitors.

Keywords: Non-steroidal anti-inflammatory drugs, cyclooxygenase, prostaglandins, COX-independent effects, cardiovascular disease, gastrointestinal (GI) problems

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a broad family of medicinal medicines used for the symptomatic treatment of rheumatic disorders. It was well recognized in early 1970s that the primary mechanism

of action of these drugs was inhibition of cyclooxygenase (COX), an enzyme that is essential to the synthesis of prostaglandins **[Díaz-González1 and Francisco *et al.* 2014].** Cyclo-oxygenase (COX), also known as prostaglandin endoperoxide synthase, was isolated. It is homogenous and enzymatically active 17. With molecular mass of 71kilo Daltons (kDa), this membrane-bound hemo-and glycoprotein was mostly detected in the endoplasmic reticulum of prostanoid-forming cells. 18 It was demonstrated that the glycoprotein has COX activity, cyclizing arachidonic acid and incorporating the 15-hydroperoxy group to create prostaglandin G2. Prostaglandin G2 hydroperoxy group is known to be reduced to prostaglandin H2's hydroxy group by a peroxidase that uses a wide range of substances to supply the necessary pair of electrons. The dimeric protein molecule has both hydro peroxidase and COX activity **[Bacchi, Palumbo, Sponta and Coppolino *et al.* 2012].** Most leukocytes express L-selectin constitutively, and upon cell activation, its ectodomain is processed by enzymes that break it and release it. Because it facilitates neutrophil rolling on the endothelial surface, L-selectin is crucial for the early recruitment of neutrophils to inflammatory foci. Research conducted in vitro and in vivo has demonstrated that soluble L-selectin can diminish intravascular leukocyte adhesion and rolling, which in turn reduces neutrophil extravasation. This effect is likely caused by soluble L- selectin competing with cell surface L-selectin for binding to endothelial ligands. In vitro, it has been demonstrated that certain NSAIDs cause human neutrophils to rapidly cleave and lose L selectin **[Díaz-González1 and Francisco *et al.* 2014**]. NSAIDs harm the gastrointestinal tract by topically damaging the mucosa and having systemic effects linked to the depletion of mucosal prostaglandins, which are generated from COX-1. Consequently, the use of enteric-coated aspirin preparations and parenteral or rectal NSAID administration for the purpose of preventing topical mucosal injury has not been successful in preventing the formation of ulcers **[Sostres, Gargallo and Arroyo *et al.* 2009].** The inhibition of prostanoid biosynthesis is the mechanism via which NSAIDs exert their anti-inflammatory, analgesic, and antipyretic effects.11, 32, 33 Arachidonic acid, a fatty acid found in cell membranes as a phospholipid ester, is the starting point for the synthesis of prostanoid. COX isozymes 11, 19, and 32 convert arachidonic acid to prostaglandin (PG) G2 and then to PGH2. PGH2 then goes through several other conversion activities to produce five bioactive prostanoid: PGD2, PGE2, PGF2α, and PGI2 (Brune and Patrignani *et al*. 2015).

Mechanism of Action

Aspirin

It binds irreversibly to COX, blocking arachidonic acid from being further metabolized. For those at high risk, aspirin is useful in preventing cardiovascular events. Aspirin principle known impact on hemostasis sis to hinder platelet aggregation by blocking the synthesis of platelet thromboxane A2, which lowers the amount of thrombus that forms on the surface of the injured artery wall (Simon LS *et al*. 1999). Salicylic acid and acetic acid can react to produce aspirin when an acid catalyst is present. With the carboxyl group on the acetic acid, the phenol group on the salicylic acid form sanester. But the yield of this reaction is quite low, pandit proceed slowly. It functions by obstructing the platelet cyclooxygenase-1 (COX-1) molecule, which keeps the platelets from activating. However, aspirin also inhibits other relevant molecules in the body, potentially decreasing it anti-clotting actions. By irreversibly acetylating the active site of cyclooxygenase-1 (COX-1), which is necessary for the synthesis of thromboxane A2, a potent aggregation promoter, aspirin has a clinically significant antiplatelet impact (Steinmeyer. *et al*. 2000).

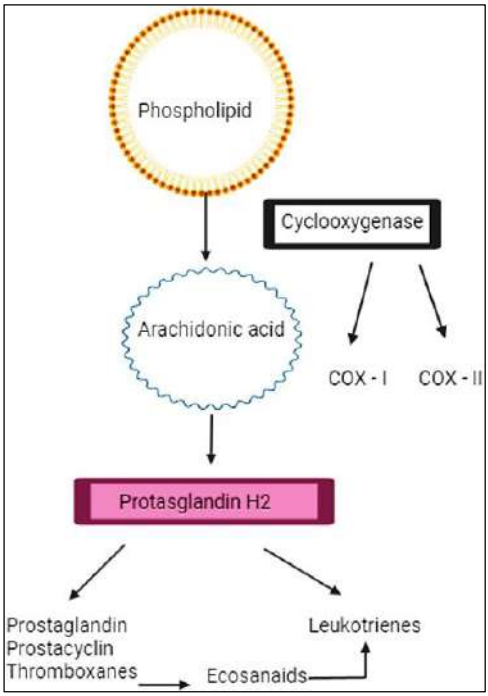

Fig 1: Pathway of eicosanoids production

Ibuprofen

It in hi bits COX-1 and COX-2 reversibly. In addition, ibuprofen scavenges $HO^{\bullet}$, $^{\bullet}NO$, and $ONOO^{-}$ radicals and can potentiate or inhibit nitric oxide formation through its effects on nitric oxide synthase (NOS) isoforms. Ibuprofen prevent the production of prostaglandins. Ibuprofen is used to treat inflammatory conditions such as osteoarthritis and rheumatoid arthritis, as well as mild to severe pain, fever, and dysmenorrhea-related suffering. Ibuprofen inhibits both COX isoforms to produce its analgesic and anti-inflammatory properties. Furthermore, ibuprofen scavenges HO radical, NO, and ONOO. Its effects on nitric oxide synthase (NOS) isoforms can either stimulate or inhibit the generation of nitric oxide. By attaching to cannabinoid receptors and inhibiting the enzyme fatty acid amide hydrolase (FAAH), which metabolizes the endocannabinoid anandamide, ibuprofen may stimulate the anti-nociceptive axis (Vane *et al.* 1998) (Smith *et al.* 2000) (Bacchi *et al.* 2012).

Celecoxib

It suppresses COX-2 specifically to prevent gastrointestinal adverse effects. Celecoxib is a diary l-substituted pyrazole with the molecular designation 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide. Celecoxib works by selectively inhibiting cyclooxygenase-2(COX-2), which is essential to the pathway that leads to pain and inflammation and is responsible for the creation of prostaglandins. Celecoxib's analgesic, anti-inflammatory, and antipyretic properties are a result of its pharmacologic activity. Moreover, celecoxib lessens the effect of aspirin on platelet function by mildly inhibiting COX-1. The anticancer effects of celecoxib are also covered below. It works by binding cadherin-11 (CDH11), which is probably important for the malignant development of cancerous cells. Additionally having sulfonamide group, celecoxib should not be used in patients who have experienced severe adverse responses to other medications that also have sulfonamide groups, such as sulfamethoxazole. Research has indicated that sulfonamides antimicrobial allergies area risk factor for sulfonamide chemical group allergies. At least as significant risk factor for an allergic reaction is a history of penicillin allerg. Therefore, healthcare professionals should be aware that these phenomena may be caused by more than just the cross-reactivity of antimicrobials and non-antimicrobials containing sulfonamide, and that some people are more prone to develop a celecoxib allergy if they have an allergy to any antimicrobial medication (Brune *et al.* 2015).

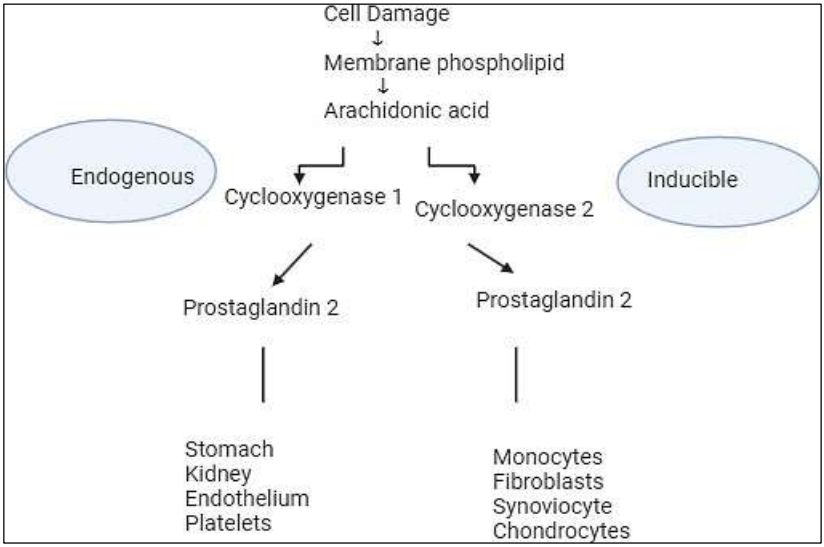

Fig 2: Cell damage using NSAIDS

Adverse Effect

Renal Toxicity

NSAIDs can either cause or worsen hypertension and interfere with the action of antihypertensive medications, such as diuretics, despite published results from experimental and clinical research showing the opposite. At sufficiently high dosages, nonsteroidal anti-inflammatory medications (NSAIDs) modify renal function decrease renal blood flow and the glomerular filtration rate, as well as resulting in salt retention. Blood pressure will increase in individuals who are sensitive to salt due to this sodium retention. Coxibs should be used with the same normal renal cautions that apply to the use of non-selective NSAIDs. Patients with hypertension, especially those on ACE inhibitors and/or potassium-sparing diuretics; those on salt substitutes; patients with diabetes, especially those with type IV renal tubular acidosis and potentially renal insufficiency; patients with volume depletion; and patients with cirrhosis and congestive heart failure should use them with caution. Nonselective NSAIDs and coxibs don't appear to have significant effects on the kidney when administered in therapeutically similar doses (Campiani *et al*. 2002).

Cancer

NSAIDs have anti-cancer activity, according to several research conducted over a long length of time (Abnet *et al*. 2009) (Chan *et al*. 2007)

(Takkouche *et al*. 2008). Following the identification of the COX-2 isoform, it was discovered that it appeared to regulate numerous cell processes and had high expression in a wide range of malignant tissues, including the colon, breast, prostate, and pancreatic (Kanaoka *et al*. 2007). Because of their potential application in the prevention and treatment of many malignancies, selective COX-2 inhibitors have been the subject of substantial research. The chance of dying from endometrial cancer may rise with regular use of NSAIDs, such as aspirin and ibuprofen, particularly for those who took the medication for more than ten years prior to receiving a diagnosis. Most people who regularly take NSAIDs may have a lower risk of colorectal cancer, although not everyone benefits from this. (Harris et al. 2005) (Rothwell *et al*. 2011) Kidney cancer risk may rise with regular use of non-aspirin NSAIDs. According to studies involving both men and women, long-term NSAID use may lower men's risk of developing cancer overall, but it may increase women's risk (Bacchi *et al*. 2012).

Topical Injury

The majority of NSAIDs, including ASA, have acidic qualities that cause mucosal injury. In the extremely acidic gastrointestinal environment, these weak acids persist in their nonionized lipophilic state. These circumstances facilitate the migration of NSAIDs into surface epithelial cells, where they break down into their ionized state and trap hydrogen ions, causing damage to the mucosa. Photo contact dermatitis has been linked to the topical NSAID gels and creams used for pain relief. With an incidence of 0.013-0.028/1000, ketoprofen gel has been the most often reported cause of this. Non-steroidal anti-inflammatory drugs (NSAIDs) like Naproxen and Ibuprofen are very effective for relieving muscle pain in various scenarios (Schoen RT *et al*. 1989) (Sostres *et al*. 2009).

Respiratory Toxicity

NSAID-induced reactions appear to be caused by the inhibition of cyclooxygenase-1 (Cox-1); this in turn activates the lipoxygenase pathway, which eventually increases the release of cysteinyl leukotrienes (Cys-LTs) that induces bronchospasm and nasal obstruction. Aspirin and other NSAIDs can induce bronchospasm and in rare cases, this reaction can lead to death in aspirin-sensitive asthmatics. This reaction is generally referred to as aspirin-induced asthma. The reported incidence varies widely affecting between 8% and 20% of adult asthmatics. Cyclooxygenase-1 (Cox-1) inhibition appears to be the root cause of NSAID-induced responses. This inhibition then triggers the lipoxygenase pathway, which increases the release of cysteinyl

leukotrienes (Cys-LTs), which causes nasal obstruction and bronchospasm. NERD, or nonsteroidal anti-inflammatory drug-exacerbated respiratory illness, is a very variable condition with a wide range of clinical presentations. It is typified by moderate-to-severe asthma and a greater incidence of chronic rhinosinusitis/nasal polyps. Aspirin or other nsaids cause upper and/or lower airway symptoms inpatients with N-ERD, which typically subside after 30 to 180 minutes. Anaphylactoid reactions, which occurs when an NSAID inhibit the cyclo-oxygenase (COX)-1 enzyme, area type of cross-reaction hypersensitivity reaction. NSAID-induced single- dose reactions that are immunologically mediated can result in genuine allergies or anaphylaxis (Bacchi *et al*. 2012).

Gastrointestinal Toxicity

The repair of active peptic ulcers is postponed by COXib therapy and nonsteroidal anti-inflammatory medication therapy. When taking NSAIDs COXibs, patients who develop stomach ulcers should cease taking the medication, start PPI therapy, and, if an infection is already present, getrid of *H. pylori*. Patients must get PPI co-therapy twice daily until the ulcer heals, which must be verified by endoscopy, if they are unable to stop taking NSAIDs. The pathophysiology of NSAID-induced gastrointestinal injury has been the subject of numerous investigations. It was thought that NSAIDs mediated the gastrointestinal injury by inhibiting prostaglandin-endoperoxide synthase 1 (PTGS1 or cyclooxygenase [COX] 1) and COX2. The introduction of exogenous prostaglandins can mitigate the effects of NSAID-induced reductions in mucosal prostaglandin levels (caused by COX1), which are correlated with stomach and small bowel injury. Compared to traditional NSAIDs, COX2 selective inhibitors are thought to be safe because COX2 isn't constitutively produced in the gastrointestinal system. The increased secretion of gastric acid by prostaglandins, the decreased secretion of mucus and bicarbonate, the reduction of cell proliferation, and the reduction of mucosal blood flow are some of the hypothesized processes of stomach injury. Therefore, it does not appear that COX inhibition is the only mechanism causing NSAID-induced gastrointestinal harm. We go over the NSAIDs' prostaglandin-independent processes and how they interact with the effects of prostaglandin changes brought on by COX inhibition. We present a model (**Fig. 1**) in which the pathophysiology of gastrointestinal injury involves multiple key components, among them COX inhibition. Our model takes into account the impact of the particular "topical" biochemical actions of NSAIDs and the resulting rise in intestinal inflammation and permeability. These cause the

damage to begin, and luminal aggressors like COX1 and COX2 inhibition exacerbate it, resulting in the formation of erosions and ulcers (Wallace *et al*. 1997) (Silen W *et al*. 1985) (Patrignani *et al*. 2011).

Cardiovascular Toxicity

Nonsteroidal anti-inflammatory drug (NSAID) consumption is linked to a higher chance of unfavourable cardiovascular events. The risk of such incidents is increased by both nonselective and cyclooxygenase (COX)-2 selective NSAIDs. NSAIDs, or nonsteroidal anti-inflammatory medicines, raise the risk of stroke and heart attack. These drugs are typically used to treat fever, inflammation-a term used to describe pain, swelling, and irritation. Analgesic, anti-inflammatory, and antipyretic properties are properties of nonsteroidal anti-inflammatory medications (NSAIDs). The decrease in prostanoid' production is how they accomplish their action. There is a significant chance of negative effects from inhibition of prostanoid. It's also commonly known that there could be an increase in blood pressure and congestive heart failure. Concerns concerning the cardiovascular safety of non-selective NSAIDs have also been highlighted. The potential cardiotoxicity of selective cyclooxygenase-2 inhibitors was brought to light increased frequency of acute myocardial infarction in clinical trials including rofecoxib. Many prospective and retrospective clinical trials, as well as meta-analyses, have examined the safety of NSAIDs in relation to cardiovascular events in recent years. In addition to decreasing the production of the vasodilator prost a cycling in the vessel wall, NSAIDs might result in salt and water retention. It has long been recognized that using NSAIDs increases the risk of high blood pressure. All NSAIDs, apart from low dose ASA, tend to have this side effect, albeit some seem to be safer than others. Industrial, the increase in blood pressure reaches the level of statistical significance only in the subgroup with medically controlled hypertension, although meta-analysis found that analgesics from the NSAID group raise mean blood pressure by 5 mmHg (Brune and Patrignani *et al*. 2015).

Neural Toxicity

A significant neurological adverse effect linked to NSAID use is aseptic meningitis. Fever, headache, photophobia, stiff neck, and other clinical symptoms are shared by drug-induced meningitis and infectious meningitis. Several hundred or thousand cells, mostly neutrophils, were pleocytosis from the cerebrospinal fluid (CSF), and other laboratory results that included high protein levels, normal or low glucose levels, and negative cultures were

equally similar. The prognosis is excellent for drug-induced meningitis, which is temporary condition. An increase in capillary permeability and vasodilatation characterizes the initial, acute response. Immune cell infiltration characterizes the second, subacute phase. Finally, a chronic phase that is typified by tissue fibrosis takes place. In the central nervous system, this final stage is lacking. Prostaglandins have a major role in the initial stage. Arachidonic acid is converted by the enzyme cyclooxygenase, which also produces thromboxane A2 and several prostaglandins that are involved in the inflammatory response. Small doses of prostaglandins injected intradermally, intravenously, or intraarterially can mimic many aspects of inflammation. When prostaglandin E2 or prostacyclin are administered, erythema develops, local blood flow increases, and leukocyte migration into an inflammatory area may be encouraged (Steinmeyer *et al*. 2000).

Reproductive Toxicity

Any negative impact chemical may have on the reproductive cycle, including the impairment of reproductive function and the induction of negative effects in the embryo, such as growth retardation, deformities, and death, is referred to as reproductive toxicity. The body's endocrine system regulates all these activities and interactions to a great degree. Reproductive toxicity is undoubtedly an issue that is current; whilst in the past, the most spectacular element of birth abnormalities received most of the attention, interest in the risks to fertility is expanding. NSAIDs suppress cyclooxygenase (COX), an enzyme required for ovulation, implantation, and placentation, which can result in reversible infertility. Moreover, NSAIDs may prevent a mature ovum from releasing, which may result in the syndrome known as luteinized unruptured follicle (LUF). Long-term NSAID use can harm testicular tissue, which lowers sperm motility, count, and seminal volume. When taking NSAIDs in the third trimester of pregnancy, there is a risk of fetal renal impairment, premature closure of the ductus arteriosus, and delayed labour and delivery. APAP and NSAIDs disrupt endocrine gland function and may have negative effects that span generations. They may specifically target germ cells, which could lead to lower-quality male and female gametes and lower fertility in those who are exposed and their offspring. Next, the review address the impact on postnatal gonadal development and adult reproductive health of exposure to either a single drug (APAP, aspirin, or IBU) or combinations of drugs during early embryogenesis. When take NASA whole, these findings may raise medical and public awareness of these reproductive health issues, especially among women who are fertile, expectant mothers, and young parents. Concerns

over the long-term reproductive health of the general public are raised by the combined effects of NSAIDs, APAP, and other endocrine disruptors, as well as by the potential for intergenerational and transgenerational consequences. The results of epidemiological research on the relationship between the use of NSAIDs or analgesics and a decreased incidence of AGD are inconsistent, and it is still unknown exactly when, how much, and what kind of medications cause congenital abnormalities. To understand the cellular and molecular effects of exposure to these medications on reproductive health, experimental models, in vitro/ex vivo rodent/human gonadal explants culture, and *in vivo* research in animal models have been created (Sostres *et al*. 2009).

Conclusion

addition offering chance to test the novel theory, the discovery of selective COX-2 inhibitors will improve the management of inflammation. Since prostaglandin formation is tightly regulated in organs like the uterus, selective COX-2 inhibitors will likely find new applications in the coming years. For instance, it's possible that the hormonal induction of COX-2 results in the production of a prostaglandin linked to parturition. Furthermore, studies have shown that aspirin is useful in the prevention of colon cancer, and that COX-2 is linked to the disease**.** Adverse effects that are not seen in research trials may manifest in daily life. An overabundance of COX-2 selectivity may also be detrimental, particularly at higher doses. Reporting on the efficacy and adverse-effect profile of COX-2 inhibitors in relation to NSAIDs that have been used successfully in the past, particularly in long-term trials, will therefore be a crucial responsibility for medical institutions. Overall, nevertheless, the initial clinical results are encouraging despite the theoretically possible adverse effects. Without a doubt, selective COX-2 inhibitors are novel medicinal discovery with potential wide applications.

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Chapter - 7
Understanding the Gut Microbiome's Role in PCOS Pathogenes is and Treatment Strategies

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Chapter - 7

Understanding the Gut Microbiome's Role in PCOS Pathogenes is and Treatment Strategies

Sohani Das and Rupesh Dutta Banik

Abstract

Five to twenty percent of women who are of reproductive age suffer with PCOS, a common and complicated endocrine condition. Clinical manifestations of PCOS encompass hirsutism, infertility, menstrual disruption, obesity, and metabolic syndrome. Although the exact cause of PCOS is unknown, four factors appear to play varying roles in the syndrome's pathophysiology: partial folliculogenesis halt, insulin resistance, elevated ovarian and/or adrenal androgen production, and disruption of the neuroendocrine axis. The human body's "second genome," the gut microbiota, interacts with the outside world to enhance immunological response and metabolism. The metabolic and clinical characteristics of PCOS have been linked to alterations in the gut microbiota, according to recent research in human and rodent models. Furthermore, dysbiosis of the gut microbiota has been suggested as possible pathogenetic component in PCOS development. In this regard, the alteration of the gut microbiota by probiotic, prebiotic, and symbiotic agents raises the possibility that these goods might be used as novel PCOS treatments. The current global PCOS treatment landscape is summarized in this review. In the absence of metformin, lifestyle modification (LSM) is regarded as the first-line therapy for all patients, regard less of their reproductive status. The primary line of treatment for long-term contraceptive (O management of individuals who do not require contraception should be oral C) tablets. Ovulation therapy is a useful treatment for people with reproductive needs.

Keywords: PCOS, infertility, gut microbiota, life style modification (LSM), ovulation therapy

Introduction

Five to twenty percent of women in reproductive age have PCOS, a widespread and complicated endocrine condition that is primary cause of

anovulatory infertility and hirsutism [1]. Nevertheless, PCOS is more than just a reproductive condition; it is linked to other metabolic diseases, including elevated cardiovascular surrogate indicators, glucose intolerance, diabetes, dyslipidemia, hypertension, and hepatic steatosis. There is currently no evidence to support a higher frequency of cardiovascular events or death in this population [12]. It was proposed in 2012 that the gut bacteria can contribute to the development of PCOS [37]. According to recent research, women with PCOS have altered gut microbiota compositions, which are linked to alterations in PCOS's metabolic and clinical characteristics [13, 18]. It is unclear what exactly causes the association between gut microbiome and PCOS, though [35]. As a result, the link and potential pathways between gut microbiota and PCOS have been covered in this study [43]. There is still much to learn about the pathophysiology of PCOS, but four factors appear to play a role in the syndrome's development: partial folliculogenesis stoppage, insulin resistance, increased ovarian and/or adrenal androgen production, and disruption of the neuroendocrine axis [5]. It is disputed by some academics that PCOS is an evolutionary contradiction [3]. The etiology and treatment of this disease have thus been extensively researched in recent years; however, because of the disease's heterogeneity and uncertainty, the pathogenesis mechanism is not fully understood and may be influenced by factors internal to the embryo, the environment, and heredity [21-2]. In order to help PCOS patients get better results, we have included the PCOS treatment strategies that have been produced recently in this study [14].

PCOS

In women of reproductive age, polycystic ovarian syndrome (PCOS) Is common endocrine disorder that affects 6% to 20% of women worldwide [8]. Polycystic ovaries, hyperandrogenism, and oligo/anovulation are at least two of its characteristics [42]. Uneven gonadotropin production (higher luteinizing hormone [LH] secretion, higher ratio of LH to follicle-stimulating hormone [FSH]), elevated testosterone levels, reduced sex hormone-binding globulin (SHBG), and persistent low-grade inflammation are among the biochemical findings of this condition [43].

The microbiota in the gut

The term "microbiota" refers to all microorganisms (bacteria, arche, protozoa, fungus, etc.) that reside in the skin, mouth, respiratory system, gastrointestinal tract and vagina [29, 33]. The microbiota is made up of around 100 trillion bacteria and has mass of approximately 1.5kg [33, 28]. The microbiota starts to form as soon as a child is born, and a variety of factors,

including age, birth type, nutrition, lifestyle, genetic predisposition, and antibiotic usage, can affect its makeup [26]. Five bacterial phyla dominate the human gut microbiota: Bacteroidetes (Bacteroides, *Porphyromonas*, *Prevotella*, etc. gram-negative species), Proteobacteria (*Enterobacteriaceae* etc. gram-negative), Actinobacteria (*Bifidobacterium* gram-negative species), and Verrucomicrobia (*Akkermansia*, etc. gram-negative species) [26, 36].

Mechanisms underlying the Association between Gut Microbiome and PCOS

According to recent research, dysbiosis of the gut microbiota may contribute to the pathophysiology of PCOS [13, 37]. There are several potential explanations for how gut microbiota contributes to PCOS development. Among these is the dysbiosis of the gut microbiota, which triggers the host's immune system. Immune system activation impairs insulin receptor function, leading to hyperinsulinemia, which raised ovarian androgen production and inhibits the growth of healthy follicles. Thus, anovulation/menstrual problem, hyperandrogenism (acne, hirsutism), and polycystic ovarian syndrome are the traditional three features of PCOS. The term DOGMA (dysbiosis of gut microbiota) is used to describe this notion. This idea is based on two primary aspects. Typical obesity and a high-fat, low-fiber diet are these variables in PCOS [37]. Women with PCOS frequently struggle with obesity, particularly central obesity, which is linked to high-fat diet [13, 18]. Thirty to seventy percent of women with PCOS are obese [13, 37]. Excess adipose tissue causes a reduction in adiponectin, an anti-inflammatory protein generated from adipocytes, and an increase in the production of tumor necrosis factor alpha (TNF-a) from adipose tissue macrophages [43]. Regardless of obesity, PCOS is linked to persistent immune system activation. In women with PCOS, genetic variables are also efficient in chronically activating the immune system; variations in genes producing pro inflammatory cytokines, such as TNF-a and interleukin-6 (IL-6), have been documented to be widespread [43]. The term "metabolic endotoxemia" refers to the rise in LPS circulation in the body, which results in alterations to the cardiometabolic system and low-grade chronic inflammation. As the source of LPS, intestinal bacteria raise intestinal permeability and trigger endotoxin translocation into the bloodstream, which results in metabolic endotoxemia [15]. Chronic endotoxemia is brought on by an overabundance of gram-negative bacteria in obese people, which raises the quantity of bacterial LPS in the blood. When bacterial lipopolysaccharide (LPS) is recognized by toll-like receptors (TLR) on the surface of enterocytes, the NF- kB pathway is triggered, which causes inflammation. Endotoxemia is also brought on by

alterations in the gut flora, which also result in decreased tight junction protein expression and increased intestinal mucosal permeability. It is well established that eating a high-fat diet increases intestinal permeability and LPS translocation [37, 15, 32]. In an investigation on the connection between intestinal permeability and PCOS, it was shown that 78 PCOS-afflicted women had greater levels of serum zonulin-a biological indicator of intestinal permeability-thanthi group of healthy women. Additionally, there was favorable link found between insulin resistance, the severity of menstruation problems, and serum zonulin levels [44]. According to Lindheim *et al*., women with PCOS had greater levels of leukocytes, lymphocytes, and lipid binding protein-a protein that binds endotoxin-than those in the control group [17].

70% of PCOS patients are insulin resistant, a condition that affects both fat and thin women [43]. In many PCOS patients, persistent low-grade inflammation results in insulin resistance. Increased levels of cytokines like TNF-a and IL-6 as well as the transfer of LPS into the systemic circulation as a result of increasing intestinal permeability cause insulin resistance in obese PCOS patients. Research have demonstrated that applying LPS directly to mice's and humans' circulations raises fasting blood glucose and insulin levels [43]. Serum testosterone levels are elevated by inflammatory insulin resistance through two different pathways two different pathways. The first mechanisms that the ovaries produce too much testosterone as a result of hyperinsulinemia [43]. Second, by lowering SHBG, hyperinsulinemia raises the levels of free (bioactive) testosterone [43]. Acne and hirsutism result from elevated testosterone levels brought on by hyperinsulinemia [9]. Moreover, hyperinsulinemia causes for insulin-like growth factor 1(IGF-1), which enhances androgen synthesis in sperm cells by preventing the synthesis of the protein that binds to IGF-1 (IGFBP-1) [43]. Elevated insulin and IGF-1 activity lead to polycystic forms and irregular menstruation the ovaries, as well as inhibit normal follicle development from tiny follicles [43]. In conclusion, three traditional PCOS traits have emerged, and hyperinsulinemia plays significant parting their development [37]. It has been noted that while a high carbohydrate diet is recognized as a risk factor for PCOS, diet may not directly cause PCOS [43]. For example, in PCOS-affected women, Dougles *et al*. could not find any correlation between the content of the diet and fasting insulin levels or the glucose-to-insulin ratio [22]. There is a dearth of information about the impact of environmental variables, such as food, on the development of PCOS, despite the overwhelming evidence that genetic factors are significant in the development of PCOS and hyperandrogenism [43].

Specifics of PCOS Pathogeneses

Hyperandrogenism in the Adrenal and Ovary Glands

There is a lot of evidence to support the theory that PCOS is an ovary intrinsic condition, with increased androgen production serving as the main cause of the disorder. In response to LH, ovarian theca cells normally create androgens. The CYP17A1 gene, which codes for the P450c17 enzyme, is expressed by theca cells. This enzyme catalyzes the rate-limiting step in the production of sex steroids, 17,20-lyase activity, as well as 17α-hydroxylase activity. Cycles in androgen production are regulated by both extraovarian and intraovarian processes. A reduction in CYP17A1 expression and a downregulation of LH receptors happen as LH levels rise, which minimizes the generation of androgens. Both testosterone and estrogen block the actions of 17,20-lyase and 17α-hydroxylase, creating a paracrine and autocrine negative feedback loop. On the other hand, the P450c17 enzyme is stimulated and LH receptor sites are up-regulated by insulin and IGFs [31]. The main cause of PCOS ovaries' hypersensitivity to LH is partial escape from LH receptor downregulation. This enhanced sensitivity to LH appears to be related to an imbalance in the intra-ovarian regulatory mechanism. Hyperinsulinemia and insulin resistance are two examples of exogenous variables that interfere with the regular intra-ovarian regulation processes [31].

Isolated theca cells or tissues from PCOS women release more androgen than cells from normal controls, according to *in vitro* research. The elevated production of androgen in PCOS theca cells is attributed to the upregulation of 17α-hydroxylase/17,20-lyase and the enzyme responsible for cholesterol side-chain breakage, indicating an inherent abnormality in the theca cell [5]. When considered collectively, these findings suggest that the ovarian cells from PCOS patients possess inherent and consistent features that shape their phenotype, even when gonadotrophin, hyperinsulinemia, and hyperandrogenism stimulation are eliminated [11].

Follicle Development in Ovaries

Gonadotropins have little influence on the ovarian mechanism that initiates follicular recruitment. Primordial follicles are kept in the quiescent state by pro-apoptotic factors (FOX) produced by oocytes, inhibitory transcription factors (LKB1/STK11/BMP4), and epithelial-mesenchymal interactions. Follicle growth and development, as well as follicular recruitment, are regulated by a group of local follicular factors (BMP6, BMP9, and GDF9). For instance, BMP15 and GDF9 work together to promote granulosa cell proliferation [31]. AMH, a member of the GFR-beta-

superfamily generated by the granulosa cells of tiny developing follicles, is another significant regulator of folliculogenesis. AMH expression is greatest in preantral and tiny antral follicles measuring less than 4 mm. AMH expression decreases and follicles become more responsive to FSH action as the expand size of >8mm. This results in enhanced follicle development and production of estrogen, the selection of the dominant follicle, and eventual ovulation in normal ovary [10]. AMH suppresses FSH-dependent follicular maturation, the selection of the dominant follicle, and the first FSH-in dependent recruitment of primary follicles from the primordial follicle pools [1].

Insulin Resistance

Acanthosis nigricans, hyperandrogenism, and uncommon types of extreme insulin resistance (IR) were first reported in 1970 (Insulin Resistance type A, Rabson-Mendenhall Syndrome, and Leprechaunism) [5]. Reduced insulin binding to its receptor and impaired insulin receptor auto phosphorylation accounted for the molecular basis of insulin resistance in these illnesses [5]. Simultaneously With Severe IR, family types of lipodystrophies were linked to hyperandrogenism [5]. As a common characteristic of these diseases, remarkable hyperinsulinemia raised the possibility that insulin may directly trigger the creation of androgens from the first time [5]. Burgenland Cols. (1980) observed that women diagnosed with PCOS exhibited elevated insulin levels during the oral glucose tolerance test, which could not be solely attributed to their obesity. Women with typical PCOS also developed acanthosis nigricans, which resulted in a phenotypic profile resembling the previously mentioned uncommon IR disorders [5].

IR is not widespread throughout all tissues in PCOS condition. Insulin's mitogenic and steroidogenic effects are intact in the ovaries, while its metabolic functions are impaired in muscle and adipose tissues [6]. Insulin boosts the sensitivity of theca cells to Land raises the expression of P450c17 and 3β-HSD2, which increases androgen synthesis. Insulin does this via acting on the ovarian insulin receptor [23]. Experiments on Rats with IR brought on by an obesity-causing diet provide good illustration of this route; in these experiments, anovulation and hyperandrogenism were enhanced by insulin receptor knockout [39]. In PCOS therapy, there is a reaffirmation of insulin's function in steroidogenesis. Any therapy that lowers blood insulin levels, such as metformin, thiazolidinedione, bariatric surgery, or weight loss by lifestyle modifications, greatly reduces anovulation and hyperandrogenemia [5].

The Neuroendocrine Axis

Since the gonadotropic stimulus closely linked to ovarian steroidogenesis, changes in LH are suggested to be one of the causes contributing to PCOS development. The main modulator of GnRH pulsatility is progesterone. Patients with PCOS are known to exhibit a GnRH-generating pulse resistance to progesterone's negative feedback, which raises the frequency and/or amplitude of LH pulses [5]. Several investigations have already linked this condition to hyperandrogenemia, showing that a 4-week course of flutamide therapy restored hypothalamic sensitivity to ovarian steroids [5]. Changes in the gonadotropic axis now seem to be a result of hyperandrogenemia. Furthermore, since LH excessive variable aspect of PCOS, it's challenging to assign LH excess key role in the pathophysiology of every PCOS patient. On the other hand, variations in LH pulsatility seem to be a predictor of the PCOS phenotype in individuals with congenital virilization [31].

Strategies for Treatment

Adjustment of Lifestyle

Insulin resistance affects between 60 and 80 percent of PCOS patients and 95 percent of obese people, elevated risk of type 2 diabetes and cardiovascular disease [30]. Regardless of the need for birth, lifestyle modification (LSM) is regarded as the first-line therapy. According to Panidis *et al*. [27], overweight people with PCOS should watch what they eat-consuming 1200-1500 kcal/day (1 kcal ¼ 4.184 kJ)-and make sure they exercise moderately for at least 30 minutes per day, five days a week. Patients can improve insulin resistance and free testosterone levels, as well as lessen menstrual disorders, excessive hair growth, acne, and other symptoms cutting back on calories, exercising, treating behavior, receiving medication treatment, having surgery to reduce BMI to a normal range, and giving up alcohol and smoking. A randomized study of the effects of exercise and/or dietary control on ovarian function and metabolic parameters in overweight women was carried out by Nybacka *et al*. [40]. The Body Mass Index (BMI) was observed to be lowered by 6%, 3%, and 5% by the combined treatments after four months, according to the authors. Domecq *et al*. [25] found that diet and exercise treatment together can lower BMI, improve insulin resistance, and lower fasting blood glucose levels. Pregnancy and live birth rates are higher in PCOS patients who lower their body mass index prior to becoming pregnant, according to many surveillance studies [14]. Lowering the BMI with LSM also lessens the negative effects of

obesity [14], such as endometrial cancer, diabetes, high blood pressure, excessive blood fat, and cardiovascular disease.

Oral Contraceptive Medication

The primary line of treatment for the long-term control of PCOS is oral contraceptive (OC) medication [41]. The primary ingredient in the tablets are progesterone and estrogen. By suppressing FSH and progesterone, estrogen hinders the development and maturity of ovarian follicles. Progesterone also suppresses ovulation by blocking LH, making cervical mucus more viscous and preventing sperm from reaching and fertilizing the egg [4]. Simultaneously, OCs have the ability to raise the concentration of sex-hormone-binding globulin (SHBG), which lowers free testosterone levels, lessens the function of androgens, and permits androgen deprivation. In addition to protecting the endometrium and enhancing hyperandrogenism and contraception, OC treatment can help prevent cancer by reestablishing the menstrual cycle [41]. The three most widely used clinical OCs at the moment are drospirenone/ethinylestradiol (Yasmin), cyproterone acetate/ethinylestradiol (Diane-35), and desogestrel/ethinylestradiol (Marvelon). According to some research, ethinylestradiol has greater impact on liver metabolism than natural estradiol. This includes impact the production of SHBG, angiotensin, and certain blood coagulation factors that are estrogen-dependent [34].

Inducement of Ovulation

Non-ovulatory or ovulatory disorder is one of the diagnostic criteria for PCOS patients. Ovulation induction is a successful treatment for PCOS patients who require fertility-promoting medications, such as gonadotropin, selective estrogen receptor modulator clomiphene citrate (CC), and aromatase inhibitor letrozole (LE). Usually used for five days beginning on day two to five of the cycle, the first dosage of CC is 50 mg/day. Usually, ovulation happens five to ten days following the course of medication. In the event that ovulation fails, the dosage may be progressively raised to 250 mg daily. In the event that ovulation is absent, the patient is classified as CC resistant Obesity, insulin resistance, higher serum testosterone levels, and advanced age are risk factors for CC resistance [14]. Third-generation aromatase inhibitors like letrozole prevent androgens from being converted to estrogens in the brain, peripheral tissues, and ovarian follicles. This leads to a positive feedback loop with the estrogen of the HPO axis, which triggers the release of GnRH naturally, encourage the secretion of FSH, and stimulates the growth of follicles. Starting on days three through seven of the

menstrual cycle, LE is given in dosages ranging from 2.5 to 5 mg/day for five days [14]. According to recent studies, letrozole aromatase inhibitors (LE) will likely take the position of clomiphene, a selective estrogen-receptor regulator, as the first-line therapy for ovulation. Additionally, LE has a distinct benefit of encouraging ovulation in obese PCOS patients [16].

Treatment with Elevated Testosterone

Adrenal zona reticularis cells, which generate testosterone, and the biological production of theca cells are linked to elevated androgen levels in PCOS patients [14]. Insulin resistance or hyperinsulinemia in women with PCOS is associated with increased adrenal gland and ovarian output [7, 38]. Side effects are a common occurrence with effective androgen treatment, particularly in individuals with PCOS who are untreated and at risk for cardiovascular disease [19]. High testosterone levels are closely linked to dysglycemia whether a person is fat or not [24], low blood pressure [20] as well as secondary arteriosclerosis [14]. Although OCs are currently the first line of treatment for excessive androgens, fibrinolysin, the recently discovered dienogest, which is another testosterone derivative with anti-androgenic activity and no affinity for GC receptors, will functionless when combined with norethindrone and GC receptors in the OC. This derivative is presently being investigation in clinical studies, however it is anticipated to be a novel type of OCs in the future [14]. Among the medications now being used in clinical settings are triptorelin and triptorelin acetate. When luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are released by the pituitary gland, GnRH first initiates a protracted period of stimulation that ends with the refractory phase. Reduced gonadotropin release can also act as a pretreatment for ovulation by lowering the synthesis of LH and androgens to the point of castration. Moreover, GnRHcan improve endometrial tolerance, thicken the uterine lining, and create a hormonally favorable environment that promotes pregnancy [45].

Insulin-Sensitive Substance

Clinically, insulin resistance varies in 60-80% of PCOS patients [14], Metformin, an insulin sensitizer, reduces the production of glycogen, which enhances sugar metabolism and inhibits follicular membrane cells. Studies have indicated that metformin can restore ovulation in approximately 30 to 50% of PCOS women when progesterone cycle therapy is used for the first six months to establish menstrual cycle rules. However, metformin was less effective than clomiphene in terms of pregnancy rate and productivity [14].

Conclusions

In conclusion, polycystic ovary syndrome (PCOS) is a complex endocrine disorder with multifaceted etiologies and pathophysiologies. Emerging evidence underscores the significant role of gut microbiota in the development and progression of PCOS. The gut microbiome influences various mechanisms underlying PCOS, including hyper and organism in the adrenal and ovary glands, disruptions in follicle development, insulin resistance, and neuroendocrine imbalances. Understanding these associations provides new insights into the specific pathways contributing to PCOS pathogenesis.

Effective management of PCOS require multi-pronged approach. Lifestyle Adjustments, such as dietary modifications and regular physical activity, are fundamental in alleviating symptoms and improving metabolic outcomes. Pharmacological interventions, including oral contraceptives and ovulation induction agents, are pivotal in regulating menstrual cycles and enhancing fertility. Treatments targeting elevated testosterone levels and insulin sensitivity further address the hormonal and metabolic dysregulations characteristic of PCOS.

Ongoing research into the gut microbiome's impact on PCOS holds promise for developing innovative therapeutic strategies. By integrating microbiota-focused interventions with existing treatment modalities, it may be possible to achieve more comprehensive and personalized care for individuals with PCOS, ultimately improving their overall health and quality of life.

Future Cope

The future scope of research on polycystic ovary syndrome (PCOS) and the gut microbiota is promising and multifaceted. Further studies should focus on elucidating the precise mechanisms by which gut microbiota influence PCOS pathogenesis, including hyperandrogenism, follicle development, insulin resistance, and neuroendocrine disturbances. Advanced genomic and metabolomic technologies can help uncover specific microbial profiles associated with PCOS, paving the way for microbiota-targeted therapies.

Investigating the impact of probiotics, prebiotics, and dietary interventions on gut microbiota and PCOS symptoms holds significant potential. Longitudinal studies are essential to understand the causal relationships and long-term effects of these interventions. Additionally, personalized treatment approaches integrating microbiome modulation with

conventional therapies could enhance efficacy and patient outcomes. Collaborative efforts between endocrinologists, microbiologists, and nutritionists will be crucial in translating these findings into clinical practice, ultimately improving the management and quality of life for individuals with PCOS.

Conflict of Interest

There is no conflict of interest related to the study.

Author Contributions

Acquisition and interpretation of data is done by Sohani Das. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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Chapter - 8
Bio-fabricated Silver Nanoparticles against Fungal Pathogens: A Review

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Chapter - 8

Bio-fabricated Silver Nanoparticles against Fungal Pathogens: A Review

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Abstract

'Zero hunger' has recently been recognized as one of the most important Sustainable Development Goals (SDG) and therefore, food security and protection of crops from pathogens have become critical worldwide issues today. 'Nanotechnology' helps in achieving this goal by innovating novel nanoparticles in the form of nano-pesticides, nano-fertilizers, and nano-agrochemicals which enhance sustainable agriculture productivity. The present study searches for environmentally sustainable ways to fulfill the agricultural demand for high yield in prevailing infection of virulent fungal pathogens. The nanoparticles show more toxicity than larger-sized molecules because of their size (1-100nm), shape, larger surface ratio, and morphology. Although numerous nano-metals have been synthesized with these novel properties, silver nanoparticles are most suitable for inhibition of the growth of many fungi like *Aspergillus fumigates*, *Trichophyton rubrum*, etc. Moreover, AgNPs are recognized as effective agrochemicals because of their compact size, easy transport, high efficiency, easy handling, and high microbial potentiality which make them preferable to farmers in the agriculture sector over other conventional products. Various fungi are now destructive, infectious, and poisonous creating highly problematic factors increasing agricultural productivity. Bio-inspired AgNPs can be successfully introduced to defend these toxigenic fungi and have a significant impact on sustainable agriculture by yielding crops. Green nanotechnology is a revolutionary method in the field of agriculture that is economical; environmentally sustainable and an alternative effective tool for controlling agricultural fungal pathogens in contrast to other conventional methods. According to the current hypothesis, smaller size and larger surface ratio enable green-synthesized AgNPs to penetrate through the cell wall and change the permeability of the cell membrane. Then AgNPs generate

Reactive Oxygen Species (ROS) which results in apoptosis and ultimately causes cell death of pathogens. This review summarizes the application of bio- inspired silver nanoparticles in sustainable agricultural fields against fungal pathogens.

Keywords: Sustainable agriculture, green-synthesis, silver nanoparticles, fungal pathogens

Introduction

In today's world, various environmental stresses like drought, uneven rainfall, saltation of areas, excessive temperature, etc. introduce a large number of virulent fungal pathogens. These pathogens invade cash crops and reduce crop yields which ultimately results in economic loss. Global climate change also adversely affect the security of food (Hasan *et al*. 2021b). According to Mokgomo *et al*., (2022), agriculture production is the main source of income of 86% of rural communities. Therefore, to overcome these worldwide prevalent fungal disease issues, an efficient, eco-friendly tool is essentially needed to be developed to avoid conventional agricultural practices.

Current trends are developing sustainable, balanced, safe, and environmentally beneficial methods through recycling strategies. In this regard, 'Nanotechnology' an innovative and revolutionized technology, offers effective monitoring of a great problem of food security by producing a powerful formulation of metal nanoparticles at the nanoscale (1-100nm). Nano-pesticides, nano-fungicides and nano-herbicides based on Nanotechnology are successfully introduced in the agriculture sector (Javed *et al*., 2022). Sarma *et al*., (2023) reported that implementation the NPs in agricultural fields has been worldwide and commercialized over the last decades.

There are various types of nanoparticles-organic nanoparticles (quantum dots, carbon nanotubes), inorganic nanoparticles (gold, silver), magnetic NPs (cobalt), semi-conductor NPs (Zinc oxide) etc. Nejatzadeh *et al*., (2021) studied that among various nanoparticles, having special properties, inorganic nanoparticles are most appropriate for sustainable crop yields. Silver nanoparticles have garnered immense interest worldwide due to their remarkable features like the least cost upon synthesis, narrow-sized distribution, surface functionalization, distinct chemical composition, biocompatibility, and vast potentiality in antifungal activity. Recently, more emphasis has been imposed on the plant-mediated generation of AgNPs because it offers sustainable and inexpensive natural and environmentally

stable alternatives over other physical and chemical processes. According to Venkatesan *et al*., (2023), AgNPs are more beneficial in application in the agricultural field than other noble metals as they have some unique characteristics like cost-efficiency, catalytic capacity, nonlinear optical behavior, and anti-fungal activity against various strains. Physical, chemical, and biological technologies are involved in silver nanoparticle synthesis. Franceschinis *et al*. (2023) demonstrated that an economical, renewable, and ecologically benign approach is green nanotechnology which is most appropriate for the production of silver nanoparticles. There are two categories of green technology-microbial-based (algae, fungi, bacteria, viruses, etc.) and plant-mediated. For the orchestration of plant-mediated nanoparticles, the whole body of plants or parts of plants (roots, leaves, peels, or fruits) can be used (Jadoun *et al*. 2021). This review illustrates the simple and environmentally safe method for silver nanoparticles and their anti-fungal activity. It has been reported that recently, plant-based biological synthesis of AgNP has become more popular than other biological resources because of its source availability.

The present work attempts to explain the antifungal capacity of bio-inspired silver nanoparticles. In identifying, the inhibition region of the microbe, anti-microbial activity of plant-mediated AgNPs can be determined. There are few reports on the application of AgNPs against various fungi and their mode of action. Therefore, in this review, we also have tried to provide updates on the application of silver nanoparticles in the sustainable practice of agriculture systemically and scientifically.

Antifungal Potentiality of Silver Nanoparticles in the Agricultural Sector

Nowadays, control of fungi in agricultural fields is a critical challenge to farmers. Although a large number of chemical fungicides come into use some ecological disappearance of pollinators, extinction of species, and toxicity to flora and fauna are associated with it. Besides this, fungal resistance against chemicals continues to grow which makes it more challenging to yield large amounts of crops. In this regard, nanoparticles are the superior alternative to current chemical pesticides in controlling fungal infections (Abdelrhim *et al*. 2021). According to Barabadi *et al*. (2023), Silver nanoparticles play a vital role in fungus control. It is reported from the Greenhouse Experiment that silver nanoparticles can increase the length of shoot and root and dry shoot weight by 11.50%, 30.67%, and 60.83% respectively of *Triticum aestivum* infected by *Bipolaris sorokiniana.* According to the literature, lignification, a most significant factor of disease

resistance is increased in using silver nanoparticles. Javeria *et al*. (2024) demonstrated that nanoparticles at high concentrations create more inhibition of fungal growth.

The size of the nanoparticles is one of the most important factors that facilitate to gain of antagonistic effect and potentiality of surface functionalization. It is reported that smaller silver nanoparticles acquire more potentiality of antifungal activity than larger ones because of a greater surface area-to-volume ratio. This property facilitates silver nanoparticles to decorate spores of fungi. Comparative anti-fungal activity of biosynthesized silver nanoparticles using plant extract as reported in the literature are shown in Table 1.

Table 1: Comparative studies of anti-fungal activation through several plant-extract-based silver nanoparticles as reported in the literature

Name of Nanoparticle	Plant Sources	Targeted Fungus	Modeof Action	References
Silver Nanoparticle	*Ixora brachypoda* Leaf extract	*Fusarium oxysporum*	Anti-fungal	Bhat M *et al*., (2021)
	Polyalthia longifolia	Alternaria alternata	Anti-fungal	Dashora *et al*., (2022)
	Aloevera	*Candida albicans*	Anti-fungal	Arsene *et al*., (2023)
	Zygophyllum album leaf extract	*Fusarium wilt*	Anti-fungal	Mossa *et al*., (2023)
	Rhazya stricta decne	*Alternaria alternata*	Anti-fungal	Sarah A *et al*., (2024)

Mode of Action

The fungal cell wall and cell membrane contain chitin, phospholipids, lipids, polysaccharides, and some proteins. Hawar SN et al, (2022) reported that silver nanoparticles having a large surface-to-volume ratio come in contact with the cell membrane by damaging the cell wall. Therefore, cell penetration into fungal pathogen is the initial step which includes three mechanisms (Figure 1):

Entrance of AgNPs through Membrane Damage: Silver nanoparticles alter fungal cell wall structure through shrinkage, and clustering and create pits and pores. This interaction between AgNPs and fungal cell walls is caused by the adsorption process which modifies the morphology of fungal cell walls and ultimately causes distortions. Then AgNPs enter the cell through the endocytosis process which aggregates them into invaginations of the cell membrane known as endocytic vesicles. Finally sorting and trafficking are caused by these vesicles.

AgNPs Enter into Fungal Cells by Transportation: Silver nanoparticles attach fungi to their surface by opsonization (phagocytosis) process in which opsonin (immunoglobulins such as antibodies, complemented proteins, etc.) is released. After that, fungi cell phagocytes NPs through ligands and receptors interaction process and form phagosome. A series of events take place-assembly of NPs to initiate action, next create cell surface extension and finally engulfing the nanoparticles.

Receptor-Mediated Absorption: Nanoparticles attach with proteins by the adsorption process. According to the literature, through diffusion, reactive oxygen species (ROS) are produced in pathogen cells which helps in the anti-fungal activity of nanoparticles. Many phagocyte receptors such as FC receptors, help NPs to interact with exposed functional groups of fungal surfaces which ultimately leads to the destruction and inactivation of fungal cells.

After penetration into the fungal cell, silver nanoparticles influence the synthesis of enzyme- glucan like N-acetylglucosamine in the cell wall. A series of anomalies then take place-cell wall thickening, hyper-vacuolization, liquefaction of cell membrane, detachment of cytoplasm from cell wall. At the molecular level, nanoparticles form various complexes by interacting biomolecules which contribute to the deformation of biomolecule structure, inactivation of catalytic protein, and impairment of nucleic acid, ultimately resulting in chromosomal aberration and DNA breakage. Moreover, AgNPs also generate ORS which results in cell death by increasing lipid per Oxidation. It is reported that AgNPs lead to the deformations of hyphae of exposed fungi to nanoparticles which finally leads to shrunken and distorted. As a result, mycelial growth shows a decreasing rate. In addition to this, maximum impact is seen on spores and their germination.

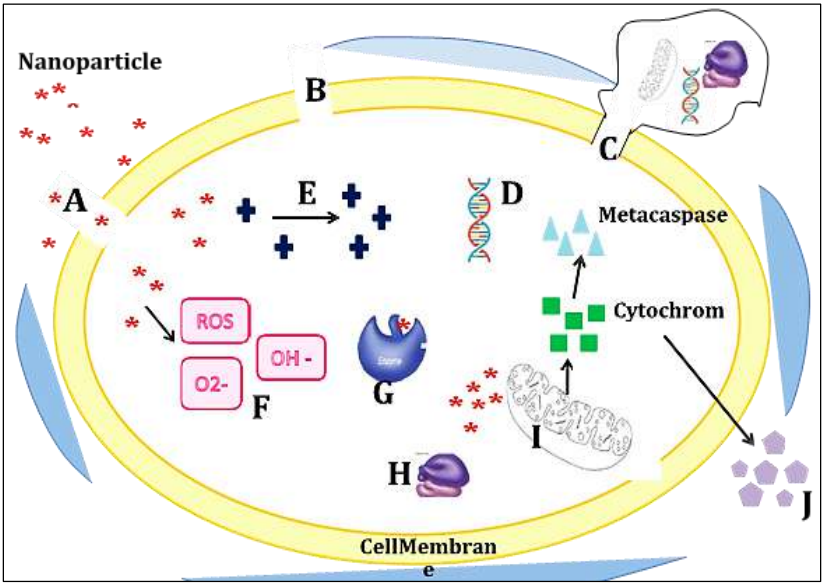

Fig 1: Mechanism of fungal cell damage by AgNPs, Where, (A) indicates Damage to cell wall and cell membrane; (B) Denotes Gap generation; (C) identifies the DNA and cell organelles releasing; (D) Fragmentation of DNA; (E) Releasing of Ion; (F) production of ROS; (G) Block of enzyme active sites (H) Depolymerization of ribosome (I) cytochrome c into cytosol leads to apoptosis and (J) biofilm formation

Future Challenges

It is challenging to acquire sufficient knowledge in the comparison of factors that are involved in the biosynthesis of silver nanoparticles. It is very crucial to determine which factor is more significant in the customization of AgNPs through formulation. Nanoparticles exhibit the most promising antifungal activity. However, there is a lack of sufficient knowledge of this mechanism. Further studies are needed to determine the toxic levels of AgNPs when it is used as fungicides in the agricultural sector. In addition to this, the impact of AgNPs on the environment as well as on aquatic life should be considered because it is associated with health risks.

Conclusions

The current review illustrates the potentiality of silver nanoparticles as fungicides and searches the possibilities of sustainable and eco-friendly applying the nanoparticles in the agricultural sector so that the problem of destroying crops by growing fungus can be easily solved. Bio-synthesized silver nanoparticles can be regarded as an avenue of worthy research in anti-fungal treatment due to their numerous functions in fungal cells-destroying

cell walls and mitochondria, ROS generation, changing gene regulation (inhibition of ergosterol synthesis) and affecting the hyphae and reproductive structure. This study enriches the already available knowledge and confirms the need for further research to overcome some limitations such as efficacy, selectivity of index, etc. Collaboration between researchers and data updating in integrating bio-inspired nanotechnology in our day-to-day lives can foster responsibility in environmental consciousness, sustainability, and technological development.

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Chapter - 9
Single Cell Protein Production from Food Wastes: A Short Review

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Chapter - 9

Single Cell Protein Production from Food Wastes: A Short Review

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Abstract

PopulationburstresultsintotheoverburdenofincreasedfoodproductionProteinbeinganessential raw material for development, growth and other physiological processes is indispensable in the diet. However, in underdeveloped communities around the globe the lack of adequate protein leads to protein deficiency and can cause serious health issues like mental retardation, kwashiorkor, malnutrition and even death. Protein sources like meat and dairy products are counted expensive to be available to all, and thus there are attempts in procuring protein from waste and cheap materials that would meet the same criteria of calorific value and metabolic efficiency. In this regard single cell proteins (SCPs) extracted from various living organisms can be used as substitute of protein rich foods, using various substrate. Also, there is increasing choice of producing SCP using food derivatives or other waste as a cheaper alternative that reduces the pollution load of environment. This review article emphasizes on the extraction of SCP from different waste sources which in turn challenges the wrath of protein deficiency in human pollutions.

Keywords: Single-cell proteins, waste material, food waste, protein deficiency

Introduction

It is estimated that by 2070, world's population is expected to reach over 13 billion people. To keep up with the way we are eating now, we would need to produce about 2250 million tons of meat and dairy products every year. (Abdullahi, N., Dandago, M.A., & Yunusa, A.K. (2021). But the way we are producing food right now won't be enough. We have to find new ways to make sure everyone has enough to eat. One promising alternative is SCP, they are microbial protein used as an alternative for protein rich foods. It is rich in amino acids like methionine, lysine etc. (Thiviya, P., Gamage,

A., Kapilan, R., Merah, O., & Madhujith, T. (2022). SCP can be used as food for animal or people ether on its own as supplements. It's becoming more popular because it's easier and faster process to produce it rather than traditional farming or raising animals. India produces a huge amount of waste every year such as fruit and vegetable waste. (Sekoai, P. T., Roets-Dlamini, Y., O'Brien, F., Ramchuran, S., & Chunilall, V. (2024). These waste materials have potential to ne uses as a substrate for production of SCP using various efficient unicellular microorganisms, they contains carbohydrate, minerals, and vitamins need for the production of substitute protein from microorganisms. (Ahmed, M.G., Gouda, S.A., Donia, S., & Hassanein, N. M. (2024).

Some microorganisms that are able to produce SCP are

Bacteria (*Cellulomonas, Alcaligenes*) Abdullahi, N., Dandago, M.A., & Yunusa, A. K. (2021).

Fungi (*Trichoderma, Aspergillus Niger*).

Algae (*Spirulina,* Chlorella) Thiviya, P., Gamage, A., Kapilan, R., Merah, O., & Madhujith, T. (2022).

Yeast (*Candida albicans, Saccharomyces cerevisiae*).

Yeast is suitable candidate for SCP production because of its high nutritional value and ability to grow on low-cost materials. They are easy to harvest and has high malic acid and lysine content. Wainaina S, Kisworini AD, Fanani M, Wikandari R, Millati R, Niklasson C, Taherzadeh MJ. 2020.

Microorganisms Utilized for SCP Production

Fungi

Fungi are key single cell protein in SCP production due to their numerous advantages-easy growth and harvest due to their large size. (5-10 micro metre) and low water activity facilitating simple recovery. (Sekoai, P.T., Roets-Dlamini, Y., O'Brien, F., Ramchuran, S., & Chunilall, V. (2024). Additionally Fungi are desirable for SCP due to their rich nutritional composition, high protein, and essential amino acid content as well as ample vitamins and lipids. Their ability to thrive at low pH level makes production cost effective and rapid. (Abdullahi, N., Dandago, M.A., & Yunusa, A.K. (2021). Progress has been made in selecting optimal fungal strains, substrate and parameters for enhanced yield. Criteria for selecting producer organism for SCP include biomass yield protein and nucleic acid content, substrate diversity utilization and host digestibility. For example, *Candida utilis* has been found as an essential compound for SCP production incorporating *C.*

utilis in dairy products. (Abdullahi, N., Dandago, M.A., & Yunusa, A. K. (2021).

Example of Fungi	Properties	Reference
Candida utilis	Highly sine and ergosterol content, short harvesting time	Abdullahi, N., Dandago, M.A., & Yunusa, A. K. (2021).
Candida intermedia	Good protein content, Efficient grow on lignocellulose material	Sekoai, P.T., Roets-Dlamini, Y., O'Brien, F., Ramchuran, S., & Chunilall, V. (2024)
Geotrichum candidum	Increased protein content, lower recovery cost	Yadav JSS, Yan S, Pilli S, Kumar L, Tyagi RD, Surampalli RY. 2015
Yarrowia lipolytica	Can utilize hydrophobic compound, fast generation time	Abdullahi, N., Dandago, M. A., & Yunusa, A. K. (2021).

Algae

Algae is a great Source of SCP mentioned by many experts. It's rich in protein comparable to sources like soy eggs and meat. Growing algae for SCP is cheap and eco-friendly needing less land and fewer resources (Yadav JSS, Yan S, Pilli S, Kumar L, Tyagi RD, Surampalli RY. 2015).

Algal protein is easy to digest once its cell wall is broken down through methods like drying and boiling. Algae also contain high quality fatty acid mineral and Omega 3 vitamins. Chlorella and spirulina are mostly used in SCP production (Abdullahi, N., Dandago, M.A., & Yunusa, A. K. (2021). They are also used as supplements and thickeners as well as animal feed. Their use as SCP is increased because they are rich in protein omega 3 fatty acids etc. *Chlorella stigmatophora* has high protein content, lower production and recovery cost, lesser harvesting time make it suitable for production. (Valverde-Pérez B, Pape ML, Kjeldgaard AF, Zachariae AA, Schneider C, Hélix-Nielsen C, Zarebska A, Smets BF. 2020) Also, other genera such as *Chlorella pyrenoidosa, Arthrospira, Chlorella sorokinia* also used as SCP sources for human feed (Abdullahi, N., Dandago, M. A., & Yunusa, A. K. (2021).

Bacteria

Algal genera	Reference
Chlorella sp.	Abdullahi, N., Dandago, M.A., & Yunusa, A.K. (2021)
Chlorella sorokiniana	Yadav JSS, Yan S, Pilli S, Kumar L, Tyagi RD, Surampalli RY. 2015
Spirulina sp.	Thiviya, P., Gamage, A., Kapilan, R., Merah, O., & Madhujith, T. (2022)
Chlorella pyrenoidosa	Yadav JSS, Yan S, Pilli S, Kumar L, Tyagi RD, Surampalli

	RY. 2015
Arthrospira sp.	Abdullahi, N., Dandago, M.A., & Yunusa, A.K. (2021).
Chlorella stomatophora	Sekoai, P.T., Roets-Dlamini, Y., O'Brien, F., Ramchuran, S., & Chunilall, V. (2024)
Nostoc sp.	Thiviya, P., Gamage, A., Kapilan, R., Merah, O., & Madhujith, T. (2022)
Punaleialla sp.	Abdullahi, N., Dandago, M.A., & Yunusa, A. K. (2021).

SCP from bacteria is favoured because it is packed with 50-80% protein, making it easy to grow using cheap materials for food and energy. Bacteria grow faster than algae and fungi making them a top choice for single cell protein. However, using Bacteria as SCP has some challenges (Thiviya, P., Gamage, A., Kapilan, R., Merah, O., & Madhujith, T. (2022).

- Bacterial cells have lots of nucleic acid, which can be harmful to both humans and animals. Removing this adds extra costs making production harder. (Sekoai, P.T., Roets-Dlamini, Y., O'Brien, F., Ramchuran, S., & Chunilall, V. (2024).
- Bacterial cells are small and light making them tricky and expensive to collect from the fermentation mixture.
- People are hesitant about eating bacteria-based SCP.
- Sone types of bacteria produce toxins that can cause food poisoning.

Some bacteria used for making SCP includes *Aeromonas* sp., *Methylomonas* sp., *Mycobacterium* sp., *Nocardia, Brevibacterium* sp., *Methylophilus* sp. (Yadav JSS, Yan S, Pilli S, Kumar L, Tyagi RD, Surampalli RY. 2015.

SCP product pruteen is made from *Methylophilus* by using methanol as substrate to prepare animal feed. (Ahmed, M. G., Gouda, S.A., Donia, S., & Hassanein, N. M. (2024).

Knio Biomed is on SCP product *Methylobacterium extroqueceme* by using methane as substrate to produce fish feed. (Yousef MK. 2012)

Yeast

Yeast has another source of SCP and has been used for along time. Yeast biomass mainly used in the pet food industry, providing supplements for dogs, cats, and fish improving their edibility. Torula yeast being commonly used for this purpose. Compared to other sources yeast has several advantages. (Wainaina S, Kisworini AD, Fanani M, Wikandari R, Millati R, Niklasson C, Taherzadeh MJ. 2020).

- It’s easier to harvest compared to large size of bacteria.
- It has higher level of malic acid and lysine.
- It can grow and thrive in acidic environment (Ahmed, M. G., Gouda, S.A., Donia, S., & Hassanein, N. M. (2024).

It has long history of traditional use.

Experiment shows that *S. cerevisiae* used efficiently to prepare SCP from food waste material such as banana peels and give satisfactory result. But adding dextrose to its substrate the productivity rate increased by 80% (Sekoai, P.T., Roets-Dlamini, Y., O’Brien, F., Ramchuran, S., & Chunilall, V. (2024).

Traditional Substrate for SCP Production

Traditional substrate used for SCP production are gas oil, methanol, n-alkenes, mostly on those substrate bacteria and yeast are grown. (Ahmed, M. G., Gouda, S. A., Donia, S., & Hassanein, N. M. (2024).

- Methanol is used to produce Single Cell Protein (SCP) because it has stable composition. It is also non-toxic and easily dissolve in water. However, in the Western world, the ICI Pruteen plant, which was the only facility of its kind, had to shut down because methanol costs were too high, (Sekoai, P.T., Roets-Dlamini, Y., O’Brien, F., Ramchuran, S., & Chunilall, V. (2024). In the Middle East, where ethanol is cheaper and fishmeal is expensive, SCP could bearable option. For SCP intended for human consumption, ethanol is considered a better choice. (Salazar-López, N. J., Barco-Mendoza, G.A., Zuñiga-Martínez, B.S., Domínguez-Avila, J.A., Robles-Sánchez, R. M., Ochoa, M.A.V., & González-Aguilar, G.A. (2022) The future success of ethanol-based SCP will depend on factors like local ethanol production, availability of agricultural carbohydrates, and political decisions about trade and economic policies n-alkanes have been tested for SCP production, but these methods are complex and many have been discontinued due to health risks from potential carcinogens. Recently, Japan and other Eastern countries have focused on developing SCP from alcohol and organic waste. (Ahmed, M. G., Gouda, S.A., Donia, S., & Hassanein, N. M. (2024).

Alternative Substrate used for Production

Waste materials like straw, citric acid, bagasse, molasses, and food scraps should ideally be recycled into the ecosystem. These materials can

significantly pollute waterways if not managed properly. Using the min Single Cell Protein (SCP) production helps reduce pollution and provide edible protein. (Thiviya, P., Gamage, A., Kapilan, R., Merah, O., & Madhujith, T. (2022). While efforts have been made to turn animal waste into feed protein, these have often failed due to technological and hygiene issues. Increasingly strict waste management regulations have made waste costly problem, though it can sometimes be used for SCPi fit's economically feasible. (Salazar-López, N.J., Barco-Mendoza, G.A., Zuñiga-Martínez, B. S., Domínguez-Avila, J.A., Robles-Sánchez, R.M., Ochoa, M.A.V., & González-Aguilar, G.A. (2022) Large-scale SCP production using waste typically involves yeast and complex bioreactors, with substrates like cheese whey and molasses. New developments, such as mushroom fermentation for SCP, show promise but are still in the early stages. In developing countries, where high-tech systems are too expensive or where skilled labor is lacking, traditional microbiological methods might benefit from these new biotechnological advances. Ahmed, M. G., Gouda, S.A., Donia, S., & Hassanein, N. M. (2024).

Industrial Waste

Waste from wood and cotton processing, paper and fuel production, and latex industries often includes polymers like cellulose, lignin, and hemicellulose. These lignocellulose wastes are common but need extensive pre-treatment-mechanical, chemical, or enzymatic- before they can be used by microorganisms to produce Single Cell Protein (SCP). This pre-treatment increases the cost of SCP production. Sekoai, P. T., Roets-Dlamini, Y., O'Brien, F., Ramchuran, S., & Chunilall, V. (2024).

Paper Waste

In 2014, global paper and cardboard production reached 390 million tons. While developed regions like Europe and North America Recycle About 60-70% of their paper, some areas have much lower rates. Paper production is expected to grow to 490 million tons annually by 2020, leading to more paper waste. Paper waste primarily consists of cellulose. (Thiviya, P., Gamage, A., Kapilan, R., Merah, O., & Madhujith, T. (2022). When hydrolyzed, it contains 60-70% sugar, mostly glucose, with some xylose, mannose, arabinose, and galactose, along with lignin, kaolinite clay, and other residues. Contamination by unwanted microbes complicates and increases the cost of SCP production from paper waste Arthrospira sp. (Abdullahi, N., Dandago, M. A., & Yunusa, A. K. (2021). Using extremophiles' microorganisms can help avoid contamination and reduce production costs by maintaining selective growth conditions.

Food Waste

Mostly vegetable and food peels are considered as typical food waste for preparing SCP. Carbohydrate-rich waste from fruits like orange, papaya, banana peels, and other agricultural residues such as sugarcane bagasse, rice husk, and wheat straw are valuable for producing Single Cell Protein (SCP). (Wainaina S, Kisworini AD, Fanani M, Wikandari R, Millati R, Niklasson C, Taherzadeh MJ. 2020 These materials are abundant, low-cost, and rich in fermentable sugars and nutrients that support microbial growth. For example, banana peels, which makeup 18-20% of the fruit, are sometimes used as feed for livestock due to their high carbohydrate and nutrient content. (Abdullahi, N., Dandago, M. A., & Yunusa, A. K. (2021). Various microorganisms, including bacteria (Cellulomonas), algae (Spirulina), molds (Trichoderma), and yeast (Candida), are used for SCP production. Yeast is particularly effective because it provides high nutritional value, can efficiently use low-cost raw materials, and is easy to harvest due to its larger size and ability to grow in acidic conditions. (Thiviya, P., Gamage, A., Kapilan, R., Merah, O., & Madhujith, T. (2022).

General Process of SCP Production

The production is carried out of the following steps:

1. Selection of Suitable Strain.
2. Fermentation.
3. Harvesting
4. Post-harvest treatment.
5. SCP processing for food. (Ahmed, M.G., Gouda, S.A., Donia, S., & Hassanein, N. M. (2024)

These steps are scenery for single cell protein synthesis

Step 1: A suitable strain needs to be selected.

Step 2: Fermentation of the Microorganism.

Step 3: Harvesting in proper condition.

Step 4: Post-harvest treatments.

Step 5: Single-cell protein processing to make the biomass edible. (Abdullahi, N., Dandago, M. A., & Yunusa, A. K. (2021).

Media Preparation

Growth media from food wastes can be prepared by 3 possible ways.

1) By washing the food waste by distilled water after that those are dried, grinded, and sifted and finally blended with distilled water and after filtration growth media is prepared.
2) In the next way peels are washed with 2% H_2SO_4 and rinsed with distilled water. Pieces are cut to reduce residue sizes and pulverized and filtered to prepared media.
3) And the last and final what is acid hydrolysis of thermal pretreatment of filtrate, pH adjustments are done to prepare media.

(Abdullahi, N., Dandago, M.A., & Yunusa, A.K. (2021).

Sterilization of Media

Growth media are sterilized at 121 °C for 15 minutes in an autoclave before inoculation (Kapilan *et al*., 2018; Mahan *et al*., 2018). While sterilizing prepared media can enhance the growth of the selected microorganism, it is not always necessary. Gervasi *et al*. (2018) reported successful biomass production in a medium made of food waste mixtures without any thermal or chemical pre-treatments.

Selection of Suitable Strain

In Single Cell Protein (SCP) production, inoculums are often isolated from food and the environment. Oshoma *et al*. (2017) isolated *Aspergillus niger* from spoiled onion bulb. The commonly used *Saccharomyces cerevisiae* can be isolated from sources like palm wine (Mensah and Twumasi, 2017) and rotten tomatoes (Milala *et al*., 2018). This yeast, when isolated from bread, yields biomass rich in essential amino acids (Ahuja and

Kumari, 2019). It has also been grown using bees' fruit peel (Haddish, 2015) and sugarcane bagasse (Samadi *et al*., 2016). *Trichoderma viride* can be sourced from soil (Anichebe *et al*., 2019), along with *Candida lusitaniae* (Shete and Raut, 2018) and *Raoultella ornithinolytica* (Al-Hadithi *et al*., 2018). *Cyberlindnera* sp., a type of Torula yeast, performed well in a banana peel medium with $(NH_4)^2$ SO_4 as nitrogen (Jiru and Melku, 2018). Researchers have also used pure culture for SCP production, such as *Aspergillus oryzae*, *Trichoderma koningii*, and *Candida tropicalis* from orange waste (Zhou *et al*., 2019), and bakers' yeast from bananas (Kapilan *et al*., 2018). These inoculums are typically grown on general-purpose media like nutrient agar or potato dextrose agar before being introduced to the production medium (Samadi *et al*., 2016; Ahuja and Kumari, 2019). The quality of SCP is influenced by the interaction between different fermentation organisms (Zhou *et al*., 2019), and factors such as inoculum size, age, incubation period, temperature, and moisture to substrate ratio (Yunus *et al*., 2015).

Incubation and Fermentation

After Autoclaving and, if necessary, enrichment, the growth medium is inoculated with microbial colony to generate biomass. Ideal fermentation temperature range from 28 to 37 °C, with incubation periods starting at 48 hours and varying by organism (Rajendran *et al*., 2018; Milala *et al*., 2018; Ahuja and Kumari, 2019). Inoculum levels affect microorganism growth (Koutsoumanis and Sofos, 2005; Xu *et al*., 2020) by influencing detection time (Bidlas *et al*., 2008), lag phase (Robinson *et al*., 2001), and enzyme production (Dinarvand *et al*., 2017; Ilgın *et al*., 2020). Lower inoculum levels require higher pH and water activity for optimal growth (Skandamis *et al*., 2007). The size and age of the inoculum impact fermentation and SCP protein content (Reihani and Khosravi-Darani, 2019). Excessive inoculation does not increase biomass protein content. Hongpattarakere and H-Kittikun (1995) found no impact on protein content of *Schwanniomyces castellii* B5285 in cassava starch with inoculum sizes above 5%. Patthawaro and Sae Jung (2019) reported no significant difference in *Rhodopseudomonas faecalis* biomass at 20% and 30% inoculum levels. Yunus *et al*. (2015) observed maximum protein yield *Candida utilis* and *Rhizopus oligosporus* biomass grown in wheat bran at 10% (v/w) inoculum size using 48-hour-old culture. Munawar *et al*. (2010) found 4% (v/v) inoculum size optimal for protein production *Candida utilis* grown fruit waste extract during submerged fermentation. Mahat and MacRae (1992) achieved maximum *Rhizopus oligosporus* mycelium production with higher protein content at a

7.5% (v/v) inoculum size. Xu *et al*. (2020) reported an optimum inoculum level of 20% in acid-tolerant *Methylocapsa acid* iphila. Inoculum size also significantly affects enzyme synthesis during solid-state fermentation (Kosseva, 2013). Alam *et al*. (2008) found 2% (v/w) inoculum size ideal for cellulase enzyme production by *Trichoderma harzianum* grown in wastewater.

Harvesting

After Incubation, the fermented broth is centrifuged at 4000-6000rpm for 20 minutes. The collected sediments are dried and then analyze for crude protein content (Samadi *et al*., 2016; Kapilan *et al*., 2018). Despite Advancements in SCP production, harvesting and purification remains challenging (Valverde-Pérez *et al*., 2020). Valverde-Pérez *et al*. (2020) explored dewatering methanotrophic biomass using forward osmosis. The protein content of harvested biomass is measured using the micro Kjeldahl method (Milala *et al*., 2018; Jiru and Melku, 2018; Rajendran *et al*., 2018) or Lowry's method (Ahuja and Kumari, 2019).

Application

1) Provides Instant Energy.
2) Promotes Healthy Eyes and Skin.
3) Excellent Protein supplement for undernourished children.
4) Rich in Vitamins, amino acids, minerals, and crude fibers.

Therapeutic and Natural Medicine Uses

1) Controls obesity.
2) Lowers blood sugar in diabetics.
3) Reduces bodyweight, cholesterol, and stress.
4) Prevents cholesterol build up.

Cosmetic Uses:

1) Maintains healthy hair.
2) Used in herbal beauty products like bio lipstick and face creams.

Poultry Uses:

1) Convenient source of protein and nutrients for cattle, birds, and fish.

Other Uses:

- Microorganisms grow rapidly, doubling biomass in 30 minutes under optimal conditions.

- Produces high-quality protein superior to that of animals and plants.
- Easy genetic manipulation allows for diverse amino acid composition.
- Uses waste products substrates, reducing pollution.
- Simple fermentation and culture conditions.
- Unaffected by environmental factors.

Commercial products of SCP

1) Microalgal Flour-used for excess protein and carbohydrates content.
2) Capsule-*Chlorella, spirulina, Haematococcus pluvialis,* are dietary supplements to increase nutrients content.
3) Dairy drinks-*Euglena* used in fermentation of yogurt as it's excellent stability in low pH beverage.
4) Juice powder-Powder enriched with *Arthrospira aquae* improves nutritional content.

Conclusion

SCP can provide high-quality protein at an affordable cost as an alternative to animal protein. Many microorganisms thrive on low-value substrates and waste, with Saccharomyces cerevisiae being the most commonly used for SCP production. This organism's biomass can contain over 60% protein, along with important nutrients like carbohydrates, fat, and minerals. However, high nucleic acid content and slow digestibility can limit SCP's use. Additionally, some individuals may develop allergic reactions. A less-discussed challenge is the spore formation by persistent microorganisms during biomass drying.

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Chapter - 10
A Review on Antibacterial Activity of Bioactive Compounds

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Chapter - 10

A Review on Antibacterial Activity of Bioactive Compounds

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Abstract

The growing concern over antibiotic resistance has sparked a growing interest in the antibacterial properties of bioactive compounds. This review examines the origins and mechanisms of these compounds, focusing on natural products from plants, marine organisms, and microorganisms. Bioactive compounds exhibit antibacterial activity through mechanisms such as inhibiting cell wall synthesis, disrupting cell membranes, and interfering with critical metabolic processes. These compounds can effectively exert either bactericidal or bacteriostatic effects. This review also explores the synergistic effect of bioactive compounds in conjunction with traditional antibiotics, which can enhance the efficacy of existing antibiotics and potentially mitigate the challenges posed by antibiotic resistance. By leveraging the unique mechanism of action of bioactive compounds, researchers can improve treatment outcomes and develop more effective strategies to combat resistant infections. However, the review also addresses the obstacles that hinder their application in clinical practice, such a standardization issues, variability in efficacy, and the need for extensive clinical trials. The review emphasizes the importance of continued research in this field and advocates for a concerted effort to explore and harness the potential of natural products in the development of innovative antibacterial treatments. Bioactive compounds, such as flavonoids, alkaloids, terpenoids, phenolics, peptides, and essential oils, have demonstrated significant antibacterial effects. Their unique properties enable them to disrupt bacterial cell membranes, inhibit enzymatic functions, interfere with replication process, or compromise cell wall integrity. Exploring the antibacterial properties of bioactive compounds not only enhances our understanding of natural antimicrobial sources but also opens up promising avenues for the creation of new antibacterial therapies.

Keywords: Antibacterial activity, bioactive compound, antibiotic, therapeutic, resistance

Introduction

Antibiotic resistance has emerged as a significant public health issue all over the world, necessitating the immediate development of novel therapeutic approaches to the treatment of bacterial infections. The disclosure and improvement of new antibacterial specialists are basic in resolving this issue, particularly given the limits of ordinary anti-microbials [Hussein *et al*. 2017; Zanwar *et al*. 2011]. In this context, the potential antibacterial properties of bioactive compounds, particularly those derived from natural sources, have received lot of attention. These compounds, which include a wide variety of metabolites produced by bacteria, plants, and fungi, each have their own distinct chemical structure and frequently possess potent antimicrobial properties [Hadi *et al*. 2017]. Secondary metabolites known as bioactive compounds are crucial to the survival and adaptation of organisms in their natural environments. They have developed as defenses against competitors, predators, and microbial pathogens. Various cultures have relied on medicinal plants for centuries for their therapeutic properties, frequently without comprehending the underlying biochemical mechanisms [Ubaid *et al*. 2017]. Research has identified numerous classes of bioactive compounds with demonstrated antibacterial effects, including alkaloids, flavonoids, terpenoids, phenolic acids, and essential oils. Advances in pharmacology, biochemistry, and molecular biology have made it easier to isolate and characterize these bioactive compounds, allowing for a deeper comprehension of their mechanisms of action against bacteria [Hameed *et al*. 2017]. Each class display unmistakable methods of activity, focusing on different bacterial cell cycle, for example, cell wall combination, protein blend, and DNA replication. Alkaloids May Inhibit Topoisomerase, an essential enzyme in DNA replication, while flavonoids have been shown to disrupt bacterial membranes and metabolic pathways. Moreover, rejuvenating oils have acquired prominence for their sweet-smelling properties as well as for their capacity to upset microbial cell layers and apply expansive range antibacterial effects [Calixto *et al*. 2017]. Bioactive compounds, naturally occurring substances found in plants, fungi, and other organisms, are being explored as potential treatments for antibiotic-resistant bacteria. These compounds can disrupt bacterial cell functions, inhibit growth, or induce cell death, enhancing the effectiveness of conventional antimicrobials. When used in combination with conventional antimicrobials, they can reduce the use of antibiotics, reducing side effects and selective pressure that contributes to resistance. Some bioactive compounds can alter bacterial cell membrane permeability, making them easier for antibiotics to enter and exert their effects. Combination therapies incorporating bioactive

compounds could lead to novel treatment regimens targeting bacteria more effectively and reducing resistance development. This approach aligns with personalized medicine principles, allowing treatments to be tailored to individual patients' unique infections [Hussein *et al*. 2017]. Bioactive mixtures as antibacterial agents are a promising approach in healthcare, addressing antibiotic resistance and promoting sustainable development. These natural compounds, derived from plant extracts and metabolites, contain phytochemicals like flavonoids, alkaloids, and terpenoids that exhibit antimicrobial activity. This approach promotes holistic approach top health and wellness, reducing the environmental footprint associated with traditional pharmaceutical manufacturing. The production of bioactive mixtures also involves less intensive manufacturing processes, reducing the use of hazardous chemicals and energy consumption. This shift aligns with the principles of sustainable development, which emphasizes the need for solutions that meet present needs without compromising future generations' ability to meet their own. By investing in research and development of natural antibacterial agents, we can address immediate health concerns while fostering a more sustainable and resilient healthcare system [Flayyih *et al*. 2017]. In the end, the antibacterial action of bioactive mixtures presents promising road for the improvement of novel antimicrobial specialists. There is significant potential to utilize these compounds' therapeutic properties in the fight against bacterial infection research continues to reveal their complexity and mechanisms of action. A comprehensive examination of the various classes of bioactive compounds, as well as their sources, modes of action, and the implications of their use in contemporary medicine, is outlined in this introduction. The accompanying segments will dig further into explicit instances of bioactive mixtures with antibacterial action, their systems of activity, and the potential for their application in treating safe bacterial contaminations.

Holy Basil: *Ocimum tenuiflorum*, also known as holy basil or Tulsi, is a revered herb in traditional medicine, particularly in ayurvedic practices, known for its antimicrobial properties. Its antimicrobial efficacy is attributed to its essential oils and extracts, which are rich in bioactive compounds like eugenol and rosmarinic acid. These Compounds inhibit microbial cell membranes, inhibiting bacteria growth and replication. Holy basil extracts have been shown to inhibit Escherichia coli, staphylococcus aureus, and Salmonella typhi, demonstrating its potential for combating serious infections. It is also effective against fungal infections, particularly Candida species, by disrupting cell wall integrity. As interest in natural and holistic health solutions grows, holy basil's potential in promoting health and

preventing disease remains a significant topic of exploration [Fakhir *et al*. 2017; Khudhair *et al*. 2017]. Tulsi, also known as *Ocimum tenuiflorum*, has antimicrobial properties due to its ability to disrupt microbial cell membranes, inhibit enzymatic functions, and interfere with microbial DNA synthesis. This multifaceted approach to combating microbial threats highlights Tulsi's potential as a natural antimicrobial agent. It may also possess antiviral properties, making it beneficial in managing viral infections, especially during flu season or respiratory illnesses. Tulsi is revered in traditional medicine systems, particularly in Ayurveda, for its ability to prevent infections and boost the immune system. It can be consumed in various forms, including essential oils, aqueous extracts, and powdered forms. As research continues to explore Tulsi's therapeutic potential, its role in infection management and health promotion is likely to become more prominent [Kamal *et al*. 2017].

Fig 1: Basil herb

Honey: Honey shows exceptional antimicrobial movement because of its one-of-a-kind organization and properties. Honey is typically acidic, with a pH between 3.2 and 4.5, which can inhibit the growth of various pathogens. Honey contains phenolic compounds and flavonoids that possess antioxidant and antimicrobial properties, helping to combat range of bacteria, fungi, and viruses. This exquisite protein, crafted by the industrious bees, grace honey with its presence and plays a pivotal role in bestowing the substance with remarkable antimicrobial qualities, skillfully undermining the integrity of bacterial cell membranes [Mohammed *et al*. 2016]. MGO, which is present in significant amounts in Manuka honey, has demonstrated notable antimicrobial properties that can help preserve food by inhibiting spoilage

organisms. Honey is frequently employed remedies for sore throat and cough due to its soothing and antimicrobial characteristics. The antimicrobial effects of honey stem from its intricate composition and unique attributes, establishing its valuable natural product for health and food preservation [Shireen *et al*. 2017].

Fig 2: Honey

Neem: Neem (*Azadirachta indica*) is widely recognized for its antimicrobial properties, which can is widely recognized for its antimicrobial properties, seeds, bark, and oil. Here's an overview of its antimicrobial activity: This is the most studied compound in neem, known for its insecticidal and antimicrobial properties. Exhibits antibacterial and antifungal activities. Known for its potential antimicrobial effects. Provide additional antioxidant and antimicrobial benefits. Neem extracts have shown effectiveness against a variety of bacteria, including Gram-positive bacteria Such as Staphylococcus aureus and *Streptococcus pyogenes* [Huda *et al*. 2017; Altaea *et al*. 2016]. Including Escherichia coli and Salmonella typhi. Neem has demonstrated antifungal properties against various fungi. Effective against *Candida albicans*. Such as Trichophyton and Microsporum species, which cause skin infections. Due to its antimicrobial properties, neem is utilized in for treating infections and skin disorders. Such as toothpaste and mouthwash to combat dental diseases. As a natural pesticide to protect crops from pathogens [Hussein *et al*. 2016]. The antimicrobial activity of neem makes it a valuable resource in both traditional and modern medicine, providing a natural alternative to synthetic antibiotics and antifungal agents. Further research is ongoing to fully understand its mechanisms and potential applications.

Fig 3: Neem tree

Main Groups of Bioactive Compounds in Plants

Bioactive compounds in plants are classified based on their pharmacological and toxicological properties, which can offer therapeutic benefits and pose health risks. This classification is crucial for professionals in fields like clinical medicine, pharmacy, and toxicology. The complex relationship between chemical structure and clinical outcomes makes it difficult to establish a comprehensive classification system. A biological classification system based on plant families and genera can provide valuable insights. However, convergent evolution, where genetically less related species produce analogous secondary metabolites, highlights the adaptability of plants in producing bioactive compounds. Understanding these factors helps professionals make informed clinical decisions and therapeutic strategies [Hussein *et al*. 2016]. The central theme of this book revolves around bioactive chemical compounds, making it essential to categorize them based on their chemical classes and biochemical pathways. Below, a concise overview of the main chemical groups of bioactive compounds present in plants is provided.

Glycosides

Glycosides are secondary metabolites found in plants and organisms, consisting of sugar moiety and a non-sugar component called an aglycone. The glycone plays a crucial role in the solubility and bioavailability of the glycoside, while the aglycone determines its biological activity and pharmacological properties. Glycosides can be classified into several major categories based on their chemical structure and biological effects.

Cardiovascular glycosides, such as digoxin and digitoxin, affect heart function and are used in heart condition treatment. Cyanogenic glycosides, found in plants like cassava and certain fruit seeds, release cyanide when hydrolyzed, posing a potential toxicity risk. Glucosinolates, present in cruciferous vegetables like broccoli and kale, have potential anticancer properties and help in detoxification processes. Saponins, characterized by the soap-like properties, have potential health benefits, including cholesterol-lowering effects and immune system modulation. Anthraquinone glycosides, found in plants like aloe and rhubarb, have laxative effects and potential antimicrobial properties. Glycosides also represent a significant category of flavonoids, plant pigments with antioxidant properties. The absorption of glycosides in the human body typically occurs after the hydrolysis of the glycosidic bond, which releases the aglycone [Ubaid *et al*. 2016]. The impact of inhibiting Na+/K+-ATP a pump in cell membranes is significant. This Inhibition is particularly evident in cardiac tissue, where it leads to enhanced contractility and reduced heart rate. These pumps are crucial for the proper functioning of cardiac cells and are predominantly located within them. Notably, certain plants from the Scrophulariaceae family, such as *Digitalis purpurea* (commonly known as foxglove), as well as those from the Convallaria Aceae Family, exemplified by *Convallaria majalis* (lily of the valley), are rich sources of cardiac glycosides [Ibraheam *et al*. 2017]. The aglycones derived from amino acids found in cyanogenic glycosides are present in various compounds. Some of these compounds can impede the utilization of iodine, potentially leading to hypothyroidism. Additionally, they can release hydrogen cyanide, a highly toxic substance that can be lethal in significant amounts. Cyanogenic glycosides are primarily found in the Rosaceae family, particularly within the *Prunus* species. Glucosinolates, which contain sulfur and impactful amino acid-derived aglycones, exhibit a complex range of effects on cytochrome P450 is of arms across different cell types, of ten reducing the hepatic bioactivation of environmental pro-carcinogens. Furthermore, glucosinolates can cause skin irritation and may also lead to hypothyroidism and goiter. The Brassicaceae family is predominantly associated with the production of glucosinolates, while most saponins, known as “soap-forming compounds,” are typically found in glycoside form [Hadi *et al*. 2017]. Aglycones can be classified as either pentacyclic triterpenoids or tetracyclic steroids. While they exhibit unique structural features, their main functional attributes are largely comparable. Saponin glycosides are substantial molecules known for their detergent-like properties and emulsifying capabilities, which arise from their hydrophilic glycone and hydrophobic aglycone components. These compounds are

recognized for their antineoplastic and immune-modulating activities. In laboratory settings, they often induce hemolysis of red blood cells; however, this effect does not seem to pose significant issues in living organisms. Certain saponins are associated with conditions such as jaundice and photosensitization. These compounds can be found across a variety of plant families [Hadi *et al*. 2017]. One notable family is Liliaceae, which includes the notably harmful plant to sheep, *Narthesium ossifragum*, commonly known as lowland asphodel. Anthraquinone glycosides exhibit a somewhat limited distribution within the plant kingdom. These compounds can be identified in species such as *Rumex crispus*, or curly dock, and various *Rheum* species, both on which belong to the Polygonaceae family. The primary effect of these glycosides is to stimulate the release of water and electrolytes, as well as to promote peristalsis in the colon.

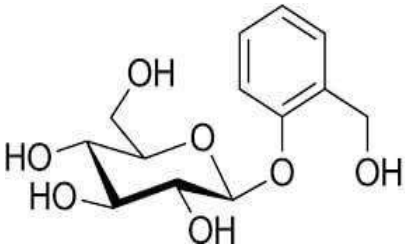

Fig 4: structure of arbutin

Flavonoids and Proanthocyanidins

Flavonoids are plant compounds with a central structure of three interconnected rings, allowing them to exhibit various biological activities and health benefits. Proanthocyanidins, which are oligomeric forms, have phenolic groups that are crucial for antioxidant properties. These compounds also have anti-inflammatory properties and potential to reduce carcinogenic risks. Isoflavones, a subgroup of flavonoids, are phytoestrogens that mimic estrogen's action, benefiting hormonal balance and health. They are found in legumes, particularly in the bean family, and contribute to the health benefits of legume diets. Flavonoids and proanthocyanidins also play a significant role in plant coloration, attracting pollinators and seed dispersers, and aiding plant reproduction and survival [Kadhim *et al*. 2016].

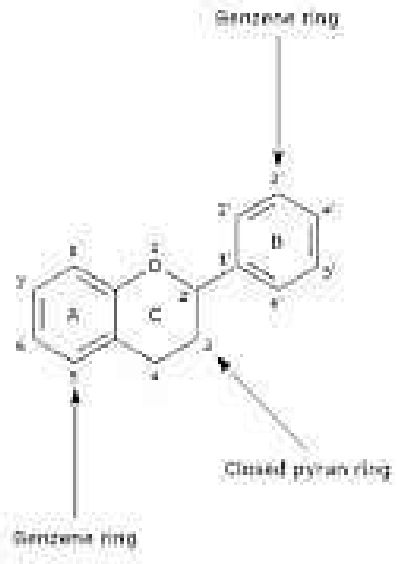

Fig 5: Structure of Flavonoid

Tannins

Tannins are polyphenolic compounds found in plants and have two main types: consolidated tannins and hydrolysable tannins. Consolidated tannins are large polymeric structures primarily composed of flavonoids, known for their antioxidant properties and pigmentation. They are used in industrial applications like tanning leather and wine production. Hydrolysable tannins are smaller polymers with several catechin derivatives attached to the sugar backbone. They are less stable and less stable, but share similar characteristics like binding to proteins and astringent properties. The size of tannin molecules affects solubility in water, with larger molecules having lower solubility. They are used in medical and pharmaceutical fields for their astringent properties. Tannins are found in various plant families, including the beech and knotweed families [Mohammad *et al*. 2016].

Fig 6: Tannin powder

Mono-and Sequi-Terpenoids and Phenylpropanoids

Terpenoids, a diverse class of organic compounds, are based on the isoprene unit, which consists of five carbon atoms. They are classified into sesquiterpenoids and monoterpenoids, with terpenoids being the most diverse group of plant constituents. Phenylpropanoids, a less diverse class, are based on a nine-carbon framework and have unique biological activities. These compounds are characterized by their lipophilic nature, which allows them to volatilize easily, resulting in strong aromas and flavors. they have been used in traditional herbal medicine for their therapeutic effects, including anti-cancer, antibacterial and antiviral properties. However, they can pose risks when concentrated, highlighting the importance of proper usage and dosage [Hameed *et al*. 2016]. These compounds are found in a variety of plant families, but Lamiaceae (the thyme family) is the family most commonly associated with them.

Diterpenoids

Diterpenoids are chemical compounds with a unique structure consisting of four isoprene units, resulting in 20 carbon atoms. They have high lipophilicity, allowing them to interact with biological membranes and influence physiological processes. Despite their flavorful properties, terpenoids are non-volatile, making them less odor-dense. There is a significant gap in research on diterpenoids compared to monoterpenes and sesquiterpenes, making it difficult to understand their safety and potential health effects. Some diterpenoids have been identified as having antineoplastic properties, potentially inhibiting tumor growth. They are found in plant species, such as coffee, and may play a role in plant defense mechanisms [Altameme *et al*. 2015].

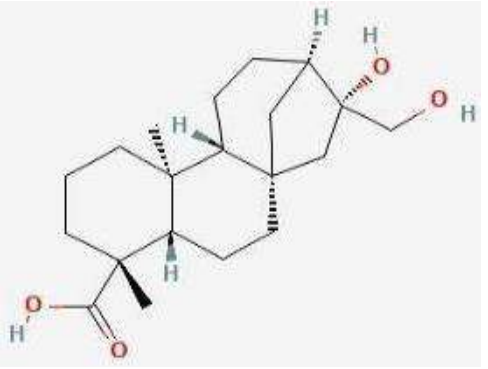

Fig 7: Diterpenoids

Future Prospects

Thereareanumberofreasonswhytheantimicrobialactivityofbioactivecompoundsinthefuture looks promising. The rise of antibiotic-resistant pathogens is driving the search for alternative antibiotics. Bioactive mixtures from normal sources, like plants, parasites, and marine creatures, are being investigated for their capability to battle safe strains [Omran *et al*. 2016]. Bioactive mixtures often exhibit a variety of active components, such as disrupting cell membranes, inhibiting enzymatic activity, and blocking nucleic acid synthesis. By integrating bioactive compounds with current antibiotics, we can create innovative antimicrobial approaches that enhance effectiveness while minimizing the risk of resistance. Recent advancements in biotechnology and computational techniques facilitate the identification and modification of bioactive compounds, paving the way for the development of targeted antimicrobial that are both less toxic and more potent. This collaborative effort is an area of active research, presenting a promising avenue for more effective treatment options [Jasim *et al*. 2015]. Compounds got from customary medication and maturation processes are being read up for their antimicrobial properties. This includes polyphenols, terpenoids, and other metabolites that have shown promise against a variety of pathogens [Hadi *et al*. 2016; Hameed *et al*. 2015]. The increasing fascination with bioactive compounds has led to the development of regulatory frameworks aimed at guaranteeing the safety and effectiveness of these substances. This progress is crucial as it opens up new possibilities for their application in clinical environments. The focus on antimicrobial agents that are sustainable and friendly to the environment is in line with initiatives aimed at improving global health. In conclusion, the future of antimicrobial bioactive compounds looks bright, with significant potential to address the pressing issue of antibiotic resistance and improve outcomes for public health. Continued investment in research will uncover new bioactive compounds and their mechanisms, leading to innovative applications in medicine, agriculture, and food preservation [Shareef *et al*. 2016; Mohammed *et al*. 2016].

Conclusion

Numerous bioactive compounds derived from plants, fungi, or marine organisms have demonstrated significant antimicrobial properties against a wide array of pathogens, including bacteria, fungi, and viruses. These compounds often show superior efficacy compared to traditional antibiotics, particularly against antibiotic-resistant strains. The antimicrobial mechanisms of these compounds are attributed to various factors, such as

disruption of cell membranes, inhibition of enzymatic activity, and interference with DNA and protein synthesis. Gaining insights in to these mechanisms is crucial for the development of new therapeutic agents. Researchhasindicatedthatcombiningbioactivecompoundswithconventionalantimicrobialscan enhance their effectiveness and reduce resistance, highlighting their potential for synergistic use in infection treatment. While many bioactive compounds exhibit strong antimicrobial activity, itis essential to thoroughly evaluate their safety profiles. Continued research into these compounds, including their potential for therapeutic formulation and understanding their structure-activity relationships, is vital. Non-toxic and biocompatible compounds are particularly promising for clinical applications. Additionally, efforts should focus on developing delivery systems and conducting clinical trials to assess their efficacy in human populations. In conclusion, bioactive compounds represent a promising avenue for the development of new antimicrobial agents, especially in the context of rising antimicrobial resistance. Their diverse mechanisms of action and potential for synergy with existing treatments merit further investigation and development.

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