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Trends in Life Science Research

Volume I

Editor:
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Dr. Pritesh Gugale
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Bhumi Publishing, India
First Edition: December 2024

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PREFACE

The field of life sciences has continually evolved, revealing the intricate mysteries of living systems and contributing to advancements that impact humanity on multiple fronts. This book, "Trends in Life Science Research" reflects the contemporary research paradigms and an exploration of the latest scientific breakthroughs shaping our understanding of life.

Life sciences encompass a vast domain, from cellular biology to ecological systems, unraveling the complex interplay between organisms and their environments. Recent innovations in biotechnology, genomics, and molecular biology have paved the way for novel approaches to addressing global challenges such as health crises, food security, and environmental sustainability. This book serves as a platform for presenting cutting-edge research and insights across diverse areas of life sciences.

The chapters included in this volume represent the collaborative efforts of researchers, academicians, and industry professionals, providing a comprehensive overview of emerging trends and technologies. Topics such as genome editing, regenerative medicine, biodiversity conservation, and the integration of artificial intelligence in biological studies highlight the multifaceted nature of modern research endeavors.

We hope this compilation will serve as a valuable resource for students, researchers, and professionals, inspiring further inquiry and innovation in the life sciences. The collective knowledge presented herein underscores the significance of interdisciplinary approaches and the transformative potential of science in addressing the challenges of our time.

We extend our heartfelt gratitude to the contributors for their scholarly input and to the editorial team for their unwavering commitment to this project. It is our earnest hope that this book will ignite curiosity and foster a deeper appreciation for the dynamic and ever-evolving field of life sciences.

- Editors

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GENETIC ADVANCES IN AQUACULTURE FOR CLIMATE CHANGE

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Abstract:

Aquaculture, a fastest growing food sector, plays an important role in providing protein nutrition to the world population. Mitigating climate change impacts and securing the availability of aquatic resources for the utilization of aquaculture farms is a great challenge in the future. Development of climate change resilient species by genetic methods, Integrated multitrophic aquaculture, recirculating aquaculture systems and utilizing more coastal sites for aquaculture is a promising field for recovering malnutrition among developing countries and help for sustainable aquaculture development. Present paper discusses the genetic advancement in the field of aquaculture and the techniques like selective breeding, Feed modification, Gene editing, Marker Assisted selection, Genomics and Epigenetic studies carried out for the improvement of race for applying molecular genetic approach in the development of resilient temperature tolerant species.

Keywords: Aquaculture Advancement, Climate Change, Resilient Species, Selective Breeding, Gene Editing

Introduction:

As reported in the 2022 edition of The State of World Fisheries and Aquaculture (SOFIA) global fisheries and aquaculture production increased from the year 2020 to 2022 by 4.4 percent. They reported that production comprised 185.4 million tonnes of aquatic animals and 37.8 million tonnes of algae. Global consumption of aquatic animal has increased twice the rate of the world population since 1961, with global per capita annual consumption rising from 9.1 kg in 1961 to 20.7 kg in 2022 (FAO, 2024). Although rise is very high only only 1% of the available resources are using for culture (Gentry *et al.,* 2017) SOFIA also reported that consumption of aquatic resources will increase by 12 percent to supply on average 21.3 kg per capita in 2032.

Climate change is a serious threat to aquaculture as it alters ecosystem by increasing temperature and natural habitat of aquatic organism especially as it displaces

coastal aquaculture. Sustainable aquaculture involves utilizing cutting edge technologies and practices that will emphasize future demands of affordable protein from water bodies.

Selective breeding, species diversification, aquaculture systems like integrated multi-trophic aquaculture, aquaponics and recirculating aquaculture system are some of the most widely accepted and adapted solutions.

Gene editing research in species used for aquaculture, namely in the area of their reproduction and development, pigmentation, growth and disease resistance. This technology can explore genetic improvement through rapid fixation of desirable alleles. Introducing sterility in aquatic organisms increases the somatic edible content by converting reproductive energy which will also reduce the rate of introgressive hybridization or escape in wild population of genetically manipulated organism. Ploidy manipulation, is successfully carried out in a range of species both in finfishes and shell fishes to utilize more edible part to increase the economic value to the farmer. (Li and Gui,2017)

Knock out genes helps to improve the genetic trait like Nanos 2 and 3 in Nile tilapia (Li *et al.,* 2014) dnd to sterility and Alb gene for albinism (Wargelius etal., 2016). To improve the production efficiency of any given aquaculture species, it is helpful to characterize genomic structure, genomic variations, and the genetic basis of economically important traits. Whole Genome sequencing (WGS) and other genetic tools have been applied in aquaculture. As reported by Robledo *et al.* (2018), GBS (Genotyping by sequencing) led to Restriction-site-associated DNA sequencing (RAD-Seq) as well as whole genome resequencing (WGR) which generated a large single-nucleotide polymorphism (SNP) data at population level which will lead to do a feasible design and use SNP arrays for SNPs genotyping. Construction of high-density genetic linkage maps for quantitative trait loci (QTL) mapping and genome-wide association study (GWAS) have been carried out using SNP arrays (Robledo *et al.,* 2018). All these studies lead for identification of many SNPs associated with economically-important performance traits, like growth rate, disease resistance, sexual determination, and tolerance of various environmental stressors.

Fish feed modification is another important step which gives effectiveness in aquatic animals. Lab grown Insect as diet for replacing fish meal in aquaculture is promising field as insects have been reported to have variable fatty acid profiles, particularly having a low level of PUFAs. PUFAs have important health benefits in humans and are required for optimal growth and development in children. (Maulu *et al.,* 2021) also insects can

accumulate eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by modifying their rearing substrates (Zarantoniello *et al.* (2020).

Jin *et al.,* 2021 reported that surrogate brood stock technology will shorten the interval of free nursery pond in hatchery. Certain alleles may be advantageous to carry during disease outbreaks of certain seasonal variations. Surrogate technology can address some of the negative consequences associated with standard gene editing such as mosaicism. It ensures that progeny will receive cells that are known to have carefully selected, targeted edits which will also help to prevent carrying a variety of different edited alleles. (Roman *et al.,* 2022). Xenogeneic transplantation has been successfully accomplished in surrogate technology

Temperature resistant species can be developed through genetic manipulation using Genetic editing (CRISPER/Cas9) (Denis *et al.,* 2024), Genetic engineering (Transgenesis) Selective breeding, Marker Assisted selection (MAS) and Genome editing (TALEN, ZFN) (Zhu *et al.,* 2024, Zituo *et al.,* 2024, Puthumana *et al.,* 2024)

Understanding the relation between environment and genetic factors is revealed by epigenetic studies in Nile tilapia (Konstantinidis *et al.,* 2021) and in Bombay duck by Liu et alk, 2022. Dorts *et al.,* 2016 reported high temperature stress causes increase of denovo DNA methyl transferase genes which do not cause cytosine methylation levels during DNA methylation.

Conclusion:

Genetic research in aquaculture is rapidly increasing. Most of the researchers highlight the improved growth rates by more than 50% using Selective breeding, genetic manipulation and genetic engineering of growth hormone genes. Enhanced disease resistance variety and climate change resilient organism mainly towards temperature and salinity by gene editing the particular genes is a promising field. Role of heat shock proteins, genetic manipulation techniques all are benefiting to improve the aquatic organisms. Studying the species-specific Omics (Genomics & Proteomics) ie mainly diving deep into molecular biology and its relation to environment will help us to explore more resilient species which will provide aquatic food security for sustainable aquaculture.

Acknowledgement:

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IMPORTANCE OF SUSTAINABILITY AND ENVIRONMENTAL SCIENCE

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Environmental science and sustainability are natural partners. Scientists gather and analyse the data to assess how our natural systems are functioning and sustainability specialists coordinate that information to generate sustainable, innovative and equitable answers to today's environmental, economic, and social justice challenges.

Environmental Science: "Environmental science is an interdisciplinary academic field that draws on ecology, geology, meteorology, biology, chemistry, engineering, and physics to study environmental problems and human impacts on the environment. It is a quantitative discipline with both applied and theoretical aspects and has been influential in informing the policies of governments around the world."

Sustainability: "Sustainability is the long-term viability of a community, set of social institutions, or societal practice. In general, sustainability is understood as a form of intergenerational ethics in which the environmental and economic actions taken by present persons do not diminish the opportunities of future persons to enjoy similar levels of wealth, utility, or welfare. The idea of sustainability rose to prominence with the modern environmental movement, which rebuked the unsustainable character of contemporary societies where patterns of resource use, growth, and consumption threatened the integrity of ecosystems and the well-being of future generations. Sustainability is presented as an alternative to short-term, myopic, and wasteful behaviours. It can serve as a standard against which existing institutions are to be judged and as an objective toward which society should move."

Brief History of Sustainability

The concept of sustainability sprouted from the movement that grew out of the notions of social justice, ecological conservation, and globalism toward the end of the 20th century. In 1983, former Norwegian prime minister Gro Harlem Brundtland was asked to run the World Commission on Environment and Development for the United Nations. Many countries were still in poverty after decades of industrialization which had come at a cost to social equity and the environment. The Brundtland Commission's report Our Common

Future defines the need for sustainable development, a holistic approach that considers the environment, economy, and equity.

Learn about the concept of sustainability and why it's important for maintaining a balance between our environment and economic growth. Sustainability is a long-term goal for our society to meet the needs of economic growth at its current speed with the least amount of impact on the environment. But it's more than preserving the natural world and its fragile ecologies. It's about enabling growth and development of businesses and government entities so that all parts of society work in harmony to ensure that future generations have the natural resources needed to survive.

Sustainability is important for preserving our planet and natural resources like water and air. Building a sustainable future and cultivating sustainable ways of living will reduce pollution and protect habitats of plants and animals. key part of sustainability involves sustainable business practices and economic development, including green technology, eco-friendly supply chains, and more. When businesses and government follow sustainable sustainable practices, it creates a ripple effect on individuals and communities to decrease greenhouse gas emissions and fossil fuels. All of this contributes

Three Pillars of Sustainability

The three pillars of sustainability (environmental, economic, and social) can help us to pave a path toward a sustainable future. Environmental sustainability: The principle of maintaining ecological integrity and ensuring that Earth's environmental systems remain balanced as natural resources such as air, water, soil, forests, and animals are being consumed by humans. Economic sustainability: The goal for humans on Earth to maintain independence and be able to get a job or acquire other resources to meet their needs. Economic systems must be in place and accessible to all. Social sustainability: The principle of ensuring that basic human needs are attainable by all and that there are enough resources available to all within a community. Strong social systems can ensure healthy, happy communities whose human rights such as labor, health care, and equality are respected.

Important of Sustainability

It is a broad discipline, giving students and graduates insights into most aspects of the human world from business to technology to environment and the social sciences. The definition of "sustainability" is the study of how natural systems function, remain diverse and produce everything it needs for the ecology to remain in balance. It also acknowledges

that human civilization takes resources to sustain our modern way of life. There are countless examples throughout human history where a civilization has damaged its own environment and seriously affected its own survival chances (some of which Jared Diamond explores in his book Collapse: How Complex Societies Choose to Fail or Survive. Sustainability takes into account how we might live in harmony with the natural world around us, protecting it from damage and destruction.

We now live in a modern, consumerist and largely urban existence throughout the developed world, and we consume a lot of natural resources every day. In our urban centres, we consume more power than those who live in rural settings and urban centres use a lot more power than average, keeping our streets and civic buildings lit, to power our appliances, our heating and other public and household power requirements. That's not to say that sustainable living should only focus on people who live in urban centres though, there are improvements to be made everywhere - it is estimated that we use about 40% more resources every year than we can put back and that needs to change. Sustainability and sustainable development focus on balancing that fine line between competing needs - our need to move forward technologically and economically, and the needs to protect the environments in which we and others live.

Sustainability is not just about the environment, it's also about our health as a society in ensuring that no people or areas of life suffer as a result of environmental legislation, and it's also about examining the longer-term effects of the actions humanity takes and asking questions about how it may be improved. Humans have, since the Neolithic Agricultural Revolution and maybe even before then, been a consumer rather than a replenisher of environmental resources. From hunter-gatherer societies that moved into an area to use up its resources in a season before setting up camp or moving on, only to return the following year to do the same, the development of a surplus economy saw permanent settlements. Slash and burn farming replaced natural wilderness often with uniform crop plantation and camps gave way to settlements, then eventually villages, towns and cities which would put pressure on the environment.

Sometimes, the environmental pressures forced people into making these changes in the first place (growing human population being one of those pressures) and often eventually they had to move on to somewhere new where the environmental could better sustain them and their practices, or make further changes to their existing environment. There was no real concept of sustainable living, even if the people of the distant past

understood that soil had a maximum fertility that could be exhausted and replenished with livestock.

It is widely acknowledged that many societies collapsed due to an inability to adapt to the conditions brought on by these unsustainable practices. Whether that was introducing alien species that upset the balance of the ecosystem, cutting down too many trees at once or even a failure to adapt to natural fluctuations in the climate, we are far more aware in the modern world about the potential damage caused by human action. Cultural change often led to survival of those societies beyond what might have been expected under the circumstances.

Though some Renaissance and Enlightenment philosophers would express concern about resources and over-population and whether these were sustainable in the long term, these people were not taken seriously at the time other than as a hypothetical question. It would take until the 20th century before we would understand the impact that we could have on our environment. Environmental damage, pollution, destabilizing soils by cutting down trees, fossil fuels and other environmental issues led to a growing concern about the environment and whether we were or could damage our own ecosystem. The United Nations was founded after World War II and in 1945, UNESCO was established to promote the importance of human culture and of science. Today, their remit is "to contribute to the building of peace, the eradication of poverty, sustainable development and intercultural dialogue through education, the sciences, culture, communication and information" The late 20th century, the science of climate change was firmly established. We knew by the 1980s about the problems of the greenhouse effect and the destruction of the ozone layer and coming very late in the century, an awareness of the notion that some of our resources - particularly fossil fuels were finite and that we should make efforts to move to renewable methods of power. It was then that we saw the social, economic and scientific birth of the environmental movement.

The Three Pillars of Sustainability

In 2005, the World Summit on Social Development identified three core areas that contribute to the philosophy and social science of sustainable development. These “pillars” in many national standards and certification schemes, form the backbone of tackling the core areas that the world now faces. The Brundtland Commission described it as “development that meets the needs of the present without compromising the ability of

future generations to meet their own needs". We must consider the future then, in making our decisions about the present.

Economic Development

This is the issue that proves the most problematic as most people disagree on political ideology what is and is not economically sound, and how it will affect businesses and by extension, jobs and employability. It is also about providing incentives for businesses and other organizations to adhere to sustainability guidelines beyond their normal legislative requirements. Also, to encourage and foster incentives for the average person to do their bit where and when they can; one person can rarely achieve much, but taken as a group, effects in some areas are cumulative. The supply and demand market is consumerist in nature and modern life requires a lot of resources every single day ; for the sake of the environment, getting what we consume under control is the paramount issue. Economic development is about giving people what they want without compromising quality of life, especially in the developing world, and reducing the financial burden and "red tape" of doing the right thing.

Social Development

There are many facets to this pillar. Most importantly is awareness of and legislation protection of the health of people from pollution and other harmful activities of business and other organizations. In North America, Europe and the rest of the developed world, there are strong checks and programs of legislation in place to ensure that people's health and wellness is strongly protected. It is also about maintaining access to basic resources without compromising the quality of life. The biggest hot topic for many people right now is sustainable housing and how we can better build the homes we live in from sustainable material. The final element is education - encouraging people to participate in environmental sustainability and teaching them about the effects of environmental protection as well as warning of the dangers if we cannot achieve our goals.

Environmental Protection

We all know what we need to do to protect the environment, whether that is recycling, reducing our power consumption by switching electronic devices off rather than using standby, by walking short journeys instead of taking the bus. Businesses are regulated to prevent pollution and to keep their own carbon emissions low. There are incentives to installing renewable power sources in our homes and businesses. Environmental protection is the third pillar and to many, the primary concern of the future

of humanity. It defines how we should study and protect ecosystems, air quality, integrity and sustainability of our resources and focusing on the elements that place stress on the environment. It also concerns how technology will drive our greener future; the EPA recognized that developing technology and biotechnology is key to this sustainability, and protecting the environment of the future from potential damage that technological advances could potentially bring.

A Sustainable Future

It is not yet clear what our sustainable future will look like but with emerging technologies and the improvement of older cleaner fuel sources, many people now look to a post fossil fuel world - including businesses. Since the 1950s, we have experienced unprecedented growth including intensive farming, a technological revolution and a massive increase in our power needs putting even greater pressure and strain on the planet's resources. We are also far more aware of the plight of the developing world and that facing our planet as we now observe both natural and human-caused disasters and the effects that these can have on the ecosystems and on human population. It's vital that we develop new, cleaner technologies to cope with our energy demands but sustainability is not just about the environment.

The biggest social activism movement related to the social development side of sustainability, has been programs such as Fair Trade and the Rainforest Alliance in encouraging good farming practices while ensuring farmers who produce luxury goods such as coffee and cocoa receive a decent living wage. Activist and sustainability professionals hope to remove trade barriers in future so that they may benefit everyone, contributing to the economic and social development core of sustainability while promoting good environmental practice.

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ANGIOTENSIN-CONVERTING ENZYME INHIBITORS AND RECEPTOR BLOCKERS USAGE IN COVID-19

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Abstract:

Current discussions surrounding the use of Angiotensin-Converting Enzyme inhibitors (ACEi) and Angiotensin Receptor Blockers (ARBs) are increasingly relevant, especially given their critical roles as lifesaving medications for patients with cardiovascular diseases and renal failure. Recent research has produced diverse perspectives on the implications of these medications, particularly in the context of COVID-19 patients. It explores how SARS-CoV-2, the virus responsible for COVID-19, interacts with hosts who are undergoing regular treatment with these inhibitors. This analysis can assist in determining their appropriateness in managing COVID-19 and evaluating whether these treatments pose any significant threat to patient health.

Introduction:

The COVID-19 pandemic represents an unprecedented public health crisis, with significant socioeconomic effects and over 3 million confirmed cases worldwide as of mid-2020. Initial clinical data highlighted that patients with cardiovascular diseases, including those with hypertension and diabetes, were notably overrepresented among those requiring hospitalization for severe COVID-19 infection. The angiotensin-converting enzyme 2 (ACE2) receptor serves as the main entry point for the SARS-CoV-2 virus. There was growing concern that medications such as ACE inhibitors (ACEi) and angiotensin II receptor blockers (ARBs), both commonly used for managing hypertension and heart failure, could potentially enhance the risk of severe infection due to their influence on ACE2 levels [1, 2, 7].

Background and Mechanisms

Recent studies suggest that chronic treatment with ACEi or ARB might elevate tissue levels of ACE2, thereby increasing the potential for SARS-CoV-2 to bind and enter epithelial cells [3, 5]. This hypothesis has raised critical questions about the implications of continuing these medications for patients with existing cardiovascular conditions during the pandemic. Several theoretical mechanisms could explain why ACEi and ARB treatment

might be risk-enhancing. Conversely, these drugs could also offer protective benefits during severe respiratory events by modulating the renin–angiotensin system (RAS) and potentially reducing inflammation and lung injury associated with severe COVID-19 [4, 6, 7, 10, 12].

Study Overview and Methodology

In the context of these concerns, a substantial study was conducted involving 1200 hospitalized COVID-19 patients across two sites in the UK. The study aimed to evaluate the association between chronic treatment with ACEi/ARB and the severity of COVID-19 outcomes, defined primarily as death or critical care admission within 21 days of symptom onset [3, 8].

The cohort included patients aged 63 years on average, with 52% being male and a significant percentage from minority ethnic groups. Notably, 74% of patients had at least one comorbidity, with hypertension and diabetes being the most prevalent [8]. Data were collected through a validated electronic health record pipeline, ensuring rapid evaluation of clinical outcomes while capturing detailed medication histories, symptoms, and other key variables [9, 11].

Key Findings

Among the 1200 patients studied, 399 (33.25%) were on chronic treatment with ACE inhibitors or ARBs. Of the total cohort, 415 (34.6%) reached the primary endpoint of either death or admission to an intensive care unit within 21 days of symptom onset. Notably, patients on ACEi/ARB were generally older and had a higher prevalence of comorbidities compared to those not on these medications. Despite this, the odds of experiencing a severe outcome (death or critical care admission) was significantly lower for those on ACEi/ARB [7, 8].

After adjusting for demographic and clinical factors, the odds ratio (OR) for severe outcomes in patients on ACEi/ARB compared to those not on these agents was found to be 0.63 (95% confidence interval [CI]: 0.47-0.84, $P < 0.01$). This suggests that chronic treatment with these medications may not only be safe but potentially beneficial among patients with COVID-19 [4, 12, 13].

A comparison of outcomes by sub-group based on ACEi/ARB usage provided additional insights, consistently revealing no increased risk associated with these drugs among patients with existing cardiovascular morbidity [11, 14].

Discussion:

The findings of this study contribute to the growing body of evidence suggesting that the risks associated with stopping ACEi and ARB medications may outweigh potential

benefits. Current clinical guidelines have recommended that patients appropriately prescribed these drugs continue their use, particularly given the benefits in managing hypertension and heart failure, which are vital in the context of a global health emergency [7, 14]. Evidence also supports the stance that cessation of ACEi/ARB could expose patients to higher risks of adverse outcomes due to the underlying conditions these medications are prescribed to manage [1, 3, 8].

Regrettably, some studies have suggested a correlation between ACEi/ARB use and increased susceptibility to severe COVID-19 outcomes, yet many of these analyses have been confounded by the underlying conditions of patients included in the studies, which often overlap with those requiring COVID-19 hospitalization [8, 9]. Comparative results from similar investigations across different cohorts indicate that chronic usage of these agents does not confer additional risk for severe COVID-19 [5, 6, 12, 14].

Conclusions and Future Research:

Based on the evidence derived from this study, ACE inhibitors and ARBs may be considered safe regarding the severity of COVID-19 outcomes among patients. It is essential for medical professionals to maintain a collaborative approach to care, ensuring that cardiovascular treatments are not unnecessarily halted, especially as new guidelines and recommendations emerge during the ongoing pandemic.

Future studies are needed to further elucidate the mechanisms by which ACEi and ARB influence COVID-19 outcomes. Randomized controlled trials may help to confirm the potential therapeutic benefits of these medications in the pandemic context and provide clarity on their roles in managing patients with both COVID-19 and pre-existing cardiovascular conditions. Emphasis must be placed on ensuring equitable access to these therapeutics across diverse populations, who may be disproportionately affected by both cardiovascular diseases and COVID-19 [9, 10, 12].

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MARKETING STRATEGIES FOR ORNAMENTAL FISHES

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Abstract:

The ornamental fish industry is a thriving area of the worldwide pet market, driven by increased consumer interest in aquariums as a decorative and therapeutic element. Success in this specialized sector depends on using efficient marketing techniques. In order to increase exposure and customer trust, this study examines cutting-edge strategies such influencer partnerships, social media participation, and digital marketing. By encouraging direct customer relationships, traditional strategies like trade exhibitions and retail partnerships support these contemporary approaches. To appeal to customers that care about the environment, a focus is put on specialized branding, sustainable sourcing, and instructional materials. Businesses may increase customer loyalty, boost outreach, and boost sales in the cutthroat ornamental fish industry by combining offline and online tactics.

Keywords: Strategies, Thriving Area, Industry.

Introduction:

A profitable and vibrant industry, the ornamental fish market meets the demand for unique and beautiful fish species in aquariums around the world. Breeders, exporters, retailers, and enthusiasts are among the many stakeholders in the business. For this industry to continue growing and to satisfy the demands of a wide range of customers, effective marketing techniques are crucial.

Overview of the Ornamental Fish Industry:

The ornamental fish market is characterized by its diversity, offering hundreds of species ranging from freshwater to marine varieties. According to industry reports, the global ornamental fish market is projected to grow significantly due to increasing interest in aquarium hobbies and the therapeutic benefits associated with keeping fish. Key markets include North America, Europe, and Asia, with the latter being a significant supplier due to favorable climatic conditions for breeding and exporting.

Supply Chain Management:

Supply chain management plays a critical role in the ornamental fish industry. It involves sourcing, breeding, transportation, and distribution of fish to retailers and end consumers. Marketing strategies within the supply chain focus on efficiency, quality assurance, and reducing mortality rates during transportation.

Breeding and Sourcing:

Breeding quality fish is a fundamental aspect of the supply chain. Marketing strategies emphasize partnerships with experienced breeders who can supply healthy and vibrant fish. Certifications and endorsements for sustainable breeding practices enhance marketability.

Transportation and Logistics:

Ornamental fish are delicate and require specific environmental conditions during transportation. Companies invest in specialized packaging, oxygenated containers, and temperature control to ensure fish arrive healthy. Highlighting these logistics innovations in marketing materials assures consumers of the quality and care involved.

Retail Distribution:

Retailers serve as the final touchpoint for consumers. Marketing strategies include attractive in-store displays, live aquariums showcasing fish in natural habitats, and informative brochures to educate customers about care requirements.

Branding and Positioning:

Branding is critical in differentiating ornamental fish suppliers in a competitive market. A strong brand identity builds trust and loyalty among consumers.

Creating a Unique Selling Proposition (USP):

Businesses emphasize aspects such as rare species, sustainable practices, or exclusive breeding techniques to create a USP. For example, companies specializing in exotic marine species often highlight their expertise in maintaining coral-safe environments.

Visual Branding:

The use of visually appealing logos, packaging, and promotional materials captures attention. High-quality images of vibrant fish are frequently employed in marketing campaigns.

Storytelling:

Sharing the origins and breeding stories of ornamental fish adds an emotional connection. For instance, a campaign detailing how certain fish species are bred responsibly in small-scale farms can appeal to environmentally conscious buyers.

Digital Marketing:

The rise of digital platforms has revolutionized marketing in the ornamental fish industry. Online strategies include e-commerce, social media, content marketing, and influencer partnerships.

E-Commerce Platforms:

Selling ornamental fish online allows businesses to reach a global audience. Websites and apps provide detailed descriptions, care guides, and videos of available fish. User-friendly interfaces and secure payment options enhance customer experience.

Social Media Marketing:

Platforms like Instagram, Facebook, and YouTube are ideal for showcasing the beauty of ornamental fish. Businesses use social media to post vibrant images, run contests, and engage directly with their audience. Live sessions offering aquarium setup tips are particularly popular.

Content Marketing:

Blogs, videos, and tutorials about aquarium maintenance, fish care, and species compatibility serve as valuable resources for hobbyists. Optimizing this content for search engines ensures higher visibility.

Influencer Collaborations:

Partnering with aquarium enthusiasts and influencers helps build credibility. Influencers share personal experiences, review products, and demonstrate fish care practices, reaching a wider audience.

Sustainability as a Marketing Strategy:

Sustainability is a growing concern among consumers. Companies that adopt eco-friendly practices gain a competitive edge.

Ethical Sourcing:

Marketing campaigns emphasize responsible sourcing of wild fish and sustainable breeding programs to protect natural habitats.

Eco-Friendly Products:

Businesses promote sustainable aquarium products such as biodegradable tank decor, energy-efficient filters, and organic fish food.

Corporate Social Responsibility (CSR):

Companies engage in initiatives like reef restoration, conservation projects, and community education programs. Highlighting these efforts in marketing materials resonates with environmentally aware consumers.

Consumer Engagement and Retention:

Building lasting relationships with customers is vital for the ornamental fish industry. Strategies include personalized services, loyalty programs, and educational initiatives.

Personalized Services:

Offering tailored recommendations based on customer preferences fosters loyalty. For example, suggesting specific fish species or tank setups suitable for beginners versus advanced hobbyists.

Loyalty Programs:

Rewarding repeat customers with discounts, exclusive access to rare species, or free consultations encourages long-term engagement.

Educational Workshops:

Hosting workshops on aquarium maintenance, fish health, and aquascaping establishes businesses as trusted authorities. Online webinars cater to a broader audience.

Challenges and Future Prospects:

The ornamental fish industry faces challenges such as regulatory constraints, disease outbreaks, and competition from alternative hobbies. Marketing strategies must address these challenges proactively.

Regulatory Compliance:

Staying updated with international trade laws and obtaining necessary certifications builds consumer trust. Marketing campaigns can highlight adherence to ethical and legal standards.

Innovations in Health Management:

Promoting disease-free fish through advanced health screening techniques enhances customer confidence.

Exploring Emerging Markets:

Expanding into untapped regions with growing interest in aquariums presents opportunities. Marketing efforts should focus on educating new consumers in these markets.

Conclusion:

The ornamental fish industry thrives on creativity, innovation, and a deep understanding of consumer preferences. Effective marketing strategies from supply chain optimization to digital outreach—are essential for capturing market share and ensuring sustainable growth. As the industry evolves, businesses that prioritize sustainability, customer engagement, and technological advancements will continue to flourish. By leveraging these strategies, the ornamental fish market can cater to the diverse needs of its global audience while promoting environmental responsibility.

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BLUEPRINTS OF HEALING: MODERN TECHNIQUES IN DRUG DESIGNING

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Abstract:

Drugs are an inevitable part of our life, required daily for various purposes and administered in varied forms. To make them available and effective, decades of research involving multiple scientific fields to discover and design a single drug, with careful planning of the drug delivering to the appropriate site in the body for it to activate and carry its function. To simplify the drug designing process to some extent, several modern techniques are employed, especially since the boom in biovinformatics field. Computerized techniques and simulations are used to construct the drug compound structure, ligand and receptor sites, protein structures, etc. to understand the mechanism of the drug when it interacts at the necessary site by simulator method. This is widely termed as Computer Aided Drug Designing (CADD). Due to this the long-term research required has been reduced to a great extent allowing researchers to produce, design and release a drug earlier in the market as well as reduced the use of animal models utilized in research. Other similar methods that mainly involve CADD are Artificial Intelligence (AI), Molecular Docking, Structure Based Drug Design (SBDD), Ligand Based Drug Design (LBDD etc. This chapter aims to give an insight of the listed methods for drug designing most of which are currently employed for drug designing research while some are still being explored to utilize its potential in the future.

Keywords: Animal Models, Artificial Intelligence (AI), Bioinformatics, Computer Aided Drug Designing (CADD), Computerized Technique, Drugs, Drug Design, Ligand, Ligand Based Drug Design (LBDD), Molecular Docking, Protein Structures, Structure Based Drug Design (SBDD), Simulations.

Introduction:

Drugs are chemical substances prepared by an authorized pharmacopoeia or formulary intended for use in healthcare for diagnosis, cure, prevention, mitigation, treatment of any abnormal condition in an individual (humans and animals) etc. classified into various categories depending on its use like antibiotics, anti-inflammatory and

analgesics, antipsychotics, barbiturates, bronchodilators, diuretics, corticosteroids, muscle relaxants, sedatives, antacids, thrombolytics and anticoagulants, antihypertensives, antineoplastics, and many more. These drugs are in market for use by the consumer as per the requirement prescribed by a medical professional. For instance, Amoxycillin and Cefixime are the two most common antibiotics prescribed to a patient during a bacterial infection, whereas Carboplatin, Altretamine, Busulfan etc. are some of the frequently used antineoplastics which are involved in cancer treatment alongside chemotherapy. But, the drugs in the market did not magically appear, it took several years even decades of planning, preparations, trials to design and discover any medicine.

Drug discovery which is defined as the process by which possible novel medications are found by encompassing many scientific fields like pharmacology, biotechnology, biology and chemistry being the main ones, whereas drug designing is the creative process of creating novel drugs using biological target information, basically creating molecules that are complimentary in shape and charge to the chemical target they interact and bind to is the most basic aspect of medication creation. Knowledge in both these fields and its appropriate implementation is necessary to prepare an efficient drug. Along with these pharmacology branches that are involved is Pharmacokinetics that is the branch of clinical pharmacology that focuses on chronic variations in the amount of a medication or drug metabolite in different bodily parts. The four processes of metabolism, excretion, distribution, and absorption can be used to characterize these alterations; Pharmacodynamics is known as the study of how medications affect the body's physiology and biochemistry. The quantity and affinity of receptors at the site of action, signal transduction, and homeostasis regulation all affect how much of a drug's pharmacologic impact is felt; and lastly Pharmacovigilance which is defined as the pharmaceutical science that deals with the collection, detection, assessment, monitoring, and prevention of the negative side effects with pharmaceutical goods and is sometimes referred to as drug safety. All these disciplines together are involved in drug development process. It is an extremely tedious, expensive, time consuming and laborious process taking at least a decade or even more to design it, conduct several stages of clinical trials and then get it approved from the FDA. Also, due to any unknown reason, even though the drug passed all clinical trial phases yet shows some side effects after release in the market, it is recalled and modified, which may require few more months or even extend to years. In general, the steps involved is designing a drug are as follows:

1. **Discovery:** Novel compounds based on the knowledge of a disease occurrence are

searched followed by testings for preliminary confirmation if the molecule is efficient to treat. If it is, further research is conducted.

2. **Development:** Here, the molecule's dosage, ADMET (Absorption, Digestion, Metabolism, Excretion and Toxicity) factor is assessed, reproducibility, side effects, route of administration, interaction with other simultaneous treatments, its suitability for different groups of people (for children, adolescents, pregnant women, gender, etc.).
3. **Preclinical Research:** In Vitro studies on animal cell lines to ensure no serious harm takes place and a dosage is set, as well as a defined protocol and requirement of the drug.
4. **FDA Approval:** Before taking the study to in vivo level, an FDA review team analyses and their approval is required to proceed.
5. **Clinical research:** Now from in vitro the study is shifted to in vivo beginning with animal models like mice, rabbits, chimpanzees and monkeys followed by trials in human volunteers. It involves at least 3 phases with a control group also called placebo group, where the categories are modified and tried on a larger group in each phase trials.
6. **FDA Review:** A New Drug Application (NDA) is submitted to the Food and Drug Administration (FDA) from the company enlisting the entire history of the molecule and complete research data statistics. The FDA review team evaluates it, with an inspection to ensure no fabricated study has been conducted.
7. **Approval:** When it is confirmed that the data is authentic, and the drug prepared is effective and suitable for consumption, the applicant company is asked to label it with the necessary information and a brand name, after which the FDA approves the medication and releases in the market.

Post Market Drug Safety Monitoring:

Though strict trials and information of the drug has been provided, FDA monitors the acceptance of the drug for a few months to a year till it is accepted by the public to ensure no side effect arises due to the medicine or incorrect prescription. If the response is not up to the mark and limitations arise, the drug is recalled from the market for re purposing

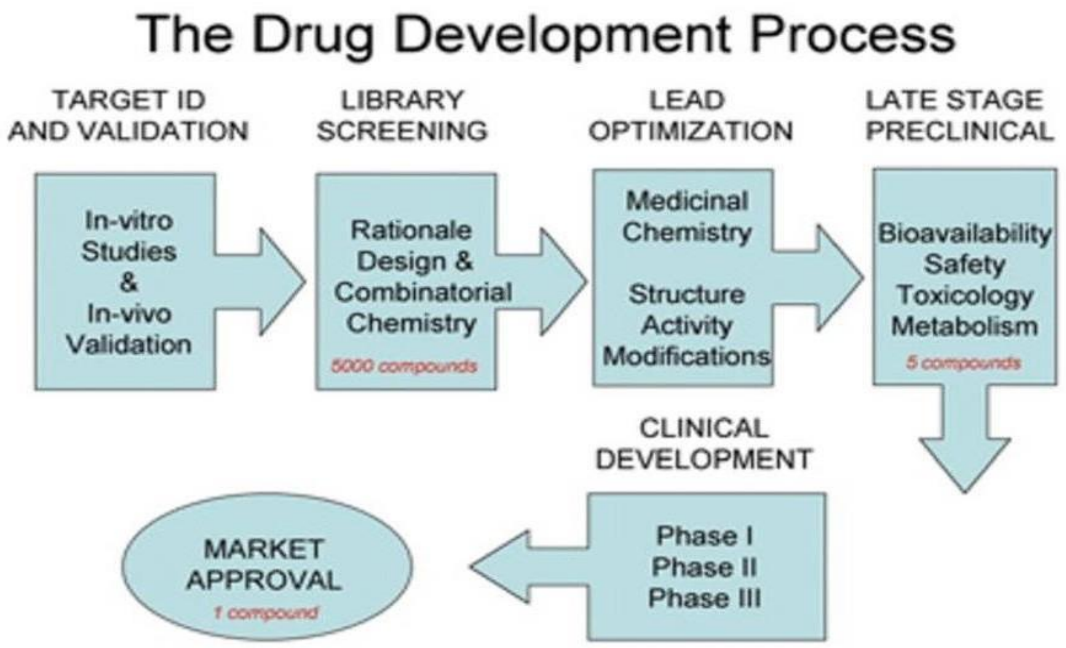


Figure 1: Flow chart of Drug Designing Process

Till date drugs were designed by the traditional methods that is obtained from a variety of sources like plants, minerals, microbes; or by serendipitous discovery which is some drugs are found by chance; ethnopharmacological approach that involves study of ethnic medicinal plants of a community; random screening; trials and errors etc. Penicillin an antibiotic discovered during World War 1, is a classic example of serendipity discovery by Alexander Fleming from the fungi *Penicillium notatum* and *Penicillium chrysogenum.* But, the major drawback of traditional techniques is it requires years of experimentation and takes a lot of time and money in its production.

To overcome this issue, new approaches mostly in silico methods are being discovered with some of them already being in use. The most widely used is Computer Aided Drug Designing (CADD), Artificial Intelligence (AI), Bioinformatics, Molecular Docking, Structure Based Drug Design(SBDD), Ligand Based Drug Design (LBDD), Hybrid methods, High Throughput Screening (HTS), QSAR, etc.

Computer Aided Drug Design (CADD):

The field of computer-aided drug design (CADD) is expanding and has many different aspects. It includes both basic and applied research in a variety of forms. Basic and applied research both combine and develop their potential in this way. Quantum mechanics and molecular modeling techniques, such as structure-based drug design, ligand-based drug design, database screening, and binding affinity analysis, which are all predicated on an understanding of a biological target, form the theoretical basis of CADD.

The fact that CAD requires less money and work than laboratory studies is one of its main benefits. The use of CADD in the drug discovery process is covered in this review.

Types of CADD

Structure-Based Drug Designing (SBDD)

One of the most effective and potent methods in the field of drug discovery is structure-based drug design, or SBDD. Along with other pertinent biological insights, it makes use of crucial data such small molecule target information, genetic sequences, binding interactions, cytotoxicity, and ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiles. Pharmaceutical businesses and R&D teams use these services widely since they are crucial for speeding up the drug discovery process. Predicting molecular structures, visualizing binding sites, performing molecular docking and virtual screening, assessing the stability of docked complexes, evaluating ADMET, and computing binding-free energy using techniques like MM-PBSA are all examples of the standard workflow

Ligand-based drug designing (LBDD)

Accurate target identification is made extremely difficult by the absence of a 3D macromolecular structure, necessitating the application of sophisticated computational methods. Because of this restriction, pharmaceutical researchers are currently facing more challenging circumstances. However, the creation of more efficient methods has strengthened the idea of high-throughput screening (HTS). To overcome these obstacles, ligand-based drug design has shown itself to be a useful strategy. There are three methods to tackle this, including Methods for similarity searches, machine learning, and mapping pharmacophore models.

Applications of CADD:

1. Molecule Screening: Using both computational and experimental techniques, CADD is essential for screening a large number of molecules against a target structure.
2. Lead Compound Optimization: It helps to optimize lead compounds according to their toxicity levels, pharmacokinetic characteristics, and binding affinity.
3. Design of Novel Compounds: By altering their structure to improve their functionality, CADD assists in the creation of novel compounds that resemble drugs.
4. Usage in Drug Modeling: Combinatorial chemistry and bioinformatics are included into CADD's usage in drug development, tackling important issues including time and cost limits.

Limitations of CADD:

The scientific community still has to address a number of issues with the SBDD approach, despite the fact that it has become a ground-breaking drug design technique. Enhancing chemogenomic compound libraries, improving screening methods, improving the quantity and quality of data, developing new tools and databases, optimizing multitarget drug structures, improving toxicity prediction algorithms, and integrating the approach for greater efficacy and compatibility are some of these challenges. Furthermore, the significance of entropy estimates and electrostatic interactions is frequently disregarded. Moreover, no one piece of software or package exists at this time that works well for particular targets and ligands, such as water molecules, and figuring out the target's most likely shape. To overcome these obstacles, more advancements and enhancements are still needed.

AI in Drug Discovery:

Artificial Intelligence is able to identify hit and lead compounds, validate drug targets more quickly, and optimize drug structure design. Together with structural and ligand-based approaches, a variety of in-silico techniques for virtual screening compounds from virtual chemical spaces offer improved profile analysis, quicker removal of non-lead compounds, and more cost-effective therapeutic molecule selection.

Molecular Docking:

The phenomenon of small molecules in the binding pockets of the protein is known as molecular docking. Molecular docking is an in-silico research method that focuses on the interaction between two or more molecular structures, such as an enzyme and a ligand. The binding of these molecules results in various interactions, such as complement cascade, inhibition or activation of the biological function of the protein. The technique enables us to determine the affinity of ceratin molecules for the relevant protein as well as to forecast and identify possible locations where these molecules bind and interact. Docking can be categorized according on the types of ligands:

- Protein small molecule (ligand) docking
- Docking of protein nucleic acids
- Docking of proteins.

Some fundamental terms used in molecular docking:

Receptor - A receptor is a polymeric structure, primarily a protein molecule, that is found on the surface of cells. It binds with particular molecules, known as ligands, which function as molecular messengers and causes a biological reaction.

Ligand **-** Ligands are molecules that attach themselves to receptors; these can be drugs, neurotransmitters, hormones, lymphokines, lectins, and antigens. However, they can also be another biopolymer or macromolecule (in the case of protein docking), or they can be another biopolymer or macromolecule.

Docking **-** the modeling method known as "docking" is intended to determine the ideal fit between the ligand and the docking site.

Dock pose- There are various positions, conformations, and orientations in which a ligand molecule can attach to a receptor. A dock stance is the name given to each of these docking modes.

Binding Mode **-** The term "binding mode" describes the precise conformation of the ligand and the receptor when they are coupled together, as well as the ligand's relative orientation with regard to the receptor.

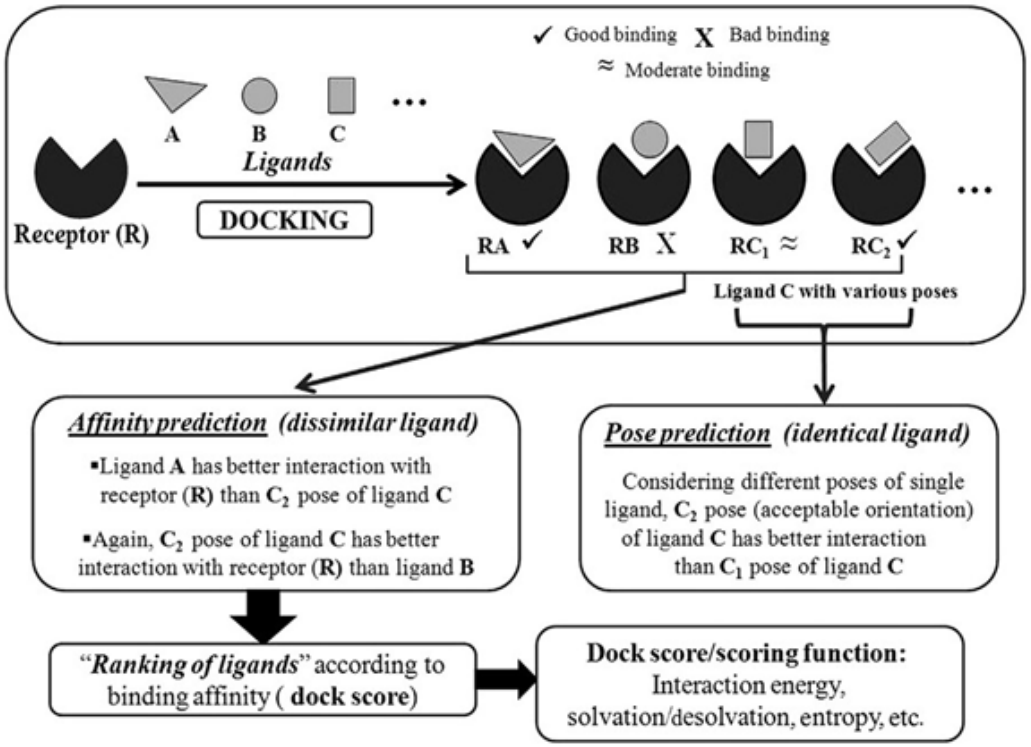
Dock Score **-** The technique of determining a certain ligand-receptor pose by counting the amount of advantageous intermolecular contacts, such as hydrophobic and hydrogen bonding interactions, is known as the docking score. This aids in determining the most energetically advantageous binding position while accounting for characteristics like as electrostatics and shape compatibility. The ligand is probably a good binder if its dock score is high.

Ranking **-** Using the expected free energy of binding as a criterion, ranking entails grouping ligands according to how likely they are to interact favorably with a particular receptor. Following the completion of docking, ligands are ranked according to their dock scores, which indicate their anticipated binding affinities. Prioritizing compounds for additional synthesis and biological testing based on their predicted level of activity is done using this ranked list.

Pose Prediction **-** Pose prediction is the process of determining a ligand's proper binding mode. Usually, this entails identifying the energetically ideal poses after conducting several experiments. Given their intrinsic flexibility, it is essential to ascertain the ligand's proper orientation and conformation.

Scoring or Affinity Prediction **-** Evaluating the optimal docking positions based on energy

scores is known as affinity prediction, or scoring. These scores are compared to determine the relative rankings of ligands. Generally speaking, scoring functions can be divided into two groups: (1) knowledge-based functions, which use observed atomic contact frequencies or distances in protein-ligand complex databases to generate statistical data, and (2) physical interaction-based functions, which concentrate on binding energetics. These functions frequently make the assumption that the binding-induced change in free energy can be divided into multiple parts: ΔGbind = ΔGint + ΔGsolv + ΔGconf + ΔGmotion. These elements stand for the distinct ligand- receptor interactions (ΔGint), interactions with the solvent (ΔGsolv), ligand and receipt confrontational changes (ΔGconf), and protein and ligand motions during complex formation (ΔGmotion).



Conclusion:

In summary, contemporary technology such as Computer Aided Drug Designing (CADD), Artificial Intelligence (AI), Molecular Docking, and others have accelerated pharmacological research and made drug design faster and more efficient. The majority of these technologies are tools for Ligand-Based Drug Design (LBDD) or Structure-Based Drug Design (SBDD), which are less expensive, more effective, and need fewer animal models. These are innovative tools to advance medication research, however there are still obstacles to overcome, including as limits in different software programs and more effective screening methods. Lead optimization, guided docking, and general drug

development already benefit from AI and in-silico methods. Ultimately, these technologies ought to facilitate quicker turnaround times, more accurate medication targets, and improved drug safety profiles.

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ADVANCEMENTS IN FERMENTER DESIGN

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Abstract:

This chapter provides a comprehensive overview of fermenters and bioreactors which are essential tools for industrial and laboratory fermentation process. Fermenters facilitate the controlled cultivation of microorganisms or cells to produce desired products as well as enable treatment of effluent released post production. This chapter starts with an overview of fermentation principle, the role of fermenters in maintaining optimal growth conditions by regulating temperature, pH, nutrient availability and aeration. It examines the basic design of fermenters and various function specific modifications and gives an overview of the entire fermenter run. This chapter provides a detailed understanding of fermenters and their role in bioprocessing and wastewater treatment.

Keywords: Fermenter, Microorganisms, Fermentation, Bioreactor, Wastewater, Effluent

Introduction:

Fermenters or bioreactors are huge closed tanks used for biochemical reactions involving microorganisms or microorganism-derived compounds. The fermenter operates on the principle of fermentation, which is carried out by the microbe itself to create desired metabolites (McNeil, 2008). However, in order to properly produce these metabolites on an industrial scale, a system must maintain optimum conditions such as temperature, pH, pressure, nutrient levels, aeration, and so on. These parameters change depending on the organism, so substantial research is required to enhance efficiency based on both the product to be produced and the microbes engaged in the manufacturing process. It is a very competitive market because industrial fermentations are lucrative businesses, thus research is critical. Regular research is conducted not just to understand how a microbe ferments, but also to enhance the fermenter's architecture in order to increase yield (Agrawal, 2024).

A fermenter should be capable of executing particular operations to ensure the smooth operation of the production line. It should allow for constant mixing/stirring of cells and media, which allows the microbe to better use the substrate by increasing the

availability of nutrients and oxygen. In the event of aerobic fermentation, it should be capable of providing adequate sterile aeration, and in the case of anaerobic fermentation, it should guarantee anoxic conditions. Most fermenters use pure cultures thus, the growth of contaminating organisms should be avoided. The design should be structurally sound to resist the high temperature and pressure conditions it must operate under, as well as large enough to handle enormous volumes of product. There ought to be a means of transferring inoculum into the vessel and removal of culture sample during the fermentation in an aseptic manner. Certain equipment, such as pH meters, should be present to monitor pH levels as product quantity increases during the fermentation process. The temperature should be kept at a preset ideal temperature set by the scientist or manufacturer at all times. In some circumstances, such as alcohol fermentation, the presence of an excess product might be harmful to the production organisms, namely yeast. As a result, a system for continuously removing alcohol produced should be included to avert yeast poisoning (Ali, 2018).

Basic Design

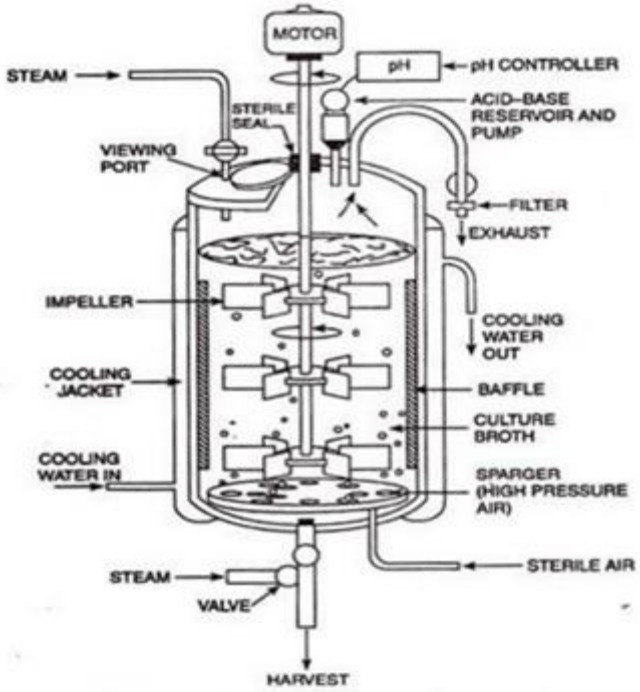


Figure 1: Basic fermenter design (Ezemba & Ekwegbalu E.A & Ezemba A.S., 2022)

Body:

1. Material: The material used to create the vessel's main body should be able to endure the steam sterilization cycles required to maintain aseptic conditions,

pressure, and corrosion, as well as being cost-effective and nontoxic. The body is primarily made of glass or stainless steel

2. Size: Laboratory fermenters range in volume from 1-2 litres to 12-15 litres. Fermenters used for research can range from 25 to 200 gallons, whereas fermenters used in industry might have a total volume of 5,000 or 10,000 gallons. However, the fermenter's total volume does not equal the vessel's complete working volume.
3. There is always about a quarter of the vessel left empty at the top to allow for medium movement and splashing, as well as space for froth production caused by gas evolution and the presence of foaming ingredients. This is known as "head space" (Steel, 1970).
4. Head Plate is the highest covering of a fermenter vessel, preventing medium overflow (Nair, 2022).
5. Inoculation port is where microorganisms cultivated for fermentation are injected into the fermenter.
6. Nutrient Inlet: Nutrients for microbial growth enter the fermenter.
7. Motor: It drives the impeller and is connected externally on top of the tank.
8. Sensor Probes: It monitors fermenter conditions to ensure optimal growth levels. This can include temperature probes, pH probes, dissolved oxygen probes, foam probes, biomass probes, and nutrition probes (Ezemba & Ekwegbalu E.A & Ezemba A.S., 2022).
9. Impeller: It is a circular disc onto which the blades of the agitating device are attached with the help of bolts. The blades can be of different types based on requirement. In the absence of an electric motor, this impeller can also work with the presence of impeller blades. It can also work on the principle of magnetic coupling. The impeller blades have to rotate at a speed so that they allow proper mixing of the medium and also do not prevent the air bubbles produced by the sparger from mixing into the medium.
10. Baffles: High-speed spinning creates a vortex that pushes liquid against fermenter walls, requiring removal. To accomplish so, four baffles are affixed to the fermenter's walls. The baffles disrupt and damage the flow of the liquid, improving the mixing process.
11. Sparger: A sparger is an aerating device with small pores that release high-

pressure air into the aqueous medium, creating tiny air bubbles. The holes can be 1/64 to 1/32 inch in size or greater. It is not economically feasible to create holes smaller than this range.

12. Foam Controlling Device: Excess foam can damage the fermenter by wetting the filters and contaminating the vessel. To avoid this, antifoam can be used alongside the media; however, it can cause aeration concerns. Foam breakers are widely used to separate foam using a spinning mechanism and return the liquid portion to the fermenter (Steel, 1970).
13. Temperature Control Device: The temperature is controlled via a cooling jacket, that is connected externally to the fermenter and helps to cool the vessel, which becomes hot due to the heat emitted during the fermentation process. This is reinforced by the use of internal coils, which enhance the surface area available for heat transfer (Ezemba & Ekwegbalu E.A & Ezemba A.S., 2022).
14. Acid/base inlet: As product formation increases, the system's pH may change, reducing production efficiency. The pH in the vessel stabilized by this inlet If the pH is excessively acidic, sodium hydroxide or ammonia is added; if the pH is too basic, sulphuric acid is used.
15. Valves: It controls the flow of gases, liquids, and other media inside a system. There are various forms of valves, and the type of valve employed is determined by the type of fermenter and its intended purpose. The valves available for use include gate valves, globe valves, needle valves, piston valves, plug valves, ball valves, butterfly valves, pinch valves, and diaphragm valves for sterility applications.
16. Exhaust Outlet: It removes byproducts and exhaust gases from the fermenter vessel (Terry, 1987).

Types of Fermentation

Microbial growth can occur in three different fermentation systems: batch, fed-batch, and continuous fermentation

- **Batch Fermentation**

  Batch fermentation involves inoculating the required microorganisms into a sterile growth medium and proceeding with no extra growth medium (Glick, 2022). During fermentation, the concentration of microorganisms, the composition of the culture medium, the internal chemical composition of the microorganisms, and the

amount of target metabolite or protein all change as a result of cellular metabolism, growth, and nutrient availability (Yamuna Rani, 1999).

- **Fed-Batch Fermentation**

  In fed-batch fermentation, an increasing amount of substrate is introduced at different stages of the fermentation, and no growth media is removed until the completion of the process. These additions that prolong the log and stationary phase cause an increase in biomass and the synthesis of stationary-phase metabolites, such as antibiotics.

- **Continuous Fermentation**

  Continuous fermentation does not remove an equal volume of wasted medium while fresh growth medium is constantly introduced during fermentation. Eventually, the total number of cells and biomass remain constant since the loss of cells due to product removal is perfectly balanced by the acquisition of new cells through division, resulting in steady state fermentation (Glick, 2022).

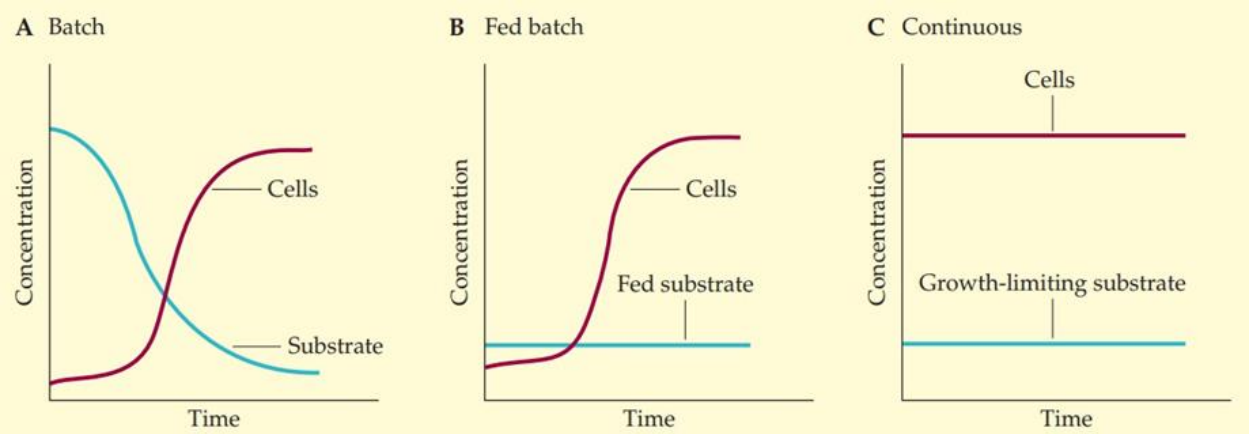


Figure 2: Graphical representation of the time course of cell concentration (mass) and the substrate in batch (A), fed-batch (B) and continuous (C) fermentations (Glick, 2022)

Types of Fermenters

Fermenters are classified into several varieties based on their intended use and the microorganisms used in the fermentation process. They are as follows:

Airlift Fermenter: It is named from the contact that happens between gas-liquid-solid or gas-liquid as a result of fermenting fluid circulation. The vessel is sectioned into two parts (Chen, 1990). The first is the riser tube, which supplies air to the fermenter from the base via a sparger. The second piece is the downcomer tube, which does not deliver air. The density difference between the liquid column in the riser and the liquid column in the

downcomer serves as the fermentation process's driving power. There are two types of airlift fermenters: internal-loop fermenters (which use a single container with a draft to carry out fermentation) and external-loop fermenters (which use an external loop to separate liquids in different channels to carry out fermentation.

They are typically employed to produce single-cell protein (SCP) (Varavinit, 1996), as the heat produced during this form of fermentation is quite intense and can easily harm the cells. Thus, the vessel allows for efficient heat removal and thus temperature control, making the use of airlift fermenters more economically feasible than stirred tank fermenters. This fermenter is also easier to scale up than the stirred tank fermenter. This fermenter also has the advantage of providing a softer environment for cell growth, making it suited for sensitive organisms.

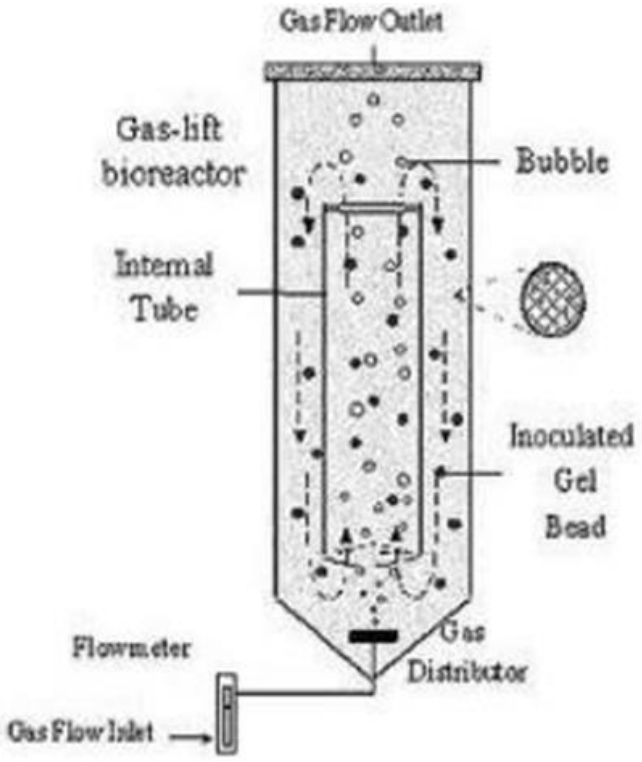


FIG 3: Airlift fermenter system (Ali, 2018)

Continuous Stirred Tank Fermenter: While it is utilized in more than 70% of fermentations, it may not always be the best option. The word continuous in the name refers to the continuous admission of substrate and the outflow of fresh product (Tang, 2015). This is done to maintain the microorganisms' steady logarithmic condition. This fermenter varies from other types of fermenters in that it operates in a constant condition, which can be accomplished in two ways: chemostat or turbidostatic. A chemostat's dilution rate is constant, and a steady state is maintained by incorporating a limiting nutrient into

the culture fluid (Mason, 1951). A turbidostat, on the other hand, maintains a constant turbidity by changing the dilution rate. The unmodified fermenter is utilized for batch manufacture of only a few virus vaccines, but the modified version is employed for a broader range of cultures. These alterations are beneficial because they serve to lower the likelihood of cell damage due to heat, contamination, or shear.

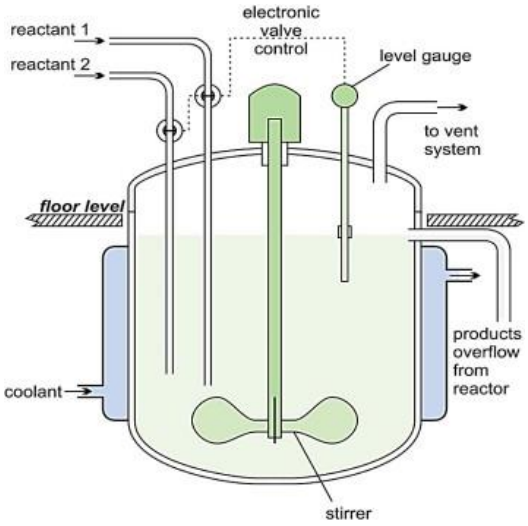


Figure 4: Continuous stirred tank fermenter

(Ezemba & Ekwegbalu E.A & Ezemba A.S., 2022)

Sparged Tank Fermenter: This is a fermenter in which non-mechanical agitation occurs. In this type of fermenter system gas is introduced from the base of the vessel by the help of a porous plate or nozzle. The bubbles of gas that are formed move through the liquid and get dispersed throughout the media by the help of the baffles connected to the side walls of the fermenter (Chandrashekhar, 2010). This fermenter has a lesser chance of contamination due to the absence of an agitation shaft, this also helps to reduce power consumption.

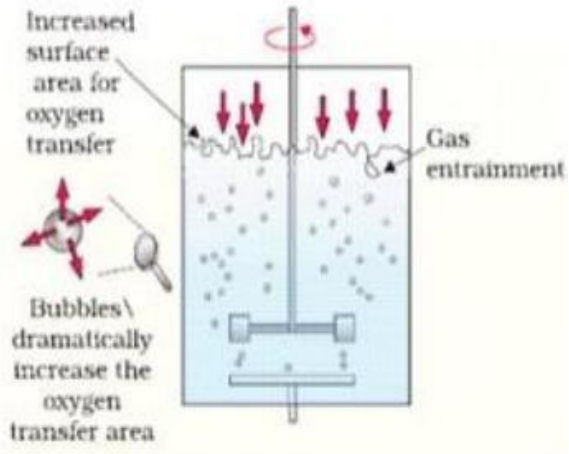


Figure 5: Sparged tank fermenter (Steel, 1970)

Tower Fermenter: Also known as the packed column fermenter, is a vertically positioned cylindrical column packed with particles of material such as wood, polythene etc which are inert in nature. The microorganisms and media are fed into the top of the column. The microbes are allowed to grow till biofilm formation subsequently followed by addition of desired substrate percolation from the bottom (Páca, 1976) (Prokop, 1969).

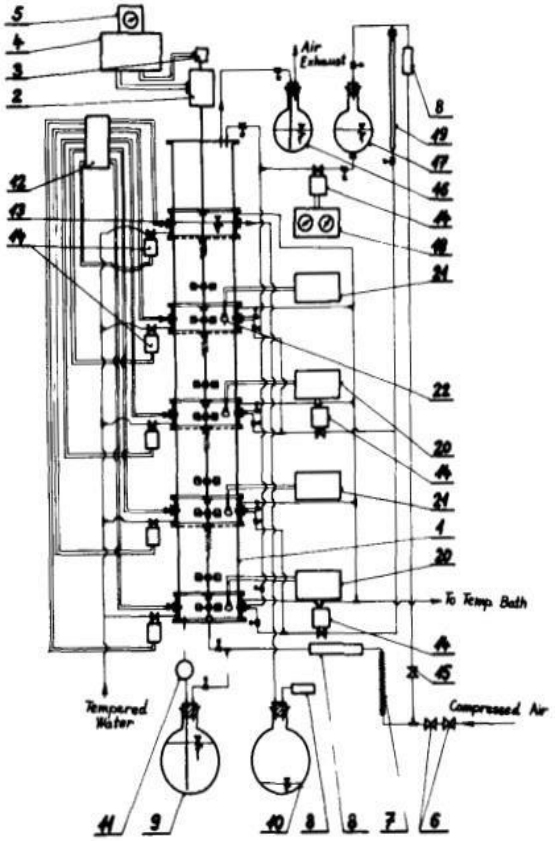


Figure 6: Tower fermenter system. 1. Body of fermenter; 2. electric motor; 3, 4. Tacho- generator feed-back control; 5. Impeller revolution measurement; 6. Automatic expansion controlled valve; 7. Rotameter; 8. Air filter; 9. Nutrient medium; 10. Harvest bottle; 11. Peristaltic pump; 12. Temperature control; 13. Control thermometer; 14. Solenoid valve; 15. Needle valve; 16. Bubbler; 17. Antifoam gauge; 18. Timer; 19. Gauge for base or acid; 20.pH controller; 21. pH meter; 22. pH probe (Páca, 1976)

Acetators: Acetators are fermenters specialized for vinegar production. Fermentation carried out by bacteria typically Acetobacter aceti, involves the aerobic conversion of substrate such as wine or spirit to acetic acid (Mariette, 1991) (Hromatka, Vinegar by submerged oxidative fermentation, 1959)

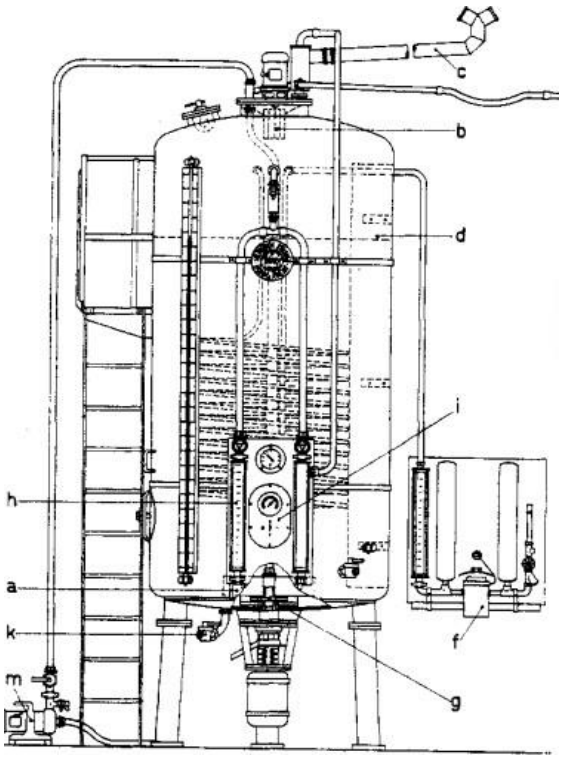


Figure 7: Acetator (Hromatka, Vinegar by submerged oxidative fermentation, 1959)

Bioprocess Run

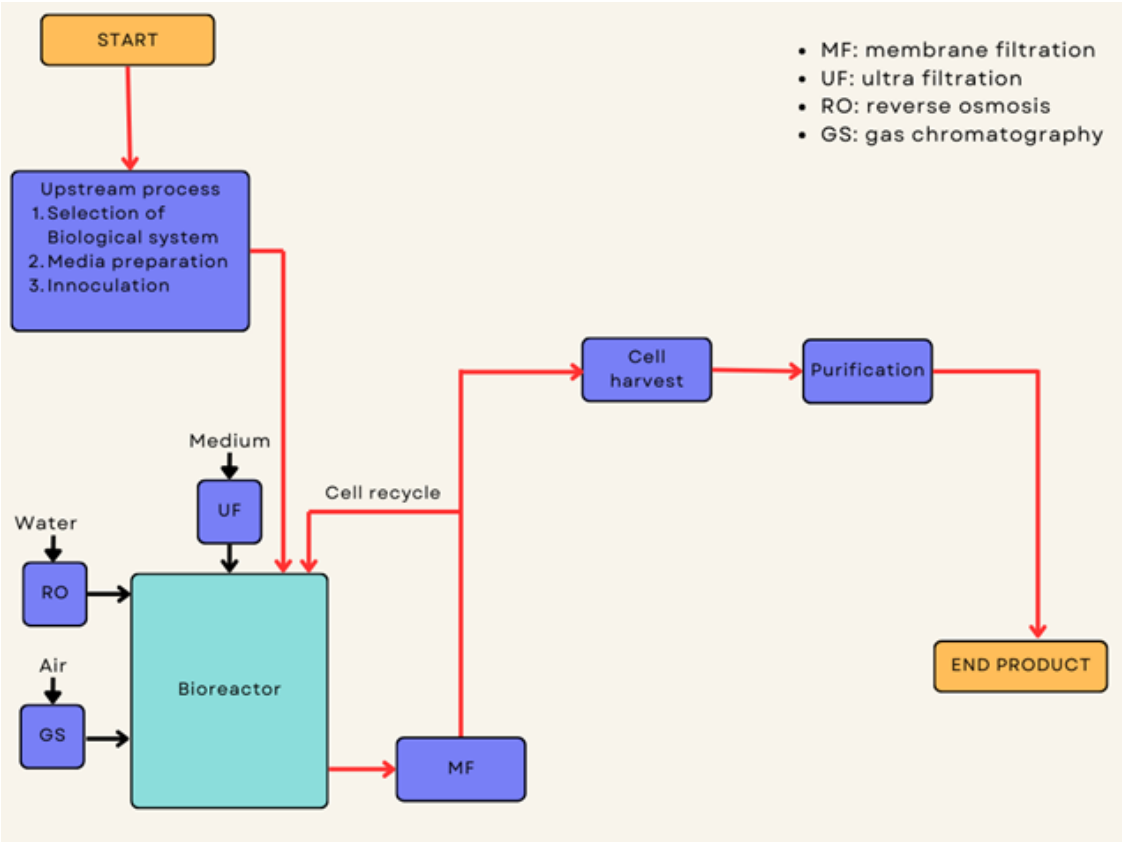


Figure 8: Bioprocess run

Types of fermenters for wastewater treatment

Aerobic Biological Treatment Fermenters

a. Fluidized Bed Reactor: A fluidized bed reactor (FBR) consists of an initially stationary bed of solid particles which is "fluidized" by an upward flow of either liquid or gas, creating a bed that behaves like a fluid. FBRs are primarily used to treat gaseous and liquid waste, and to some extent solid wastes. They are an advanced method for recalcitrant pollutant removal (Werther, 2000) (Bello, 2017).

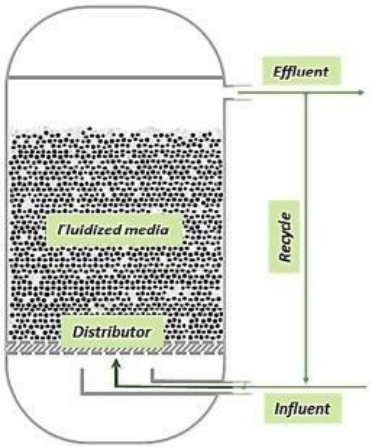


Figure 9: FBR for wastewater treatment (Bello, 2017)

b. Membrane bioreactor: A membrane bioreactor (MBR) combines the activated sludge process (ASP) with a microfiltration or ultrafiltration membrane. It works to treat effluent in a similar manor to ASP but without the need for secondary clarification and further tertiary steps. This is due to the fact that the microorganisms used for aerobic biological degradation is separated from the treated wastewater by the membrane (Marrot, 2004)

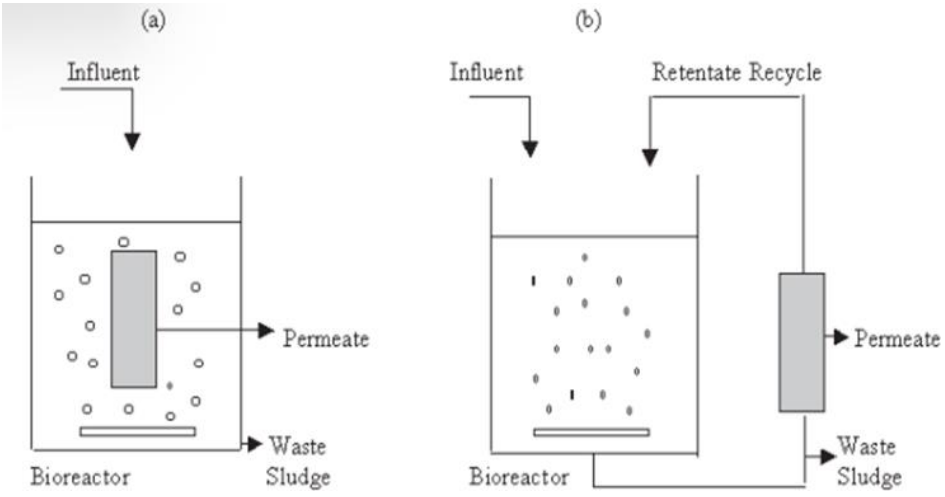


Figure 10: MBR system: (a) submerged MBR, (b) side-stream MBR configuration (Melin, 2006) (Melin, 2006)

c. Rotating Biological Contactor: A rotating biological contactor (RBC) an alternative for ASP, consist of closely spaced large flat discs that are mounted upon a haft and are either completely or partially submerged in the wastewater. The continuously rotating shaft enables the formation of biofilm by microorganisms colonizing the discs and subsequently degrading organic matter (Hassard, 2015) (Cortez, 2008).

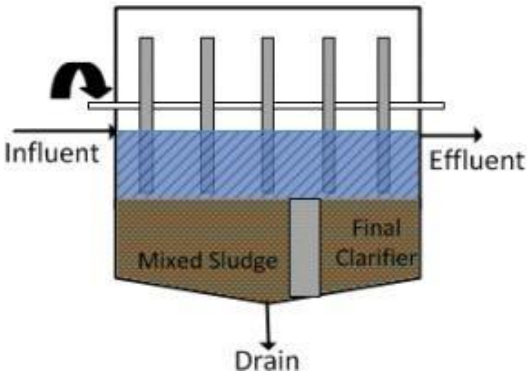


Figure 11: RBC system (Hassard, 2015)

Anaerobic Biological Treatment Fermenter

a. Packed bed reactors: Also known as anaerobic filter consists of a fixed matrix (made of reticulated foam polymers, granite, quartz, stones, sand, plastics and granular activated carbon which provides a surface of attachment that supports the growth of a biofilm of anaerobic microorganisms. Dissolved pollutants are absorbed by the biofilm as upward flow of wastewater occurs (Afandizadeh, 2001). It can be used to remove dye from textile industrial effluent (Afandizadeh, 2001) and also for the recovery of metals from wastewater (Obanijesu, 2004).

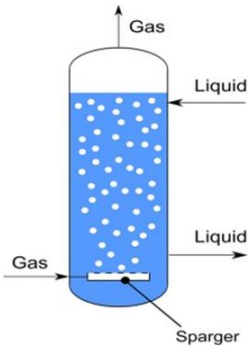


Figure 12: Packed bed fermenter (Ali, 2018)

b. UASB: Upflow anaerobic sludge blanket reactor (UASB) allows the upward flow of wastewater through an activated sludge bed in the lower part of the reactor and upper part consists of three-phase i.e, solid, liquid and gas separation system which allows biogas collection (Lettinga, 1991) (Chong, 2012)

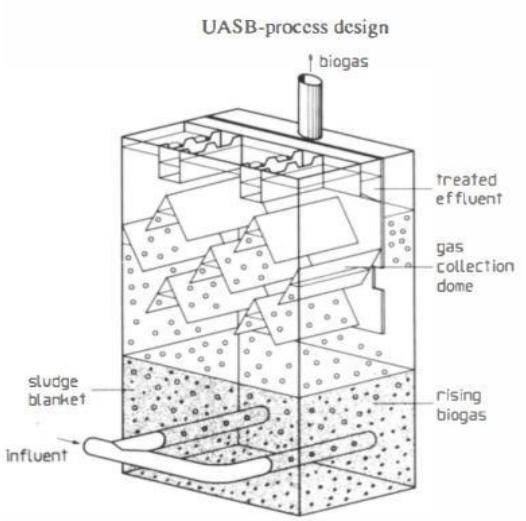


Figure 13: UASB reactor (Lettinga, 1991)

c. **Anaerobic baffled digestor:** It is an advanced version of an anaerobic RBC bioreactor, the wastewater passes over and under a series of staggered vertical baffles as it flows from inlet to outlet (Barber, 1999). It is shown to remove a large percentage of oil (Hassan S. &., 2013)and greatly reduce the COD of the wastewater.

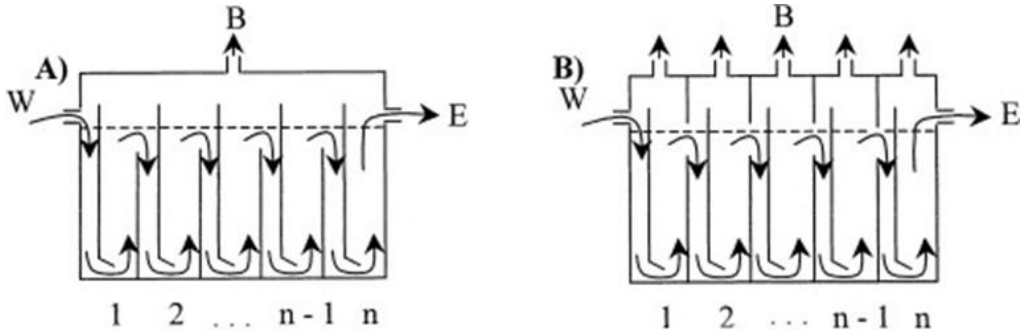


Figure 14: Anaerobic baffled digestor (Barber, 1999)

d. **Contact Digestor:** Functioning similar to activated sludge process, consist of a tank and stirred tank under anaerobic conditions. The stirred tank digester output is settled and a part of this is returned to the digester allowing longer retention time for methanogenic organisms and concentration of sludge (Bertin, 2004).

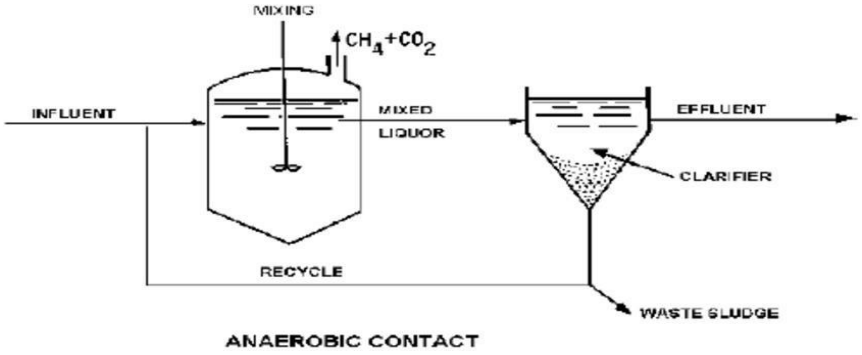
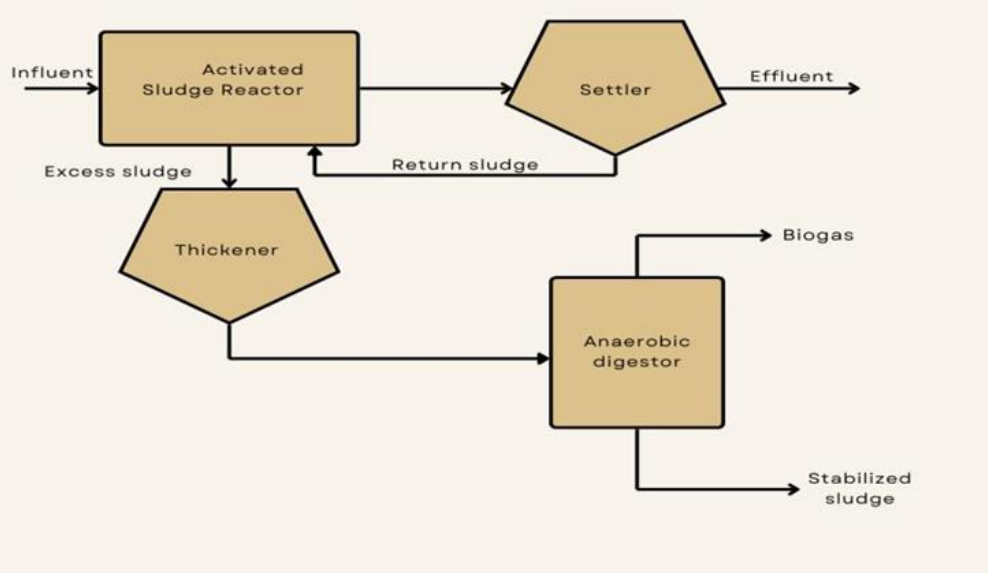


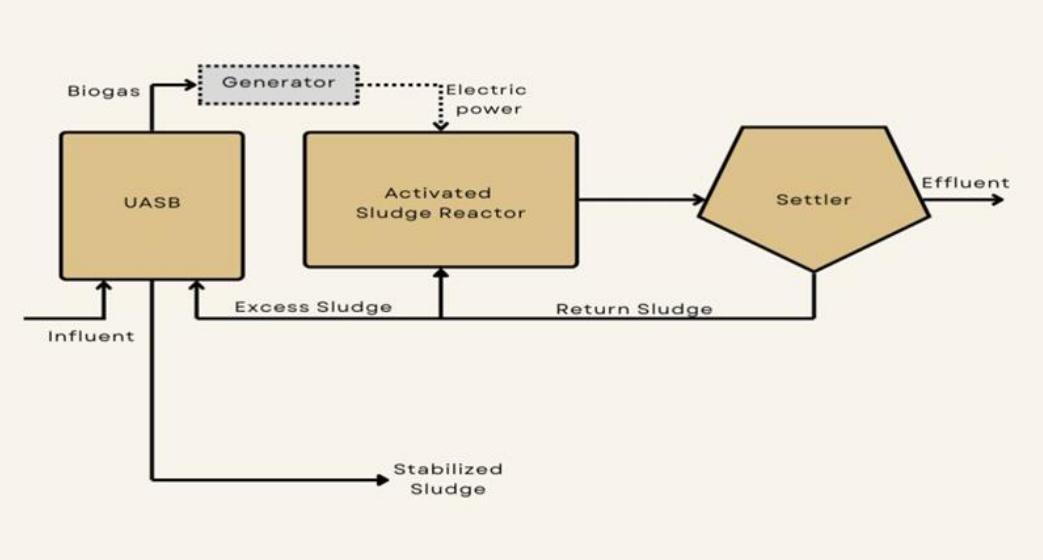
Figure 15: Contact digestor (Hassan S. R., 2013)

Wastewater Treatment Run

A) Aerobic Wastewater Treatment



B) Anaerobic Wastewater Treatment



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A REVIEW OF MEDICINAL PLANTS IMPORTANCE APPLICATIONS

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Abstract:

Medicinal plants have played a crucial role in traditional healing practices across various cultures. These plants are rich in bioactive compounds that offer a wide range of therapeutic benefits, including antimicrobial, anti-inflammatory, antioxidant, anticancer, and pain-relieving effects. As interest in natural therapies continues to rise, there has been a significant push for scientific exploration to validate the medicinal properties of plants. Modern pharmacological research is increasingly focusing on identifying and understanding the molecular mechanisms behind the effectiveness of these plants, with numerous plant-derived compounds being investigated for their potential in pharmaceutical development. However, the use of medicinal plants presents several challenges, including the inconsistency in plant composition, concerns regarding safety, and the lack of standardized methods for extraction and preparation. Additionally, the sustainability of plant resources is a growing concern, as overexploitation and habitat loss pose a threat to many species used for medicinal purposes. Progress in fields like biotechnology, ethnobotany, and clinical trials is helping to integrate traditional knowledge with modern scientific techniques, ensuring a more evidence-based and sustainable approach to plant-based therapies.This abstract underscores the growing significance of medicinal plants in modern medicine, the necessity for thorough scientific research, and the need to balance traditional wisdom with contemporary technological advancements to advance the use of natural health products.

Keywords: Medicinal Plants, Therapeutic Uses, Export, Import, Pharmaceutical Industries

Introduction:

Medicinal plants play an essential role in both environmental health and human well-being. Their significance extends beyond providing therapeutic benefits to human health; they also offer critical ecological advantages, such as maintaining biodiversity, enhancing ecosystem services, and promoting sustainable development. These plants have

been central to many traditional healing practices and are increasingly contributing to modern pharmacology as sources of bioactive compounds used in drug development.[1]

1. Medicinal Plants and Their Environmental Importance

Medicinal plants provide substantial ecological benefits that contribute to environmental sustainability, including:

- **Biodiversity Conservation**: Medicinal plants are vital for preserving genetic diversity in ecosystems. Many of these plants are endemic to specific regions, meaning they are found only in particular habitats. Conserving these species is crucial for maintaining ecosystem health and biodiversity.[2]
- **Soil Erosion Control**: Certain medicinal plants, like varieties of mint and ginger, help stabilize soil with their root systems. These plants are particularly important in preventing soil erosion in areas prone to degradation, such as hillsides or riverbanks.
- **Habitat for Wildlife**: Medicinal plants provide essential habitat and food for wildlife, including insects, birds, and small mammals. By supporting pollinators and other species, they help maintain ecological balance and biodiversity.
- **Regulation of Climate**: Medicinal plants contribute to climate regulation by absorbing carbon dioxide and influencing local microclimates. Forests rich in medicinal plant species help stabilize temperature and humidity levels, creating a favorable environment for surrounding plant and animal species.
- **Water Conservation**: Some medicinal plants, such as aloe vera and agave, are drought-resistant and can thrive in arid conditions. These plants help conserve water by requiring minimal irrigation and contribute to maintaining the water cycle in ecosystems.

2. medicinal Uses of Plants

Medicinal plants have been used for thousands of years to treat various ailments, offering a wide range of therapeutic benefits. Their bioactive compounds, including alkaloids, flavonoids, terpenoids, glycosides, and phenolic acids, possess distinct medicinal properties. Below are some categories of medicinal uses:[3]

A. Antimicrobial and Antiviral Properties

- **Garlic (*Allium sativum*)**: Known for its powerful antibacterial and antiviral properties, garlic is used to treat infections, including the common cold, respiratory infections, and food poisoning. Allicin, the active compound in garlic, plays a key role in its antimicrobial effects.

- **Echinacea**: Commonly used to boost the immune system and reduce the duration and severity of cold symptoms. Echinacea stimulates immune function and has anti-inflammatory effects.

B. Anti-inflammatory and Pain-relieving Properties

- **Turmeric (Curcuma longa)**: The active compound curcumin in turmeric has potent anti-inflammatory effects. It is widely used to treat conditions like arthritis, inflammatory bowel disease, and general pain.
- **Ginger (Zingiber officinale)**: Ginger contains compounds called gingerols, which provide natural pain relief and have anti-inflammatory properties, making it effective for conditions like osteoarthritis.

C. Antioxidant Effects

- **Green Tea (Camellia sinensis)**: Rich in polyphenols, especially epigallocatechin gallate (EGCG), green tea has strong antioxidant properties that neutralize harmful free radicals in the body. It is linked to preventing chronic diseases such as heart disease, cancer, and age-related degeneration.
- **Acai Berry (Euterpe oleracea)**: Acai berries are rich in anthocyanins, which offer antioxidant benefits that protect cells from oxidative stress, support heart health, and reduce inflammation.

D. Cardiovascular Benefits

- **Hawthorn (Crataegus spp.)**: Used to support heart health, hawthorn helps improve circulation, treat mild heart failure, and lower blood pressure. Its flavonoids enhance blood vessel function.
- **Ginseng (Panax ginseng)**: Ginseng has adaptogenic properties that help the body manage stress, improve circulation, and lower cholesterol, contributing to overall heart health.

E. Anti-cancer Properties

- **Ashwagandha (Withania somnifera)**: Known as an adaptogen, ashwagandha helps the body cope with stress and has shown anticancer properties by inhibiting tumor growth and enhancing immune health.
- **Neem (Azadirachta indica)**: Neem has demonstrated anti-cancer properties, with studies indicating that it may inhibit the growth of cancer cells and protect against the side effects of chemotherapy.

F. Digestive Health

- **Peppermint (Mentha piperita)**: Peppermint has soothing effects on the digestive system, helping relieve symptoms of irritable bowel syndrome (IBS), indigestion, and nausea. Its carminative properties ease bloating and gas.
- **Fennel (Foeniculum vulgare)**: Traditionally used to support digestion, fennel helps relieve bloating, cramps, and indigestion and contains fiber that promotes gut health.

G. Skin Health

- **Aloe Vera (Aloe barbadensis)**: Aloe vera is used for its cooling and healing effects on the skin, treating burns, wounds, acne, and other skin irritations due to its moisturizing and anti-inflammatory properties.
- **Calendula (Calendula officinalis)**: Known for its anti-inflammatory, antifungal, and antibacterial properties, calendula is often applied topically to heal skin wounds, rashes, and ulcers.

H. Mental Health and Stress Reduction

- **Lavender (Lavandula angustifolia)**: Lavender is recognized for its calming effects, commonly used in aromatherapy to reduce anxiety, stress, and promote sleep.
- **Chamomile (Matricaria chamomilla)**: Chamomile is widely known for its mild sedative effects, helping to alleviate anxiety and improve sleep, making it ideal for those experiencing stress or insomnia.[4]

3. The Challenge of Sustainable Use and Conservation of Medicinal Plants

Despite their many benefits, the use of medicinal plants faces several challenges:

- **Overharvesting**: Increased demand for medicinal plants in global markets leads to the overexploitation of certain species, putting them at risk of extinction. For example, ginseng and certain orchids are vulnerable due to unsustainable harvesting practices.
- **Loss of Habitat**: Deforestation, agricultural expansion, and urbanization threaten the natural habitats of many medicinal plants, making them susceptible to environmental changes. This loss of habitat reduces the availability of these plants and further endangers them.
- **Conservation Efforts**: Efforts are underway to conserve medicinal plant species through sustainable farming practices, cultivation, and the establishment of plant reserves. Organizations are promoting sustainable harvesting methods and cultivating rare species to ensure that medicinal plants are available for future generations.[5]

Medicinal plants are an invaluable resource, contributing significantly to both environmental health and human wellness. They offer a diverse range of therapeutic benefits, from antimicrobial and anti-inflammatory effects to supporting cardiovascular health and cancer prevention. However, it is essential to balance their medicinal potential with the need for conservation and sustainable practices. Protecting these plants through research, sustainable harvesting, and conservation efforts is critical to ensuring that they continue to benefit future generations and contribute to global health. By integrating traditional knowledge with modern scientific advancements, we can safeguard medicinal plants and unlock their full potential for promoting health worldwide.

The global trade of medicinal plants has become an increasingly important industry, driven by the rising demand for natural products across the pharmaceutical, wellness, and beauty sectors. These plants are vital to the production of herbal medicines, dietary supplements, essential oils, and other health-related products. The international exchange of medicinal plants not only contributes to the economic development of exporting and importing nations but also fosters the growth of traditional and modern medicine.[6]

Global Export and Import of Medicinal Plants

1. Major Exporters of Medicinal Plants

A number of countries around the world are major exporters of medicinal plants due to favorable climates, rich biodiversity, and strong traditions in herbal medicine.

- **China**: As one of the largest exporters globally, China supplies a wide range of plants for traditional Chinese medicine (TCM) and international markets. Popular exports include ginseng, licorice, and ginger. China plays a key role in the global trade of medicinal plants, with this sector being integral to its agricultural economy.[7]
- **India**: India is another significant exporter, owing to its diverse medicinal plant species and its deep tradition in Ayurvedic medicine. Commonly exported plants from India include turmeric, ashwagandha, aloe vera, and neem. Indian herbal products are highly sought after in markets across Europe, North America, and Asia.
- **Indonesia**: With its tropical biodiversity, Indonesia is a major exporter of plants such as ginger, turmeric, and pandan leaves, which are used both in traditional medicine and modern herbal products.
- **Brazil**: Known for its extensive rainforests, Brazil exports medicinal plants used in Amazonian medicine. Notable exports include acai berries, guarana, and copaiba oil, which are prized for their antioxidant and energizing properties.

- **Thailand**: Thailand contributes significantly to the medicinal plant trade with exports such as lemongrass, turmeric, and pandan leaves, which are popular both locally and internationally in wellness markets.

2. Major Importers of Medicinal Plants

The demand for medicinal plants spans across many countries, with some nations acting as key importers for the production of herbal products and pharmaceuticals.[8]

- **United States**: The U.S. is one of the largest importers of medicinal plants, fueled by increasing demand for herbal supplements, organic products, and natural remedies. Common imports include ginseng, turmeric, ginger, and echinacea. The U.S. imports medicinal plants from countries like China, India, and South Africa for both traditional and modern medicine applications.
- **Germany**: With a long-standing tradition in herbal medicine, Germany is a significant importer of medicinal plants. Popular imports include chamomile, valerian, and ginseng, which are used in teas, supplements, and essential oils.
- **France**: France imports a variety of medicinal plants for use in herbal medicine, teas, and cosmetics. Key imports include lavender, peppermint, and echinacea, which are in high demand for wellness and beauty products.
- **Japan**: Japan imports a substantial amount of medicinal plants, especially for Kampo (traditional Japanese medicine). Popular imports include ginseng, ginger, and licorice, which are also increasingly used in wellness and beauty products.
- **United Kingdom**: The UK is a major importer of medicinal plants for alternative medicine, herbal teas, essential oils, and supplements. Popular imports include valerian, ginseng, and turmeric.
- **South Korea**: South Korea imports medicinal plants, particularly for use in traditional Korean medicine (KTM). Ginseng, ginger, and ginkgo are highly sought after in the country's growing health and wellness sectors.

3. Challenges in the Global Trade of Medicinal Plants

While the medicinal plant trade offers significant economic opportunities, there are several challenges:

- **Overharvesting**: High demand for medicinal plants has led to overexploitation, especially for species like ginseng and certain orchids, which risks depleting natural populations.

- **Sustainability**: The sustainability of the medicinal plant trade is a major issue, as excessive harvesting without adequate regeneration can lead to species depletion. Countries are increasingly focusing on cultivation and domestication as alternatives to wild harvesting.[9]
- **Regulatory Issues**: Inconsistent regulatory standards across countries create challenges. In some regions, the lack of proper oversight allows for illegal trading of endangered species or the entry of low-quality products into international markets.
- **Climate Change**: Climate change poses a threat to the availability of many medicinal plants, particularly those dependent on specific climates or geographic regions. Changes in temperature, rainfall, and environmental conditions may disrupt the availability and distribution of these plants.[10]

4. Trends in Medicinal Plant Trade

Several emerging trends are shaping the global medicinal plant market:

- **Growing Demand for Natural Products**: The global demand for natural and plant-based products continues to rise, driven by consumer preferences for wellness, beauty, and holistic health solutions. This trend is increasing the global demand for medicinal plants.
- **Sustainable and Ethical Sourcing**: There is a growing focus on sustainable sourcing practices within the medicinal plant industry. Many companies and consumers are prioritizing organic or fair-trade-certified plants to ensure environmental sustainability and social responsibility.
- **Integration with Modern Medicine**: Medicinal plants are becoming more widely studied for their therapeutic potential and are increasingly integrated into pharmaceutical development. This trend is creating new opportunities for exports, especially in research-driven markets.
- **E-commerce Growth**: The rise of e-commerce has boosted the global trade of medicinal plants, enabling companies to access plant-based products and consumers to purchase herbal supplements and remedies from international sources.

The medicinal plant formulations industry, a key part of the broader herbal medicine and natural products sector, has seen significant growth. Between 2024 and 2030, the global herbal medicine market is projected to grow at a CAGR of 7-9%, driven by increasing consumer preference for natural remedies, awareness of plant-based health

benefits, and the growing popularity of alternative medicine, particularly in developed countries.[11]

Markets and Regions

- **Asia Pacific**: India, China, Japan, and South Korea lead the market, with traditions in Ayurveda and Traditional Chinese Medicine. These countries serve both domestic and international markets.
- **North America**: The U.S. and Canada are seeing rising demand for herbal products, with manufacturers producing a variety of supplements, essential oils, and natural cosmetics.
- **Europe**: Countries like Germany, France, and the U.K. are major players, where consumers demand high-quality herbal products that meet strict regulatory standards.
- **Latin America**: Brazil, Argentina, and Mexico are emerging markets with rich biodiversity and growing interest in medicinal plants.
- **Africa**: African countries, with a history of traditional medicine, are increasing production and export of medicinal plant-based products.

Notable Global Companies

- **Himalaya Drug Company** (India): Focuses on Ayurvedic products, including supplements and personal care.
- **Blackmores** (Australia): Known for herbal supplements and natural remedies.
- **Nature's Way** (USA): Specializes in herbal supplements and wellness products.
- **Weleda** (Germany): Offers holistic plant-based wellness and beauty products.
- **Traditional Medicinals** (USA): Renowned for herbal teas and wellness products.
- **Dabur** (India): A leader in Ayurvedic medicine and health supplements.
- **Schwabe Pharmaceuticals** (Germany): A major player in plant-based pharmaceuticals.
- **Nature's Sunshine Products** (USA): Focuses on herbal supplements and preventive care.
- **Arjuna Natural Ltd.** (India): Specializes in herbal extracts for pharmaceuticals and nutraceuticals.
- **Organic India** (India): Known for organic herbal teas and supplements with a focus on sustainability.

Emerging Trends

- **Sustainability**: Companies are focusing on ethical sourcing and eco-friendly practices.
- **Standardization**: Ensuring consistency in herbal formulations, especially in regions like Europe and North America.

- **Research and Innovation**: Increased investment in clinical trials to prove the benefits of medicinal plants.
- **Adaptogens and Stress Management**: Herbs like ashwagandha and ginseng are gaining popularity for their stress-relieving properties.
- **Personalized Wellness**: Customizing herbal remedies based on individual health needs.

Challenges [12]

- **Regulatory Hurdles**: Navigating differing regulations across countries is complex.
- **Quality Assurance**: Ensuring product purity and consistency remains a key challenge.
- **Market Competition**: The rising popularity of herbal products intensifies competition.
- **Supply Chain Issues**: Climate change and environmental factors impact the sustainable sourcing of plants.

The medicinal plant formulations industry offers significant growth potential but requires careful management of quality, regulations, and sustainability for long-term success.

The trade in medicinal plants plays a vital role in the global economy, supporting both the pharmaceutical and wellness industries. Countries such as China, India, and Brazil are major exporters, while developed nations like the U.S., Germany, and Japan are leading importers. However, sustainable practices and conservation efforts are critical to ensuring that the benefits of the medicinal plant trade continue for future generations. With a focus on ethical sourcing, cultivation, and scientific research, the industry can thrive while maintaining biodiversity and promoting human health globally.

Medicinal plants have been utilized for centuries to treat various health conditions, and their cultivation remains a key component of the global health and wellness sector. The processes of collecting and cultivating these plants differ by region, influenced by local climate, culture, traditional knowledge, and market demand. Here's an overview of the key aspects related to the collection and cultivation of medicinal plants worldwide:

1. Global Significance of Medicinal Plants

Medicinal plants are a cornerstone of global healthcare systems, particularly in traditional medicine systems like Ayurveda in India, Traditional Chinese Medicine (TCM) in China, and various indigenous healing practices worldwide. Beyond traditional uses, many modern pharmaceutical drugs are derived from these plants, contributing significantly to the global market value of herbal medicine.

2. Collection of Medicinal Plants

The collection of medicinal plants refers to their harvesting for medicinal purposes. There are two main methods:

- **Wild Harvesting**: Many plants are still gathered from natural habitats, especially in biodiversity-rich regions. This method is typical for plants used in traditional remedies.
- **Cultivation**: To prevent overharvesting and ensure a steady supply, many medicinal plants are now cultivated. This method helps control the quality and quantity of the plants.[13]

3. Cultivation of Medicinal Plants

Cultivating medicinal plants involves specific agricultural practices tailored to the growth needs of medicinal species. Key factors include:

a. Climate and Soil Conditions

Medicinal plants require distinct climates and soil types:

- **Tropical and Subtropical Plants**: Herbs like turmeric, ginger, and moringa prefer warm climates with well-drained, fertile soil.
- **Temperate Plants**: Varieties like ginseng, echinacea, and valerian are suited to cooler, temperate environments with moist soil.
- **Altitude-Specific Plants**: Certain plants, such as high-altitude medicinal species, demand specialized conditions for optimal growth.

b. Soil Preparation and Planting Techniques

- **Soil Fertility**: Medicinal plant cultivation demands rich, well-drained soil. Organic farming methods are commonly used to avoid synthetic chemicals.
- **Propagation Methods**: Different plants require different propagation techniques, such as seeds for ginseng or cuttings for lavender.

c. Crop Management

- **Watering and Irrigation**: Regular watering is critical, but excessive moisture should be avoided to prevent root diseases. Drip irrigation is often used for even water distribution.
- **Pest and Disease Management**: To maintain plant quality, organic pest control methods are typically used, such as neem oil and other natural pesticides.

4. Global Trends in Medicinal Plant Cultivation

The global demand for medicinal plants has surged, influenced by alternative medicine trends, herbal products, and wellness-focused consumption. Key trends include:

- **Increased Market Demand**: The popularity of herbal medicines and supplements, especially in the food and beverage sectors, has led to higher demand for medicinal plants.
- **Sustainability and Conservation**: To combat overharvesting, sustainable farming practices like crop rotation and organic cultivation are increasingly adopted.
- **Investment from Pharmaceutical and Nutraceutical Industries**: Large companies are expanding their investment in the cultivation of medicinal plants for both therapeutic and commercial use.[14]

5. Regional Approaches to Medicinal Plant Cultivation

a. Asia

- **India**: Known for its deep connection with Ayurvedic practices, India cultivates plants like turmeric, tulsi (holy basil), and ashwagandha. The country is also working on cultivating and conserving herbs traditionally collected from the wild.
- **China**: TCM relies heavily on medicinal plants, such as ginseng, ginger, and astragalus. China leads large-scale cultivation to meet domestic and global demands.
- **Southeast Asia**: Countries like Indonesia and Thailand cultivate plants like pandanus, lemongrass, and ginger, often for both local consumption and international export.

b. Africa

- **Sub-Saharan Africa**: This region boasts a wealth of medicinal plants, such as moringa, baobab, and rooibos, often used in treating various ailments. Though wild harvesting is still common, efforts are growing to cultivate these plants sustainably for export markets.
- **South Africa**: Known for indigenous medicinal species like buchu and devil's claw, South Africa is prioritizing sustainable cultivation to support the global herbal medicine market.

c. Europe and North America

- **United States**: The U.S. has a growing herbal industry, particularly in states like California, cultivating plants like echinacea, valerian, and peppermint using organic and sustainable methods.
- **Europe**: Countries such as Germany and France have long traditions of phytotherapy and cultivate plants like St. John's Wort, lavender, and chamomile. The EU enforces strict regulations to ensure the quality and safety of these plants.[15]

6. Challenges in Medicinal Plant Cultivation

Several factors pose challenges to the cultivation of medicinal plants:

- **Overharvesting**: The increasing demand for certain plants leads to the depletion of wild populations.
- **Climate Change**: Changing weather patterns can impact the growth of plants, with some species struggling to adapt to new temperature and moisture conditions.
- **Market Volatility**: Prices for medicinal plants can fluctuate due to supply issues, pest problems, or changing consumer preferences.
- **Regulatory Differences**: Varied regulations on the cultivation and trade of medicinal plants across countries complicate international trade.

7. Sustainable Practices and Future Trends

To ensure the continued availability of medicinal plants, several initiatives are focused on sustainability:

- **Conservation Initiatives**: Governments and organizations are working to promote sustainable cultivation through certifications, biodiversity conservation, and the establishment of seed banks.
- **Research and Development**: Ongoing research into the properties of medicinal plants has led to innovations in cultivation techniques, the development of improved plant varieties, and the integration of these plants into modern medicine.
- **Agroforestry**: Incorporating medicinal plants into agroforestry systems, where they are grown alongside trees and other crops, offers a sustainable solution that supports biodiversity and provides farmers with diversified income.[16]

The cultivation and collection of medicinal plants is an intricate process that is crucial to global healthcare. The increasing demand for these plants, driven by both traditional practices and modern wellness trends, requires careful attention to sustainable practices, biodiversity conservation, and innovation in agricultural techniques. Ensuring the health of ecosystems and the availability of medicinal plants will be essential in meeting future demands while safeguarding these invaluable resources.

Conclusion and The Future of Medicinal Plants

The future of medicinal plants is influenced by several dynamic factors, including the increasing global demand for natural products, climate change, biodiversity loss, and technological advancements. As society becomes more focused on plant-based solutions for health, wellness, and pharmaceutical applications, the role of medicinal plants is expected

to expand significantly. However, challenges such as sustainability, conservation, and environmental changes must be addressed. Below is an in-depth exploration of the critical factors shaping the future of medicinal plants.

1. Increasing Demand for Natural Remedies

The shift toward natural and alternative medicine is a defining trend in recent years. As people become more health-conscious, many are turning to herbal remedies, dietary supplements, and plant-based pharmaceutical solutions. This shift is driven by several factors:

- **Growing awareness of plant-based medicine**: As wellness culture rises, many seek alternatives to conventional pharmaceuticals due to concerns about side effects and the desire for holistic health solutions.
- **Cultural practices and traditional systems**: Healing systems such as Ayurveda, Traditional Chinese Medicine (TCM), and indigenous medicine continue to gain global recognition, further increasing the demand for plants associated with these traditions.[17]

As both traditional and modern healthcare systems increasingly rely on medicinal plants, their role is expected to expand, with new opportunities for growth in global health markets.

2. Sustainability and Conservation Challenges

While demand for medicinal plants grows, so too do the challenges related to sustainability and conservation. Overharvesting of wild plant populations, coupled with habitat loss, threatens many valuable species. Key concerns include:

- **Biodiversity loss**: As specific medicinal plants are harvested at unsustainable rates, wild populations face depletion, threatening entire ecosystems and pushing some species toward endangerment.
- **Sustainable harvesting practices**: To prevent further depletion, large-scale cultivation of medicinal plants is becoming increasingly important. This shift to cultivated sources helps to protect wild populations from overexploitation.
- **Conservation efforts**: Governments, NGOs, and businesses are increasingly focused on conservation initiatives that promote the cultivation of endangered species and establish protected areas to preserve biodiversity.

Sustainable cultivation and conservation practices will play a pivotal role in securing the future supply of medicinal plants, and the establishment of global guidelines for sustainable harvesting is essential.

3. Impact of Climate Change

Climate change poses a significant threat to the cultivation of medicinal plants. Variations in weather patterns, such as unpredictable rainfall, temperature extremes, and changing seasons, will affect plant growth. Key effects of climate change include:

- **Altered growing seasons**: Many medicinal plants require specific environmental conditions for optimal growth. Changes in temperature and rainfall patterns could reduce the productivity of certain species.
- **Geographical shifts**: As temperatures rise, some plants may no longer thrive in their current regions, while new areas may become suitable for cultivation. This could open opportunities but also disrupt current growing practices.
- **Pests and diseases**: Warmer climates can promote the spread of pests and plant diseases, further threatening the cultivation of medicinal plants. Addressing this will require sustainable pest control strategies and improved agricultural practices.

Adaptation strategies, such as the development of climate-resilient plant varieties and improved agricultural techniques, will be essential to mitigate the impact of climate change on medicinal plant cultivation.

4. Advances in Biotechnology and Plant Breeding

Biotechnology is expected to play a crucial role in shaping the future of medicinal plants. Key innovations include:

- **Genetic improvement**: Biotechnology enables the development of high-yield, disease-resistant, and climate-tolerant medicinal plants. Genetic modification and advanced breeding techniques can enhance the potency and chemical composition of plants, making them more effective for medicinal use.
- **Phytochemical analysis**: Advances in analytical tools, such as high-performance liquid chromatography (HPLC), allow scientists to better understand the chemical profiles of medicinal plants. This research could lead to the discovery of new bioactive compounds with therapeutic potential.
- **Tissue culture and propagation**: Plant tissue culture techniques enable the production of uniform, high-quality, disease-free plants in large quantities, ensuring a consistent supply for medicinal use.

As biotechnology advances, it will help secure a sustainable, high-quality supply of medicinal plants, addressing both the rising demand and the environmental challenges that accompany it.

5. Integration into Modern Healthcare Systems

While medicinal plants have long been central to traditional healthcare, their integration into modern medical practices will continue to increase. Several factors will drive this process:

- **Regulatory frameworks**: Establishing strong regulations for the cultivation, production, and distribution of herbal medicines is essential to ensure safety, quality, and efficacy. Many countries are strengthening their regulatory frameworks, and global harmonization of standards will be crucial for international trade.
- **Clinical research and validation**: Ongoing scientific research and clinical trials will help substantiate the therapeutic claims of medicinal plants. As the body of evidence grows, these plants are likely to be more widely accepted by healthcare professionals and integrated into mainstream medicine.
- **Collaboration between traditional and modern medicine**: An integrated approach that combines traditional knowledge with modern pharmacological science holds great promise. Partnerships between traditional healers and modern medical practitioners will bridge the gap between these systems, facilitating the development of effective treatments.

As medicinal plants gain greater acceptance in global healthcare, they will likely play a significant role in integrative medicine, combining time-tested healing practices with cutting-edge scientific advances.

6. Global Trade and Market Growth

The global market for medicinal plants is poised for significant growth. Factors contributing to this expansion include:

- **Rising demand for herbal products**: As consumers seek natural health alternatives, the market for herbal medicines, supplements, and essential oils is expanding. Additionally, plant-based beauty and wellness products are in high demand.
- **Global acceptance of traditional medicine**: There is growing recognition of the value of traditional healing systems, driving demand for medicinal plants from regions such as India, China, and Africa.

- **Export opportunities**: Countries with diverse ecosystems and rich biodiversity, such as India, China, and Brazil, have the potential to become major exporters of medicinal plants. This can create economic opportunities, but it also requires the implementation of sustainable practices to ensure long-term viability.

Global trade in medicinal plants will continue to increase, but it will depend on efficient supply chains, sustainability certifications, and adherence to international standards to meet the growing demand while preserving plant species.

The future of medicinal plants is promising, but it comes with significant challenges. As demand for plant-based remedies continues to rise, sustainable cultivation, climate resilience, and the integration of modern scientific advancements will be essential. With ongoing research, innovation, and responsible stewardship, medicinal plants will remain a vital resource for global health. By blending traditional knowledge with modern science, medicinal plants can continue to contribute to healthcare and wellness, benefiting both human populations and the ecosystems that sustain them. Collaboration between governments, researchers, and the private sector will be critical in ensuring the long-term success of medicinal plant cultivation and conservation, meeting global health needs while safeguarding biodiversity.

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HEAVY METAL CONTAMINATION IN ENVIRONMENT AND HUMAN HEALTH - A PATHOLOGICAL AND BIOCHEMICAL APPROACH

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Abstract:

Heavy metals are well known environmental pollutants and they gets accumulated in the various organs of the body including humans along with other species. Many heavy metals occur naturally but very few are derived by different anthropogenic activities and leads to environmental pollution that may prove lethal to humans. Cadmium (Cd) causes long term health effects as they enter the body through inhalation and ingestion and its biological half-life in humans is 10-35 years. Cd causes injury to almost all the tissues and disturbs the antioxidant system in the body. Cd also alters the protein synthesis and also effects the glycogen concentration. The effects of Cd were studied in mice as they were administered an acute dose of Cd and their effects on different tissues were observed. Even the antioxidant status and the other biological parameters were also measured. Cd treated mice showed many pathological changes in the cells. The antioxidant enzymes were disturbed in the treated group but peroxidation in the cells was enhanced. Cd caused neurotoxicity, nephrotoxicity, hepatotoxicity, renal toxicity, cardiovascular toxicity and even hemotoxicity in humans as well as other species.

Keywords: Heavy Metals, Cadmium (Cd), Oxidative Stress, Toxicity, Antioxidant Enzymes and Histopathology.

Introduction:

Environmental pollution is enhancing day by day due to many anthropogenic and human activities. Pollution mainly is caused due to many heavy metal accumulations in air, water and soil that not only effects humans but also aquatic and terrestrial species. Heavy metals such as zinc, copper, manganese, iron are essential to the body in very small amounts as they play as important role in the biological mechanisms of the cell.

Cadmium is one heavy metal that is present in the environment due to its variety of uses in different processes, firstly as a painting and plating compound for about 100 years. Cd tends to present in the environments and gets piled up in the soil, then it enters into the plants and make its entry into the food chain (Chavez *et al.,* 2015). Many foods that mainly contribute to daily intake of Cd includes cereals and bread (34%), leafy vegetables like spinach (20%), potatoes (11%), stem and root vegetables (6%) and fruits (5%). When Cd reaches blood, it gets structured in various tissues such as kidneys, liver and skeleton (Nordberg and Nordberg 2022). Cd gets inhaled into body from workplace and non-occupational exposure is not at all enough to cause any harmful health effects in humans.

Cd has major impact on kidneys as the tubular region is the area of Cd deposition that leads to tubular damage (Akesson *et al.,* 2005). This may further impact the uptake of vitamin D by the kidney that may provoke osteoporosis which has deleterious effects on the bones (Kjellstrom 1992). Cd has incredible effect on the reproductive system, where this metal interferes in different aspects (Pizent *et al.,* 2012) as it disturbs cell adhesion in the testis that alter the migration of germ cells in the seminiferous epithelial cells. It also reduces testicular growth rate, hormone levels, sperm count and motility of the sperms along with the strong influence on the antioxidant status in the tissue (Thompson and Bannigan 2008).

The cardiovascular system also gets affected by Cd toxicity as it causes hypertension due to inhibition of endothelial nitric oxide synthase and elimination of acetylcholine-induced vascular relaxation (Gallagher and Meliker 2010). Even, the lungs are one of the main target organs for Cd intoxication as it leads to pulmonary fibrosis, obstructive lung disease that result in depressed lung function (Li *et al.,* 2022). Many studies also claim the effect of Cd on pancreatic β cells resulting in reduce insulin production (Edwards and Prozialeck 2009) and also effect the brain functions (Vijaya *et al.,* 2020).

The major mechanism by which Cd causes damage is oxidative damage by changing the Ca concentrations inside the cells. Cd doesn't directly produce reactive oxygen species (ROS) as it cannot undergo Fenton-like reactions, but on the other, it can replace redox-active metal ions and can inhibit the activity of many antioxidant enzymes (Liu *et al.,* 2009). Cd toxicity may cause cell death by apoptosis via various pathways like that of intrinsic one (Wang *et al.,* 2019) and it may enhance the inflammation markers like THF-α and NF-kB along with autophagy in the skin epidermal cells (Chiarelli and Roccheri 2012).

The aim of the present study was to study the effect of heavy metal, Cd on various organs of mice on the basis of biochemical and histological studies. This work was correlated to the outcome of cadmium intake in humans and other species.

Methodology

1. **Animals:** Albino mice (20-25g) were procured from Central Research Institute and were acclimatized in laboratory conditions. They were kept in translucent cages and were finally divided into 3 groups. All the mice were fed with standard mice feed and were given free access to R.O. water. This research was conducted after the approval of Institutional Animal Ethical Committee (Reg No. 107/99/CPCSEA/2013-03).
2. **Acquisition of Chemicals:** Cadmium chloride ($CdCl_2$) was purchased from S.D FINE CHEM LIMITED, Mumbai. Cadmium was given in the form of cadmium chloride that was dissolved in distilled water and directed to mice orally through gavage.
3. **Experimental Outline:** The animals were segregated into 3 groups with 6 mice in each group for the conduct of this study (Fig. 1).

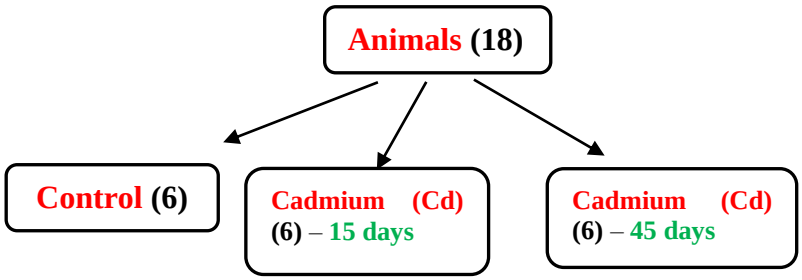


Fig. 1: Division of animals for the experiment.

Group I – Control mice, they were given normal feed and water.

Group II – Mice were administered with an acute dose of 6 mg/kg bw of cadmium (Cd) orally and were kept for 15 days.

Group III - Animals were subjected to Cd at a dose of 6 mg/kg bw orally for 1st day and were left on normal diet for 45 days.

4. **Tissue sample collection:** Animals were sacrificed after the completion of the experiment. Different tissues (Heart, Kidney, Liver, Lung, Pancreas and Testis) were removed, cleaned from adipose tissue and they were washed with cold water. Then were further processed for biochemical and histological analysis.

i. **Biochemical Estimations:** All the tissues were homogenized with 3 ml of phosphate buffer, centrifuged at 12,000 rpm for 20 min and the supernatant was collected and used for different biochemical studies.

1) **Measurement of Malondialdehyde (MDA):** Lipid peroxidation (LPO) in the tissues was evaluated according to Wilbur *et al* (1949) based on the concentration of malondialdehyde (MDA) which is the most important end-product of lipid peroxidation.
2) **Measurement of Superoxide Dismutase (SOD):** Superoxide dismutase was quantified by an indirect method that involves the scavenging of superoxide radicals by its enzyme. It was measured by a sensitive method of Das *et al* (2000).
3) **Measurement of Catalase (CAT):** Catalase is an enzymes that is present in all the organisms and helps to prevent oxidative stress in the body. The amount of this enzyme was analyzed by the method proposed by Aebi (1983).
4) **Measurement of Glycogen content:** Glucose is a source of energy to the body and it is stored in the form of glycogen in liver and muscles. The amount of glycogen present in different tissues was assessed by the method given by Montgomery (1957).
5) **Measurement of Cholesterol content:** Cholesterol is one of the important steroid that is present in the body. It can be estimated by the method of Zlatkis *et al* (1953).
6) **Measurement of Total Proteins:** The proteins were quantified by the method of Lowery *et al* (1951) that is one of the sensitive and most often used colorimetric methods.

ii) Histopathological study: Various tissues (Heart, Kidney, Liver, Lung, Pancreas and Testis) of both treated and control mice were cleaned, washed, cut into small pieces and were fixed in Bouin's fixative for 24 hours. Then the tissues were washed with 70%alcohol followed by various grades of alcohol, cleared in xylene and embedded in paraffin wax. They were sectioned at 5-6µm and stained with hematoxylin and eosin for pathological analysis that was studied with light microscopy.

Results:

Cadmium treatment results in lot of damage in different tissues of mice as it gets assimilated in the tissues and effects both biochemical and histological features. Cadmium significantly reduces body weight as compared to control mice (Fig. 2). The reduction was more at 45 days than at 15 days in toxic group.

The heavy metal treated group showed significant decrease in different organ weights in comparison to control group (Fig. 3). It also showed day's dependent decrease in Cd group.

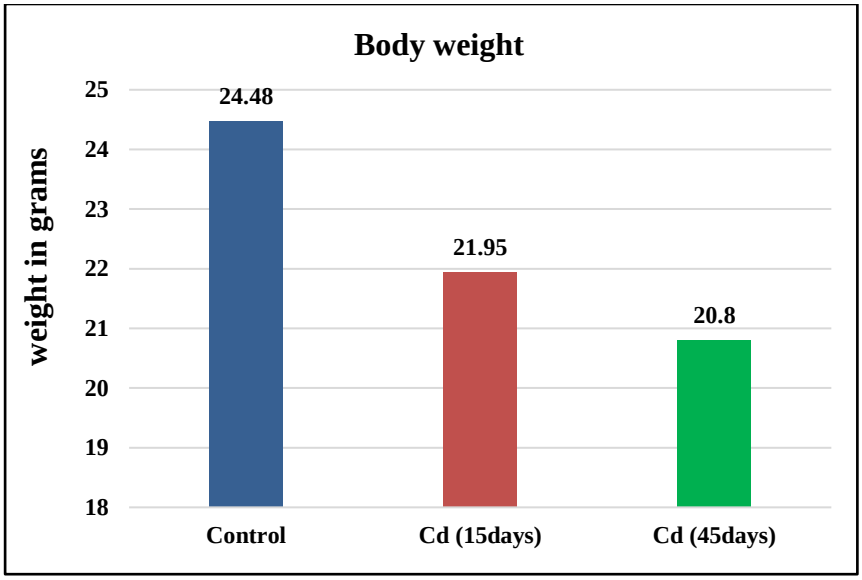


Fig.2: Body weight in all the groups during the experiment

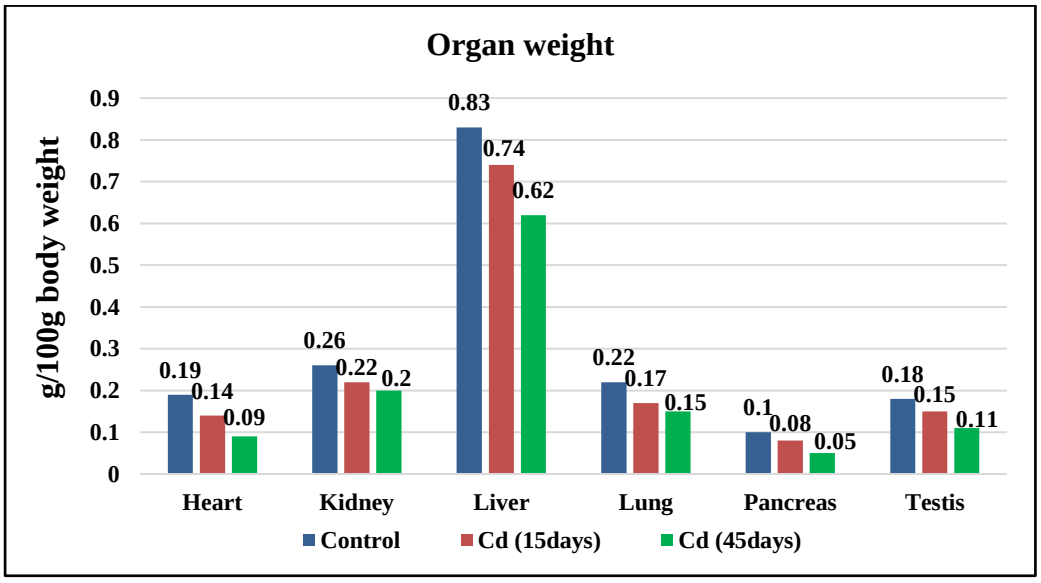


Fig. 3: Organ Weight (g/ 100g body weight) in control and treated groups.

Lipid peroxidation was one of the most parameters to check the damage caused by Cd. It was studied by quantifying MDA in different tissues that showed significant decrement in Cd treated groups as compared to control group (Fig.4). It may be due to oxidative damage caused by reactive oxygen species induced by heavy metal Cd.

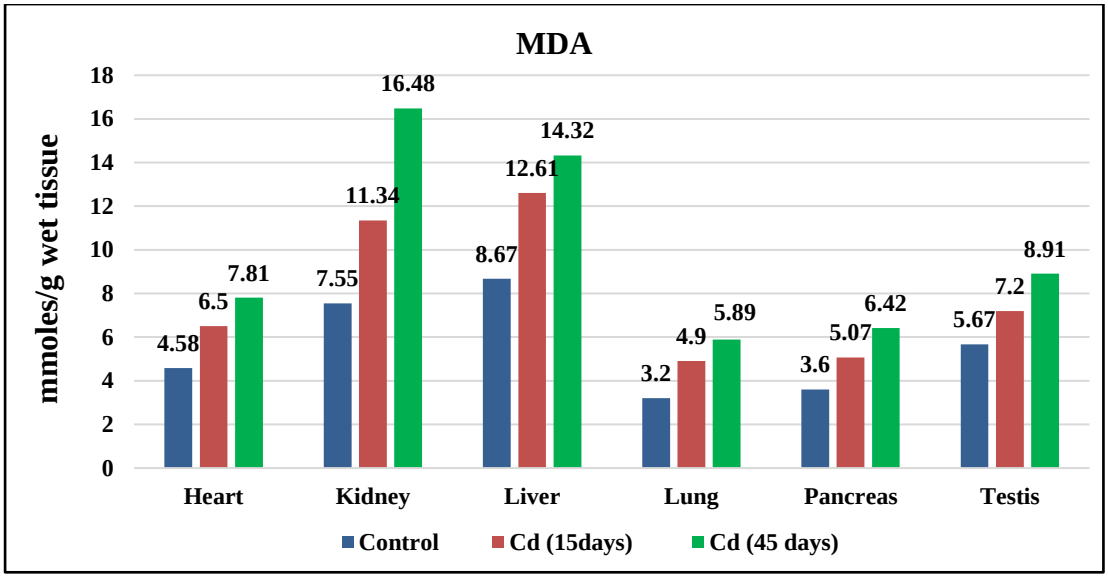


Fig. 4: MDA content (mmoles/g wet tissue) in different tissues of all the treated groups

Antioxidant enzymes like SOD (Fig. 5) and CAT (Fig. 6) are also affected by Cd toxicity. These enzymes were significantly decreased in Cd treated mice at 15 and 45 days in comparison to control mice. SOD is an enzyme that break down harmful oxygen molecules in the cells as Cd causes changes in the antioxidant status of a cell in almost all the tissues of the body. Catalase detoxifies hydrogen peroxide after breaking it into water and oxygen and this process is declined in toxic group in according to control values.

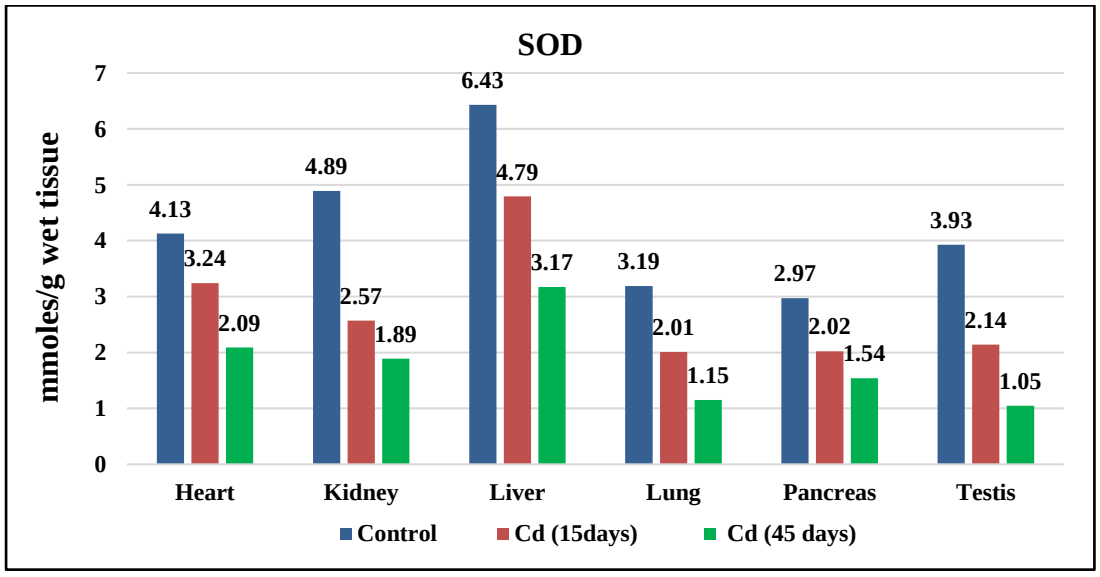


Fig. 5: SOD activity (mmoles/g wet tissue) in different tissues of all the treated groups.

The other biochemical parameters were also affected by Cd toxicity. The glycogen content is source of energy and this was also significantly reduced in Cd group as compared

to control group (Fig. 7). This decrease was seen in all the tissues of the mice and at both the treatment days i.e. 15 and 45 days.

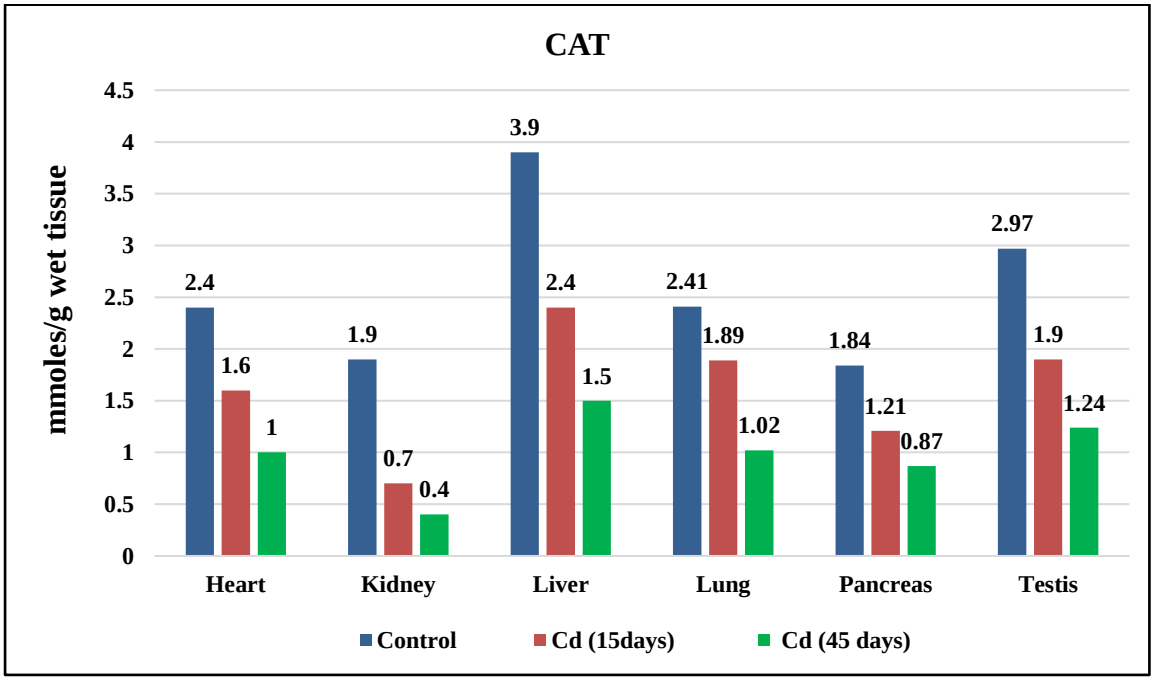


Fig.6: CAT activity (mmoles/g wet tissue) in different tissues of all the treated groups

Total cholesterol showed significant decrease in all the organs of toxic group as compared to control group (Fig. 8) but Cd showed increment in cholesterol level of liver tissue. Proteins are integral part of cell membranes and cells. The concentration of proteins significantly effects the biological functioning of the cells. The total proteins were also depleted in all the tissues of Cd treated groups in comparison to control (Fig. 9).

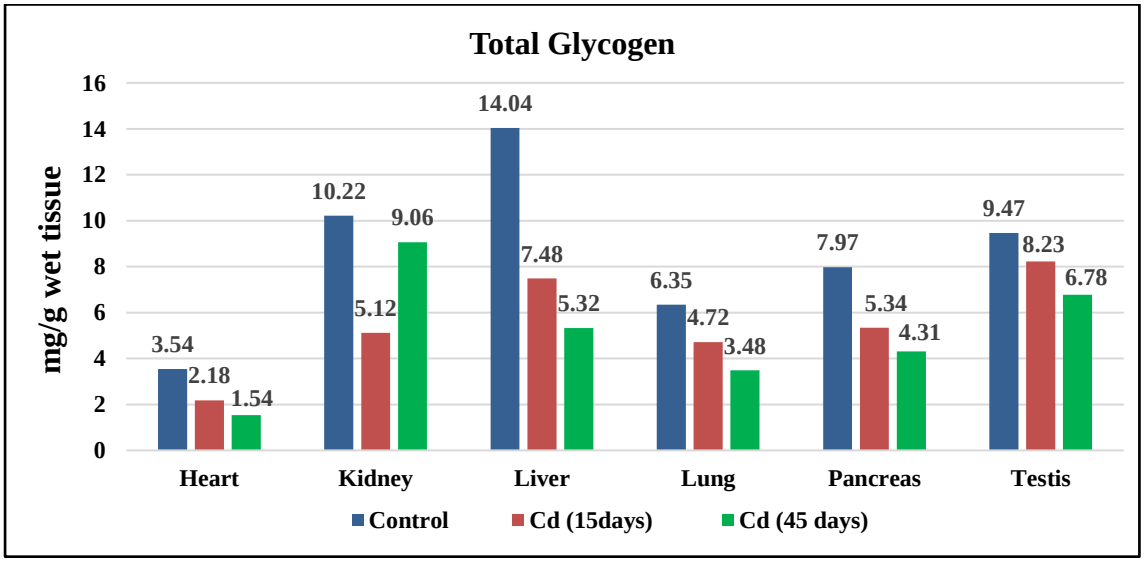


Fig. 7: Glycogen content (mg/g wet tissue) in different tissues of all the treated groups.

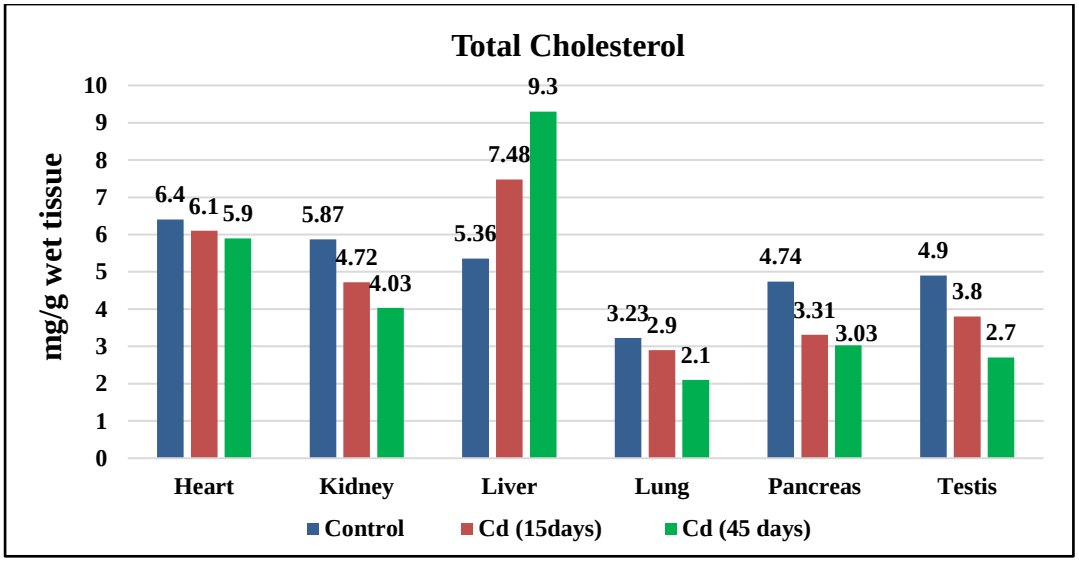


Fig. 8: Cholesterol content (mg/g wet tissue) in different tissues of all the treated groups

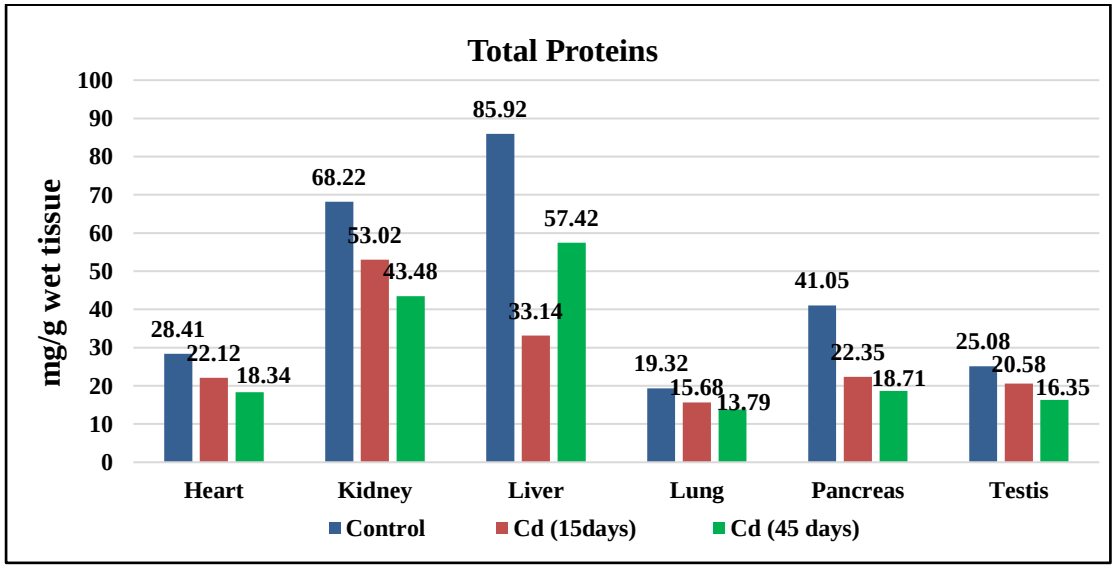
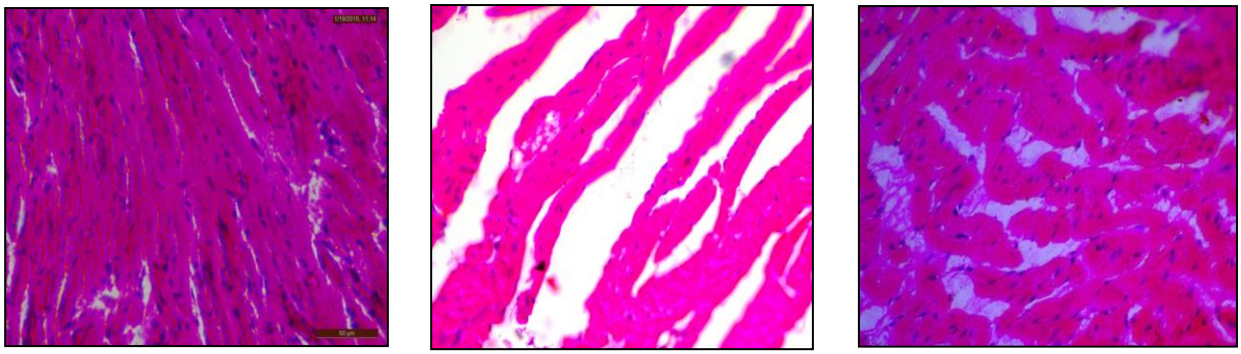


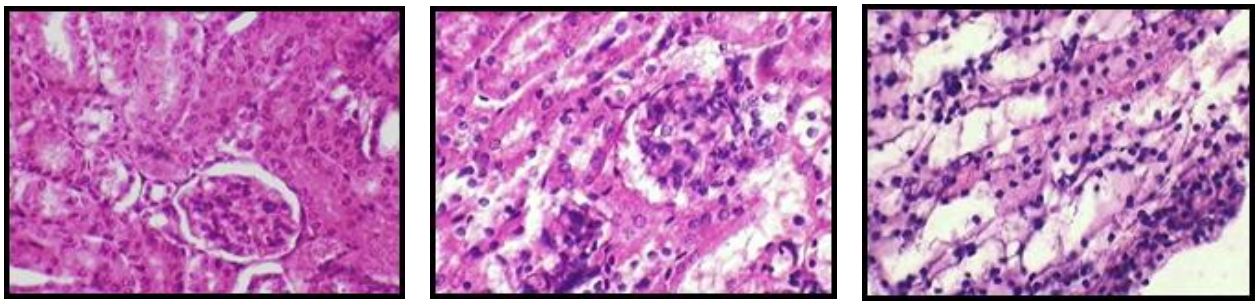
Fig. 9: Total Proteins (mg/g wet tissue) in different tissues of all the treated groups.

Histological observations of all the tissues (heart, kidney, liver, lung, pancreas and testis) showed hyperemia, degeneration of cell walls, binuclei and multinuclei cell formation along with vacuolation, lymphocytic infiltration etc. in Cd treated group (Figs. 10-15). Cd caused drastic pathological changes in all the tissues of mice which can be correlated with the effects in human beings.



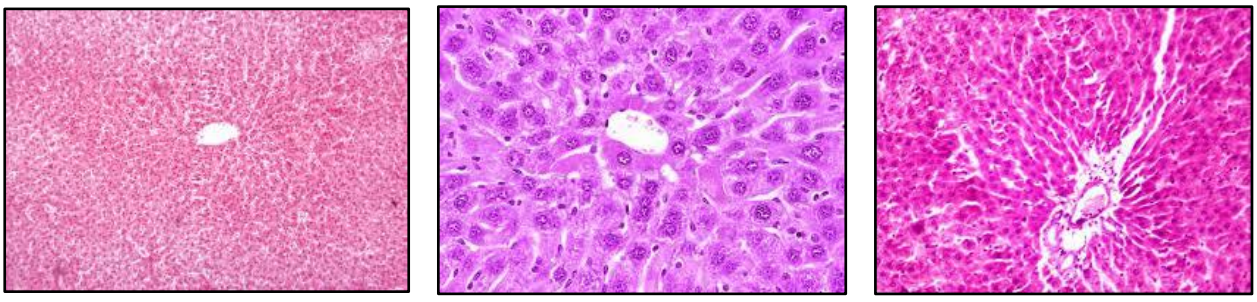
A **B** **C**

Fig. 10: Photomicrograph of heart tissue: A- Control, B-C – Cd treated heart tissue that showed vacuolar degeneration of cardiac myocytes, perivascular edema of cardiac muscle cells mild disorganization of myocardial fibers. X400



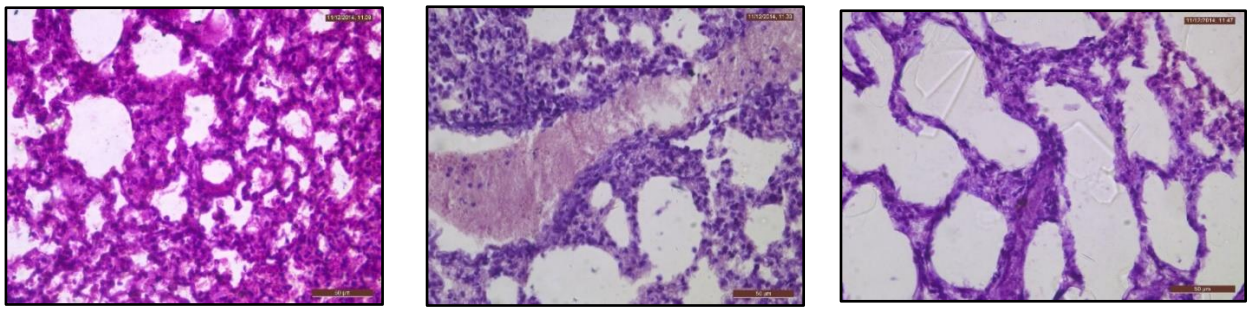
A **B** **C**

Fig. 11: Photomicrograph of kidney tissue: A- Control, B-C – Cd treated kidney tissue that showed degeneration of cellular wall and cytoplasm, binuclear cell formation, hyperemia and vacuolation. X400



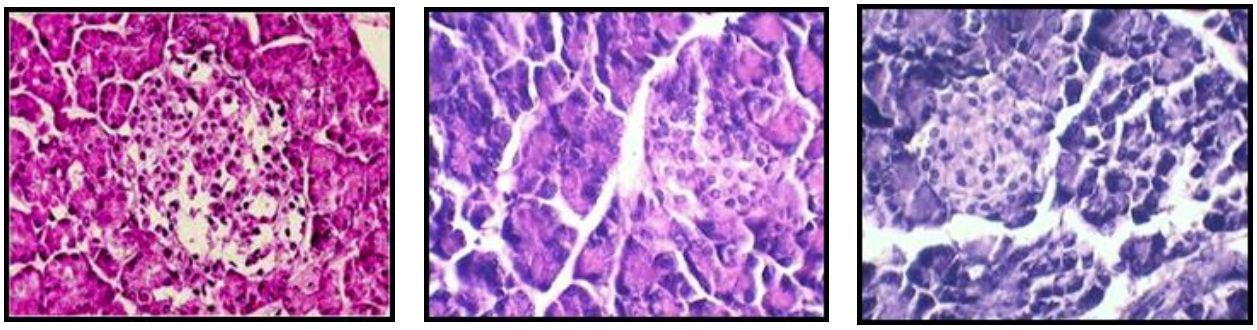
A **B** **C**

Fig. 12: Photomicrograph of liver tissue: A- Control, B-C – Cd treated liver tissue that showed lymphatic infiltration, binuclear cell formation, hyperemia and vacuolation. X400



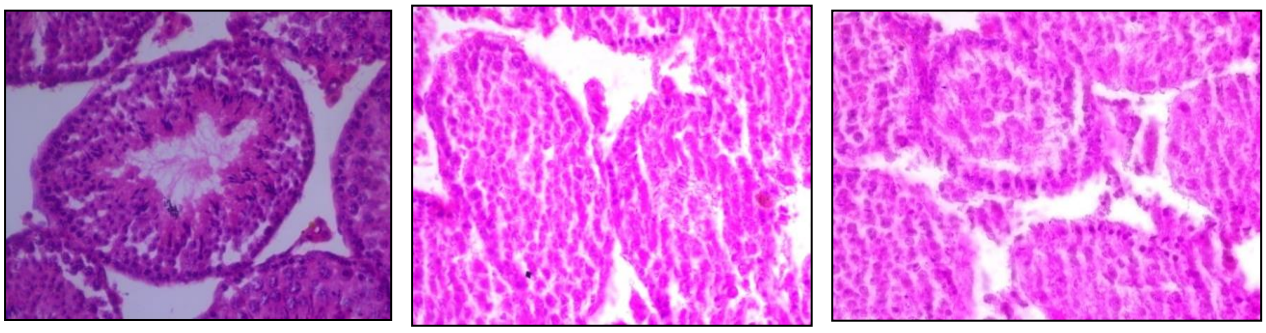
A **B** **C**

Fig. 13: Photomicrograph of lung tissue: A- Control, B-C – Cd treated lung tissue hyperemia and hyperemia, vacuolation in-between alveoli. X400



A **B** **C**

Fig. 14: Photomicrograph of pancreas tissue: A- Control, B-C – Cd treated pancreas tissue hyperemia, reduction of islets of Langerhans and degeneration of pancreatic acini. X400



A **B** **C**

Fig. 15: Photomicrograph of testis tissue: A- Control, B-C – Cd treated testis tissue hyperaemia and clumping of numerous seminiferous tubules, atrophied testicular tissue and dead cells. X400

Discussion:

Cadmium (Cd) is a toxic heavy metal that induced many biochemical and pathological changes in the different tissues of mice. Cd gets accumulated in all the tissues and causes damage to the cells along with altered biochemical pathways. This may lead to decrease in overall weight of mice as well as organ weight in toxic group.

The direct targets of lipid peroxidation are the cell membranes as they are composed of phospholipid bilayers with extrinsic proteins and this may further lead to various detrimental effects such as increased membrane rigidity, osmotic fragility, cell membrane destruction and cell damage that further influences the fluidity of the cell membrane along with DNA (Zama *et al.,* 2007). The intensity of the Cd intoxication not only depends on the route but also on the dose and duration of the exposure to the metal (Djokic *et al.,* 2014). So, lipid peroxidation constituted a major consequence of Cd- induced oxidative damage and it can be correlated with the exposure levels to Cd.

The most important defense mechanisms that help in neutralizing free radical chain reactions and protect the cell against their toxic effects are mostly connected with the antioxidant effect of SOD, CAT, GPx and GST. With the long term exposure to Cd, the activity of SOD, CAT and GST was reduced as many of the essential metals from their active sites and were replaced by other nonessentials metals by making the enzymes less active (Winiarska-Mieczan 2018). Cd may change the protein conformation by interacting with the enzyme and finally changing its functional activity that causes deleterious effects due to the aggregation of superoxide radicals. So, the decreased SOD activity in Cd treated group might be due to its inhibition by the immense production of ROS (Jamakala and Rani 2015) as noticeable by the LPO levels in the present study.

CAT is an inducible cytosolic enzyme which serves to protect the biological system against reactive oxygen species, rapidly converting hydrogen peroxide to non-toxic oxygen and water (Hussein *et al.,* 2014). There was significant decline in CAT level in Cd treated group. This decrease was attributed to the possibility of high production of ROS and their increased intracellular accumulation which further exceed the detoxification capacity of antioxidant enzymes, finally resulting in subsequent development of tissue injury (De Castro *et al.,* 2000).

Glycogen is an imperative source of energy for the general metabolism of the body (Desai *et al.,* 2015). A reduced insulin release/activation of gluconeogenic enzymes may also results in decrement of tissue glycogen (Demir *et al.,* 2006) Glycogen can be depleted

in response to some physiological processes or nonchemical stresses such as temperature and hypoxia (Yuness, 2005). Cholesterol is an essential part of cell membrane and helps in the maintenance of cellular homeostasis along with the communication between and with cells (Liu *et al.,* 2010). There was decrement in the cholesterol level in response to Cd treatment.

Proteins are the constituents of cell membranes and are manufactured by the ribosomes of the cells. The decrease may be correlated to with the decreased protein synthesis due to hepatic dysfunction under heavy metal exposure and there is a direct effect of chronic renal diseases that are associated with heavy metal toxicity can cause excessive loss of proteins (Barbier *et al.,* 2005). Moreover, Cd retards the protein synthesis by binding itself to sulfhydryl group (SH) of many enzymes and this may further cause inhibition of many enzymatic activities (Waisberg *et al.,* 2003).

Cd results in histological alterations in all the tissues of mice and this can be correlated in humans and other species. There is direct link between the Cd ingested and the amount of Cd accumulated in the proximal tubules of kidneys that induced cellular damage and results in pathological changes. Even, Cd results in cell death by apoptosis in cardiomyocytes, kidneys, testes and bones resulting in DNA fragmentation (Ma *et al.,* 2021). Cd also causes stress in endoplasmic reticular, mitochondria that disturbs the ATP generation and protein synthesis. All the tissues samples showed pathological signs that are induced by Cd. The weight of all the tissues was directly related to the histological changes in the mice.

Conclusion:

Many heavy metals are present in the environment. Some of them like Iron, Zinc and Cobalt have biological significance as they act as essential nutrients in the body but other heavy metals such as Arsenic, Cadmium, Mercury, Lead and Nickel are highly poisonous and are carcinogenic agents that leads to oxidative stress as they interfere with the various physiological and biological assets in distinct organs of the body. Cd also causes damage of proteins and DNA in a long run which influences DNA repair enzymes, cell to cell proliferation and invigorate cancer growth. In this current era, though it is impossible to restrict the intake of heavy metals whether through food or water so it's the need of the time to follow healthy life style with the intake of antioxidants as to reduce the effect of these heavy metals, if any in the body.

Acknowledgements:

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PHYTOREMEDIATION FOR THE MANAGEMENT OF ORGANIC POLLUTANTS

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Abstract:

In light of climate change, the growing threat of organic pollution to the environment is a serious obstacle to the sustainability of ecosystems. In response to this phytoremediation is an advanced approach for environmental detoxifying pollutants using plants and some microbes, this technology harnesses the capabilities of plants to mitigate pollution effectively and sustainably. The study highlights the critical need for action by describing the complex relationship between organic contaminants and their detrimental effects on ecosystems. Persistent organic pollutants (POPs) in particular show characteristics of long-range transport and bioaccumulation, which can cause extensive environmental contamination. Phytoremediation is characterized by its cost-effectiveness and the property of metabolizing hazardous organic contaminants into nontoxic or less toxic forms. Climate change affects plant development and pollutant uptake by changing soil moisture levels, temperature, and precipitation patterns. This has an impact on how well the phytoremediation systems perform. The success of phytoremediation in strengthening ecosystem resilience and mitigating climate change effects has been limited due to shifting climatic conditions and low development rates. In this review, different phytoremediation methods and possible approaches are discussed.

Keywords: Phytoremediation, Persistent Organic Compounds, Climate Change, Carbon Sequestration

Introduction:

There are significant environmental risks as a result of rapid industrial development and population growth. Since ancient times, industrial processes including mineral mining, gas emissions, pesticide application, and the creation of urban garbage have added significant amounts of contaminants to the soil, water, and atmosphere biotopes (Nedjimi, 2021). A critical risk to environmental health and ecosystem sustainability is the convergence of climate change with growing organic pollution. In the last few years, a greater understanding has been observed regarding the uptake and metabolism of organic

xenobiotic chemicals by plants, especially chlorinated solvents, some pesticides, and explosives compounds (Lu *et al.,* 2018). Phytoremediation is a green, nature-inspired technique that employs plants in the battle against pollution. This technology is considered efficient, cheap, and environmentally suitable (Ashraf *et al.,* 2019). Phytoremediation is the utilization of suitable plant species in polluted areas to reduce the toxic levels of pollutants (Chatterjee *et al.,* 2013). Phytoremediation is more cost-effective than mechanical and chemical methods of remediating hazardous compounds from soil, sediments, surface, and groundwater environments (Tripathi *et al.,* 2020). Almost all plants are capable of absorbing, breaking up, and transforming environmental pollutants found in soil, water, or air. Phytoremediation uses the natural healing powers of nature to remedy itself rather than traditional methods, which frequently involve many anthropogenic interventions and may pose risks to the environment (Trapp & Karlson, 2001).

Organic pollutant types and their impact on the ecosystem

In recent decades, there has been an increase in the use of green technologies and approaches to reduce the amount of dangerous organic compounds discharged into the environment, (Parul *et al.,* 2020). Many different types of contaminants are still contaminating the earth's ecology. There are many contaminants in various locations. Some endure a long time and are resistant to environmental deterioration (biological, chemical, and photolytic reactions) (I. Ali *et al.,* 2009). When assessing chemical hazards and risks, persistent organic compounds or persistent organic pollutants (POPs) are ranked as a priority group of substances. These chemicals "have time" to travel great distances and reach far-flung locations around the globe because they are persistent.(Scheringer *et al.,* 2012). POPs are pollutants consisting of pesticides, industrial chemicals (PCBs, polychlorinated biphenyls, PBDEs, PFOS, etc.), and by-products of industrial processes (dioxins and furans) (Jacob & Cherian, 2013).

United Nations Environment Program (UNEP) recognized some (POPs) which are aldrin, dieldrin, heptachlor, chlordane, hexachlorobenzene, mirex, endrin, chlordecone, toxaphene, lindane, hexa- and penta-bromodiphenyl ethers (commercial octabromodiphenyl ether), tetra- and penta-bromodiphenyl ethers (commercial pentabromodiphenyl ether), hexabromobiphenyl, pentachlorobenzene, polychlorinated biphenyls (PCBS), α- and β-hexachlorocyclohexane, αand β-endosulfans (technical endosulfan and its isomers), perfluorooctane sulfonic acid and its salts (PFOS), perfluorooctane sulfonyl fluoride (PFOSF), DDT, pentachlorobenzene, hexachlorobenzene,

polychlorinated dibenzofurans (PCDFS), polychlorinated dibenzo-dioxins (PCDDS), polychlorinated biphenyls (PCBs), hexachlorobutadiene, chlorinated naphthalenes, pentachlorophenol hexabromocyclododecane (HBCD), short-chained chlorinated kinds of paraffin (A. Singh & Kumari, 2021). Before discussing the environmental effects of POPs. We should consider the notorious effects of POPs mostly POPs are highly lipid soluble with semi-volatilities (Pv between 10^{-4} and 10^{-11} atmosphere at 25C) properties. Besides, some POPs are also water-soluble such as PFOS(Al-Rashdan & Helaleh, 2013). Furthermore, POPs' persistent nature prevents them from degrading in the environment, which causes bioaccumulation in living things. (Gaur *et al.,* 2018). This bioaccumulation happens as these toxins travel up the food chain, posing a serious risk to ecosystems and human health (H. Ali & Khan, 2019). Exposure to these pollutants can lead to many major health issues, including cancer, heart disease, obesity, reproductive and neurological disorders, hormone disruption, learning difficulties, and diabetes. In addition, these contaminants cause abnormalities in the developing female fetus (Alharbi *et al.,* 2018). Moreover, Long-distance transit of certain POPs can result in extensive distribution, harming regions far from their initial origins and underscoring the global scope of the environmental challenges related to these compounds (Jones, 2021).

Organic pollutants have considerable adverse environmental effects on other living creatures as well as diverse media including air, soil, or water (Aktar *et al.,* 2009). Anthropogenic activities, such as practices for intensifying agriculture (Mózner *et al.,* 2012). Unlimited urbanization and rapid industrialization are causing serious environmental pollution by organic pollutants (Anyakora *et al.,* 2013). Urban runoff's organic compounds and nutrients have a detrimental effect on global warming (Tang *et al.,* 2021). All things considered, phytoremediation is a viable and long-term approach to combating environmental contamination and aiding in the recovery of ecosystems impacted by human activity (P. Sharma & Pandey, n.d.).

Mechanism of Phytoremediation

Remediation of water and soil pollution is one of the main problems our civilization is currently facing in maintaining ecosystem processes and functioning. Many physical, chemical, and biological techniques have been used to clean up environmental pollution, but their uses are restricted because of the expenses and labor involved, the risks to safety, and the damage they do to ecosystems (H. Ali *et al.,* 2013). Alternative phytoremediation is the term used to describe the process of using plants to remove contaminants from soil,

water, and air (Cunningham *et al.,* 1995). Plant roots contain enzymes called peroxidases and laccases, which are essential to phytoremediation because they convert organic pollutants into innocuous substances (Angélica Rodríguez Dorantes, 2012) also, microbial biodegradation uses microorganisms, specifically fungi and bacteria, to break down and change organic contaminants into less hazardous forms (El-Sheekh & Mahmoud, 2017). Many types of pollution, such as those caused by metals, herbicides, explosives, and oil, can be cleaned up by plants (Abdel-Shafy & Mansour, 2018).

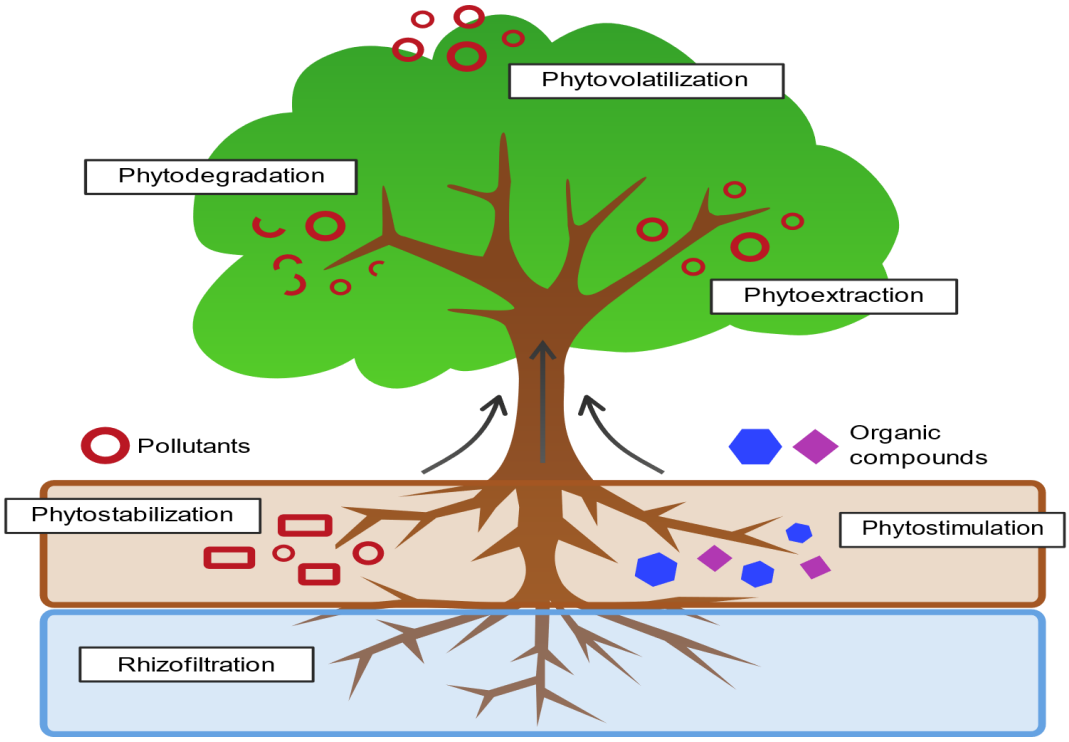

Fig. 1: Phytoremediation techniques in the phyllosphere and rhizosphere (Lee *et al.,* 2020).

Additionally, plants aid in preventing contaminants from being carried away from places and into other areas by wind, rain, and groundwater. An affordable, non-destructive in situ technique called phytoremediation can be utilized for the contamination of soil removal. Given the tropical climate's favorable plant growth and microbiological activity, there is great potential for this technique (Etim, 2012). Soil conditions, bioavailability, and the type of contamination all affect the mechanisms and effectiveness of phytoremediation (Petruzzelli *et al.,* 2015). Plants may clean up or remediate contaminated environments in a variety of ways. The main methods for preventing toxicity are found in the root system, which is also where most pollutants are absorbed by plants. The source system offers a vast surface area for absorbing and storing non-essential pollutants along with water and nutrients needed for growth (Kumar Yadav *et al.,* 2018). There are seven methods for the

removal of environmental pollutants with the help of phytoremediation namely phytoextraction, phytovolatilization, phytodegradation, phytostabilization, biodegradation, rhizofiltration, as shown in Fig. 1.

Phytoextraction

Because phytoextraction removes pollutants from the soil and moves them to the above-ground portions of plants, it affects both the phyllosphere and rhizosphere of plants (Pandey & Bajpai, 2019). This method is also referred to as phytoaccumulation, phytoabsorption, or phytosequestration (Farraji *et al.,* 2020). The term "phytoextraction" describes how pollutants are absorbed, moved, and accumulated by plants. Harvest products can be discarded or reused; they concentrate the contaminants. Heavy metals are the ideal application for this technology (Ogundola *et al.,* 2022). By absorbing pollutants from polluted soils and collecting them in the phyllosphere, this technique makes use of hyperaccumulator plants, which can concentrate 100 times more metals than nonaccumulating plants (Srivastava, 2017).

Phytovolatilization

To eliminate pollutants from soil and water, phytovolatilization uses transpiration as well as phytotransformation. Plants absorb the pollutants through their roots during this process. The pollutants are subsequently carried to the leaves to the phyllosphere, where they are eliminated as a volatile material through transpiration. Before being transpired, a large number of the chemicals are broken down or detoxified (Jeevanantham *et al.,* 2019). For the most part, this method works well with organic pollutants (Shmaefsky, 2020b). But inorganic pollutants such as mercury have also been cleaned up via phytovolatilization, which reduces the metal to its elemental state. In other research, phytovolatilization was utilized to eliminate selenium and arsenic from soil and water (Shmaefsky, 2020a).

Phyto and biodegradation

The degradation of pollutants by plants is called phytodegradation while the degradation of contaminants in the root zone is called rhizodegradation. Rhizodegradation is also known as phytostimulation because pollutants are degraded in the rhizosphere while being stimulated by microorganisms found there (Yang *et al.,* 2023). Plant roots offer an additional surface area for the growth of microorganisms and the transport of oxygen. Along with the biodegrading enzymes that plants produce, microbes in the rhizosphere develop and break down contaminants using the metabolites and exudate of plants (Asemoloye *et al.,* 2019). Organic substances can undergo phytodegradation either inside the plant or in its rhizosphere. This technique may remove a wide range of different

substances and types of compounds from the environment, such as volatile compounds in the air, petroleum and aromatic compounds in soils, solvents in groundwater, and solvents in soil contaminations, such as petroleum, PAH, BTEX, TNT and pesticides, Phyto, and biodegradation are commonly employed. (Feng *et al.,* 2017).

Rhizofiltration

Rhizodegradation can be combined with hemofiltration to treat surface water, groundwater, and wastewater remediation. The rhizosphere serves as a filter in this bioremediation application, absorbing and breaking down water pollutants (Hanus-Fajerska & Koźmińska, 2017). Simply this is the process of using plant roots to adsorb and absorb contaminants from water and aqueous waste streams, primarily metals (S. Ali *et al.,* 2020). In a hydroponic system, where pollutants are absorbed by the roots or other rhizosphere organs, plants are frequently grown. The plants were gathered like the phytoextraction approach after they reached the saturation limit of the pollutants (Mahrukh & Ali, 2023).

Phytostabilization

The immobilization of soil compounds or the stability of the soil to stop erosion is known as phytostabilization (I. Alkorta *et al.,* 2010). Utilizing root accumulation or rhizosphere-based precipitation, phytostabilization seeks to keep pollutants out of the vadose zone. By doing this, the movement of pollutants through soil dispersion, leaching, and erosion from wind and water is averted (Teodoro *et al.,* 2020). It is not necessary to dispose of hazardous materials when using this technology. Additionally, this technology is a useful tool for protecting surface and groundwater resources. This technique can also lessen the amount of water available in the system and soil erosion (Carvajal-Flórez & Santiago-Alonso Cardona-Gallo, 2019).

Plant species selection for phytoremediation

The capacity of various plants to absorb and accumulate particular pollutants varies (S. Sharma *et al.,* 2015). Look for plants with a history of accumulation of specific pollutants that need to be cleaned up (Weidlich *et al.,* 2020) Determine the potential environmental effects of introducing a specific plant species to an area by examining which plants are better at eliminating organic contaminants and absorbing heavy metals. Don't use invasive species that could cause native plants to be displaced or disturb nearby ecosystems. (O. V. Singh & Jain, 2003). Considering the plants that have high biomass. Deep-rooting plants are commonly utilized in phytoremediation (Prabakaran *et al.,* 2019). Choose the plants that have adapted to local climates. (Munang *et al.,* 2010). Evaluate the environmental

conditions of the location, including the soil type, pH, moisture content, temperature, and solar exposure. Choose plants that have the greatest adaptation to the site's environment so they can grow and treat the area effectively (Kim *et al.,* 2015).

Plants used as phytoremediants

Plants	Scientific Names	Contamination Removed
Sunflowers	*Helianthus annuus*	Lead, arsenic, and uranium (Adesodun *et al.,* 2010).
Indian Mustard	*Brassica juncea*	Lead, cadmium, zinc (Kutrowska *et al.,* 2017).
Alfalfa	*Medicago sativa*	Cadmium, lead (Kutrowska *et al.,* 2017)
Poplar Trees	*Populus spp.*	VOCs, hydrocarbons (McCormick *et al.,* 2019).
Willow Trees	*Salix spp.*	Heavy metals, and pollutants in soil and water (Bajraktari *et al.,* 2019).
Water Hyacinth	*Eichhornia crassipes*	Excess nutrients, heavy metals (Bajraktari *et al.,* 2019).
Duckweed	*Lemna spp.*	Excess nutrients, heavy metals, and organic pollutants (Ziegler *et al.,* 2016).
Bamboo	*Bambusoideae spp.*	Heavy metals (Bajraktari *et al.,* 2019).
Ferns	*Pteridophyta spp.*	Heavy metals (Bajraktari *et al.,* 2019).
Cattails	*Typha spp.*	Heavy metals, and organic pollutants (Ziegler *et al.,* 2016).
Seaweed	*Algae spp.*	Heavy metals are pollutants in seawater (Pan & Wang, 2012).
Switchgrass	*Panicum virgatum*	Hydrocarbons, and heavy metals (Alacabey & Zorer Çelebi, 2020).

Conclusion:

Phytoremediation has gained significant attention as a sustainable, cost-effective approach for the remediation of contaminated environments, utilizing plants to remove, degrade, or immobilize pollutants. This bioremediation strategy is particularly effective for addressing soil, water, and air pollution. Recent research highlights the potential of synergistic interactions between plants and microorganisms, which can enhance the efficiency of phytoremediation processes. Among the microorganisms studied, Pseudomonas *putida* stands out due to its versatility in degrading a wide range of organic

contaminants, such as hydrocarbons, pesticides, and heavy metals. Its ability to thrive in the rhizosphere and metabolize toxic substances makes it a valuable tool when used with phytoremediation systems. P*. putida* facilitates pollutant degradation by breaking down contaminants into less toxic or non-toxic forms, while also promoting plant growth through improved nutrient cycling and enhanced stress tolerance. The interaction between P*. putida* and plants can significantly increase the efficiency of pollutant removal, particularly in the case of complex contaminants that are otherwise challenging for plants to do alone. This microbial-plant combined effect accelerate the detoxification process and improves overall remediation outcomes. However, the effectiveness of this combined approach depends on factors such as plant species, microbial strain, environmental conditions, and the nature of the contaminants. Despite its promising potential, several challenges still remain. These include optimizing plant-microbe interactions, ensuring the stability and persistence of microbial populations in field conditions, and scaling up the technology for large-scale applications. Additionally, the long-term impacts of such bioremediation strategies on ecosystem health and sustainability need further investigation.

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MICROPLASTIC AND NANOPLASTIC, AN EMERGING THREAT TO HUMAN BEING

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Abstract:

Plastics have an important aspect in everyday day to day life including technology, medicine, treatments and domestic appliances. These plastics are thrown away by the consumers after a very first single use, which become a huge threat to nature as they will end up in river, lakes, ponds, landfills, oceans and other waterways. These small plastics are discarded in vast number each day and the breaking down of the plastic from Micro to Nano sizes has led to worries about how harmful these plastics are to the nature and humans, while there are several earlier studies reported the effect of Microplastics (MP) and Nanoplastics (NP) have on the nature, but there is spare exploration about their impact on the human body at sub cellular or molecular level. Microplastics are usually produced designedly or generated when large synthetic polymer products, such as plastic packaging material or other products are not properly disposed of or treated. These are synthetic, high-molecular weight composites that have been micronized into plastic patches smaller than 5 μm in size. Such materials have a low biodegradation rate and thus, remain substantially in the environment and negatively affect the mortal.

Keywords: Microplastic, Nanoplastic, Polymers, Synthetic, Landfills, Environment

Introduction:

Plastics are made of synthetic accoutrements that have experienced several chemical processes and physical reactions. The main process used are polymerization and poly condensation, during which the core elements are fundamentally transformed into polymer chains.

The term Microplastics was first introduced by Thompson et al. in 2004 to report on small plastics on marine environment. According American Chemical Society plastics particle and fibers smaller than 5μm and lager than 1μm are defined as microplastics. Microplastics can be classified according to their source and scrap size. Broadly, microplastics can be distributed into primary and secondary microplastics. Primary microplastics are intentionally created plastic patches such as consumer-care products

(e.g., detergents and cosmetics, glitters) or industrial products. Whereas Secondary microplastics are products containing plastics, such as plastic waste and fibres or plastic products that have perished after being exposed to the outside environment. In addition, the chemical constituents of environmental microplastics are diverse and include polymers, such as polyethylene (PE), polypropylene (PP), polystyrene (PS), PE terephthalate, polyvinyl chloride (PVC) and polyvinyl alcohol.

Microplastics due to their tiny sizes fluently ingested. They easily persist in the environment for long time since they are refractory to biodegradation and move upwards in the food chain. In addition, as microplastics persists in micro-level to nano-level sizes, they are virtually impossible to remove once released into the environment. Due to these characteristics, microplastics pose implicit hazards to humans and the environment. As a representative illustration of the threat posed by microplastics, they can cause physical and mechanical harm (e.g., cause abnormalities in internal organs) to marine organisms when they inaptly ingest microplastics. Ecotoxicity may be caused by the polymer itself, unreacted monomers, (e.g., residual catalysts or reaction by-products), additives (e.g., stabilizers), or other contaminants substances in the polymer matrix (e.g., dyes, lubricants, or plasticizers). In addition, microplastics can enter into the human body when they are not filtered out during sewage-treatment processes, or they can flow into the sea, thereby posing pitfalls for the ecosystem and humans. Various examples of damage caused by microplastics as well as nanoplastic have been reported, such as Microplastics accumulation in the bodies of sexually reproducing living organisms (leading to malnutrition), inflammation, reduced fertility, and mortality. Accumulation of microplastics also detrimental to lower species where it is question of survival. The threats that microplastics present to the human body have not yet been clearly identified. However, previous reports have shown that ultrafine microplastics absorption resulted in complex toxicity in zebrafish and that microplastics under 100 nm in size can reach almost all organs after entering the human body. Therefore, concerns exist regarding the negative effects of continuous microplastic accumulation in the human body.

There are three primary routes for Microplastics to end up in the human body; inhalation, ingestion and dermal contact. Inhaled airborne microplastics appear from civic dust, which include synthetic textiles and rubber tyres. Microplastics are ingested as they are prevalent in the food chain and water supplies, while the skin membrane was too fine for microplastics and nanoplastics to pass through especially nanoplastics, it is possible for

them to enter through wounds, sweat glands and hair follicles. Although all three routes contribute to the total amount of microplastics present in the human body. Most investigations of microplastics have been based on observations of synthetic microbeads (i.e., primary microplastics) intentionally manufactured for industrial or household use. However, unintentional causes of microplastics pollution should also be paid attention to, such as secondary microplastics that comprise a relatively large proportion of plastic particles in the environment. It is also well known that the physicochemical properties of nanomaterials play important roles in their toxic properties and lethality. Primary microplastics currently used in toxicology studies are mostly uniform in size and shape, whereas secondary microplastics exist in a variety of sizes and shapes, making it difficult to assess their actual health risks.

Several studies have shown that microplastics can affect various systems in the human body, including the digestive, respiratory, endocrine, reproductive, and immune systems. The digestive systems are affected when microplastics are ingested, and physical irritation to the gastrointestinal tract may eventually cause inflammation, resulting in various gastrointestinal symptoms. Microplastics may cause changes in the intestinal microbiome, resulting in an imbalance between beneficial and harmful bacteria, which can lead to various gastrointestinal symptoms, such as abdominal pain, bloating, and changes in bowel habits. In addition to their physical effects on the digestive system, microplastics can cause chemical toxicity, which involves the absorption and accumulation of environmental toxins such as heavy metals and polycyclic aromatic hydrocarbons. Microplastics may cause oxidative stress in the airways and lungs when inhaled, leading to respiratory symptoms such as coughing, sneezing, and shortness of breath due to inflammation and damage, as well as fatigue and dizziness due to a low blood oxygen concentration. Microplastics interfere with the production, release, transport, metabolism, and elimination of hormones, which can cause endocrine disruption and lead to various endocrine disorders, including metabolic disorders, developmental disorders, and even reproductive disorders (i.e., infertility, miscarriage, and congenital malformations). Recent report showed that microplastics exposure in new-borns and infants could increase due to the use of feeding bottles and medical devices, and bio monitoring data provide indirect evidence of microplastics exposure in infants and children.

Following exposure, uptake is plausible via ingestion and/or inhalation. The toxicity assessments of Microplastics on human are mainly focusing on gastrointestinal and

pulmonary toxicity, which involves oxidative stress, inflammatory reactions, metabolism disorders. Based on the findings of recent Studies, further research is needed to investigate the potential mechanisms of Microplastics toxicity in human. Moreover, it is important to understand whether microplastics can be further degraded after ingestion under the acidic conditions in the gut or inside the lysosomes of the cells. Hence the long-term fates of the ingested Microplastics in human body needed further investigation. Unfortunately, the accurate assessment of human exposure to microplastics remains a scientific challenge due to the lacks of validated methods, certified reference materials, and standardization across the analytical procedures used.

Discussion:

- The article followed a structured methodology that included the routes of human exposure to MPs and NPs sources, mechanisms of potential health risks, the organs and tissues involved, research gaps.
- MPs and NPs are considered an emerging environmental contaminant, and so there is a dearth of convincing evidence on adverse human health effects
- It is evident that the health risks of MPs and NPs need to be further explored, including toxicological testing not only based on environmentally relevant concentrations but also on individual properties.
- Develop standardized and validated methods for quantifying, characterizing, analyzing and evaluating the potential human health risks of MPs and NPs exposure.

Conclusion:

- While, MPs and NPs are widely studied in the context of the marine environment, we have only recently recognized the potential human exposure pathways.
- Based on the findings of recent studies, further research is needed to investigate the potential mechanisms of MPs and NPs toxicity in human.
- Notably, most of the reported studies were conducted using polystyrene due to its ease in synthesis and processing into NPs, while there are different type of plastic is prevalent.
- Unfortunately, the accurate assessment of human exposure to MPs and NPs remains a scientific challenge due to the lack of validated methods, certified reference materials.

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PHYTOCHEMICAL STUDIES ON MEDICINAL PLANTS OF CENTRAL WESTERN GHATS OF KARNATAKA INDIA

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Abstract:

The fruits of *Artcoacrpus gomezianus* and *Zizyphus rugosa* are collected from different altitudes of Central Western Ghats which differ in their topography, vegetation, and social cultural and food habits of the local people. The objective of the study was to phytochemical screening of secondary metabolites. About 500gm of air dried powdered material of unripe fruit of *Zizyphus rugosa* and *Artocarpus gomezianus* were subjected to soxhelect extraction with petroleum ether, chloroform, 95% ethanol and distilled water for 18 hours in the order of increasing polarity of solvents. The condensed crude extracts were used for preliminary screening of phytochemicals. The screening of secondary metabolites reveled presence of alkaloids, phenols, flavonoids, tannins, steroids and saponins in the fruit samples of all regions. The quantitative determination of secondary metabolites shown that the alkaloids were dominant at all the sampling stations followed by lignin, phenols, flavonoids, tannins and steroids. However, the amount of shifting among the flavonoids and phenols in the middle lower and middle higher altitudes. Though alkaloids were dominant secondary metabolites and steroids were the lowest, with respect to concentration of individual alkaloids, remarkable variations were observed. Among the six secondary metabolites, the concentration of steroids, tannins and phenols were high at the lower and higher altitude and low in middle altitudes. In contrast to phenols, tannins and steroids, the concentration of flavonoids was more in the middle and lower in higher altitudes and lowest in higher and lower altitudes. The concentration of alkaloids and lignin did not show any relationship with variation in altitudes. The qualitative separation of secondary metabolites by thin layer chromatography method revealed that alkaloids, flavonoids and glycosides have three distinct spots with different hRf values. Steroids revealed two spots and saponins revealed one spots along with different hRf values. The fruits of *Zizyphus rugosa* showed the + ve response for alkaloids, phenols and flavonoids and –ve response for tannin, lignins, steroids and saponins. The phytochemical screening of

secondary metabolites reveals that alkaloids, phenols and flavonoids showed the +ve response whereas tannin, lignins, steroids and saponins showed –ve response. The quantitative analysis of alkaloids, phenols and flavonoids reveals that the alkaloid was dominant in both two yearly average values. Further, alkaloids are followed by flavonoids and phenols in their concentration.

Keywords: *Artocarpus gomezianus,* Central Western Ghats, Phytochemicals, *Zizyphus rugosa.*

Introduction:

The bioactive constituents of the plant are many, a large number of plant species have been screened for their pharmacological and chemotherapeutic properties and still a vast wealth of edible medicinal plants have to be exploited for their edible and medicinal properties. 75% to 80% of the world population depends on the crude plant drugs problems and it is due to their poor economic conditions (Sukh Dev, 1997). At least 25% of the perception drugs issued in the USA and Canada contained bioactive compounds which are derived from plants (Vimalavady and Kadavul, 2012). In addition to primary metabolites, the secondary metabolites like tannin, saponins, photobatalins, flavaonoids, terpinoids, cardiacylycosides and alkaloids are found in medicinal plants (Krishnaiah *et al.,* 2009).The qualitative and quantitative variation of secondary metabolites are not only varied between the different regions of the same plant but also varied in the different parts of the same plants and also fresh and harvested products (Krishnamurthy and Sarala, 2014; Krishnamurthy and Asha, 2011; Mallikharjuna *et al*., 2007).

The wild edible plants were screened for bioactive compounds not only in India but also in other countries (Krishnaniah *et al*., 2009; Edeoga *et al.,* 2005; Chhetri Paudel Himal *et al*., 2008). The crude extracts of wild edible plants were studied with referenced o the antioxidant, anthelmintic and insecticidal and also other pharmacological action. Based on the pharmacological action the various investigators tried to isolate and elucidate structural of the particular compound respond for that activity In addition, a few workers estimated secondary metabolites in the different parts of the medicinal plants Seventy five to eight per cent of the world population depends on the crude plant drugs preparations to tackle their health problems and it is due to their poor economic conditions (Sukh Dev, 1997). At least twenty five percent of the perception drugs issued in the USA and Canada contained bioactive compounds which are derived from plants (Vimalavady and Kadavul, 2012). In addition to primary metabolites, the secondary metabolites like tannins,

saponins, photobatalins, flavonoids, terpinoids, cardiacglycosides and alkaloids are found in medicinal plants (Krishnaih *et al.,* 2009). The qualitative and quantitative variations of secondary metabolites are not only varied between the different regions of the same plant but also varied in the different parts of the plants and also fresh and harvested products (Krishnamurthy and Asha, 2011 and Mallikharjuna *et al*., 2007). Medicinal plants were screened for bioactive compounds not only in India and but also in other countries. Number of investigators initiated phytochemical studies in India (Rai Vaishall *et al*., 2013; Vimalavady and Kadavul, 2012; Yadav and Agarwala, 2011; Bindu Hima, 2012; Sherwani *et al*., 2012; Mallikharjuna *et al*., 2007; Krishnamurthy and Asha, 2011) and other countries (Krishnaiah *et al*., 2009; Edeoga *et al*., 2005; Chhetri Paudel Himal *et al*., 2008).

Material and Methods:

Study area

Western Ghats extends about 1600 km starting in North from Tapti River and going down to Kanyakumari in South. The Western Ghtas, run parallel to the Western coast of India about 40 km away on the average from the seashore. The average elevation ranges between 900 and 1500m and some places go beyond 2000m. The region is rich in flora and fauna. The natural vegetation is interrupted by plantation of areca, coffee and paddy fields. The entire region receives heavy rain fall during monsoon and they year is not clearly demarcated into different season. The fruits of *Artocarpus gomenzianus* were collected from different regions between 20 and 2000 m altitudes. The study area of Kuvempu University Campus is 230 hectares, it is on the latitude of 13º 42' 20" N and longitude of 75º 13' 22" E. The campus is surrounded from the north by Gonibeedu, south by B.R. Project the east from Nellisara and from the west by Umblebylu. The area receives the rainfall between 741.1 mm and 1500.48 mm/year. The year is divided into three district seasons summer, winter and rainy. The vegetation type is moist deciduous type. The *Zizyphus rugosa* which has been grown widely in the Kuvempu University Campus has been identified and selected for study (Map 1).

Collection of fruits

The fruits of monkey jack (*A. gomezianus*.) are collected from different places of Western Ghats. The unripe and ripe fruit of sweet jujube which is collected are shade dried and subjected for extraction with different solvents. The extracts are subjected for qualitative and quantitative phytochemicals analysis (Fig 1 and Fig 2).

Phytochemistry

Preliminary screening of secondary metabolites

Extraction

The shade dried fruit material is powdered using mixer grinder and subjected to Soxhlet extraction with petroleum ether, chloroform, 95% ethanol and distilled water for 18 hours in the order of increasing polarity of solvents. The condensed extracts are used for preliminary screening of phytochemicals (Mallikharjuna *et al.,* 2007).

Qualitative, quantitative and separation of secondary metabolites of alkaloids, phenols, flavonoids, tannins, lignins, steroids, glycosides and saponins were determined by using standard methodology (Dey and Harbour 1987; Evans 1989Mallikharjuna *et al.,* 2007, Sarala and Krishnamurthy 2014,2019).

Results and Discussion:

A. gomezinaus

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, tannins, lignins, steroids, glycosides and saponins. The successive extracts of fruit of *A. gomezinaus* have revealed the presence of alkaloids, phenols, flavonoids, tannins, lignins, steroids, glycosides and saponins thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their qualitative estimation, quantitative separation, isolation, purification and structural elucidation of secondary metabolites for pharmacologically active chemical compounds.

Preliminary phytochemical screening

The fruit samples which have prepared for phytochemicals are subjected for extraction in different solvents (Petroleum ether, chloroform, ethanol and aqueous). The known extracts are tested for various phytochemical (alkaloids, phenols, flavonoids, tannins, Steroids, Glycosides and Saponins). The observations are detailed (Table 1).

Alkaloids, phenols, flavonoids showed +ve response in both ethanol and aqueous extract, but it showed –ve response in petroleum and chloroform extract. Tannins, steroids showed +ve response petroleum ether, chloroform extract but it showed –ve response in ethanol and aquous extract. But in case of glycosides and saponins –ve response in petroleum ether and chloroform extract, but +ve response in ethanol and aqueous extract (Table 1).

Z. rugosa

Phytochemical screening

The unripe pulp samples which are prepared for phytochemicals are subjected for extraction in different solvents (Petroleum ether, chloroform and ethanol). The known extracts are subjected for the detection of phytochemicals (alkaloids, phenols, flavonoids, tannins, steroids and lignins). The observations are detailed in Table 2.

Alkaloids

Wagner's test and Mayer's test showed positive response in petroleum ether, ethanol and aqueous extract but negative response in chloroform extract. Drangendorff's test showed positive response in ethanol and aqueous extract, but negative response in petroleum ether and chloroform extracts.

Phenol

Phenol test and Ellagic acid test showed the positive response to all the extracts except ellagic acid test which showed negative response for chloroform extract.

Flavonoids

Zinc hydrochlorid acid test and shinoa's test showed negative response in petroleum ether and chloroform extract, but positive response showed in ethanol and aqueous extracts. In case of Alkaline reagent and flavonoids test showed negative response in ethanol and aqueous extracts but showed positive response in petroleum ether and chloroform extract.

Glycosides

In case of killer- Killiani test showed negative response in petroleum ether and chloroform extract but positive response in ethanol and aqueous extract.

However all the extracts showed negative response to tannins lignins, steroids and saponins.

Quantitative estimation of phytochemicals

The percentage of phytochemicals at different altitudes /places of fruits of *A. gomezianus* are detailed in Table 3.

Alkaloids

The percentage value of alkaloids varied between 7.7±0.38 and 37.00±3 at lower middle and higher coastal altitudes. The study did not reveal any ascending or descending pattern Through, the percentage of alkaloids was higher at all the regions, the percentage of alkaloids varied remarkably among the different regions.

The number of investigators estimated secondary metabolites in the different parts of the medicinal plants. Krishnaiah *et al*. (2009), Krishnamurthy and Ash (2011), Mallikharjuna *et al*. (2007) and Evans (1989) recorded the percentage of alkaloids between 0.28 ± 0.12 and 5.63 ± 0.20 in *B. tinctoria* and *P. edulis* respectively. Krishnaiah *et al.* (2009) reported the percentage of alkaloids between 0.24 ± 0.03 and 0.52 ± 0.12 in *E. officinalis* and *A. indica* respectively. Edeoga *et al*. (2005) reported the percentage alkaloids between 0.34 ± 0.1 and 1.04±0.20 from the medicinal plants. Mallikarjuna *et al*. (2007) observed the variation of quantity of alkaloids in different parts of the *S. potatorum* and they recorded percentage of alkaloids between 1.3 and 2.2. The quantity of alkaloids may vary not only in the parts but also varied between fresh and preserved plant parts.

Phenols

The percentage phenol was ranged between 0.59 ± 0.19 and 1.50 ± 0.11 at lower middle and higher, higher altitudes respectively. The percentage of phenols are comparatively higher at higher and coastal regions when compare to the middle altitudes. Similar to alkaloids, the percentage of phenols varied among the different altitudes.

Doss (2009) recorded percentage of phenol between 0.16 ± 0.10 and 12.85 ± 0.28 from different medicinal plants. Krishnaiah *et al*. (2009) reported variation of percentage of phenol between 0.024 ± 0.13 and 0.719 ± 0.23 medicinal plant of Malaysia. Edeoga *et al*. (2009) observed variation of percentage of phenols among the medicinal plants. Mallikarjuna *et al.* (2007) reported variations of phenols among from the different parts of the *S. potatorum*.

Flavonoids

The values of flavonoids ranged between 0.41 ± 0.21 and 1.28 ± 0.33 at lower coastal and lower higher altitudes respectively. The moderate values of flavonoids were recorded at lower and higher middle latitudes. The low percentage of flavonoids are recorded at lower and higher altitudes The percentage of flavonoids are studied by number of investigators. The previous studies reported flavonoids content below 1%. Mallikharjuna *et al.* (2007) reported variations of flavonoids content in the different parts of the *S. potatorium.*

Tannins

The concentration of tannin ranged between 0.16 ± 0.02 and 0.23 ± 0.05 at lower middle and lower and higher altitudes respectively. At the same time the lower and higher altitudes recorded maximum percentage of tannins of 0.23 ± 0.05 and 0.23 ± 0.02

respectively. The quantitative variation of flavonoids at different sampling stations was very narrow. The higher percentage of tannin was reported from the medicinal plants of Nigeria and Malaysia (Hossain Mokarram *et al*., 2010).

Steroids

Steroids ranged between 0.16 ± 0.02 and 0.23 ± 0.05 at lower middle and lower altitudes respectively. The quantitative variation of steroids was very narrow at different sampling stations. It ranges between minimum and maximum values of steroids were very narrow.

Lignins

The values of lignins ranged between 1.76 ± 0.37 and 5.61 ± 2.87 at lower middle and higher altitudes respectively. The range between minimum and maximum values of lignins was very wide. The higher value of lignins was recorded at lower and higher altitudes respectively.

The quantitative values of secondary metabolites are summarized below on the bases of variation with respective altitude and qualitative variation of individual secondary metabolites.

Place/Altitude	**Concentration of secondary metabolites**
Ashwatpura/22MSL	Alkaloids>Lignins>Phenols>Flavonoids>Tannins> Steroids
Padubidare/147 MSL	Alkaloids > Lignins > Phenols > Flavonoids > Tannins ≥ Steroids
Banajalaya/579 MSL	Alkaloids > Lignins > Flavonoids > Phenols > Tannins ≥ Steroids
Navanagere/590 MSL	Alkaloids>Lignins>Phenols>Flavonoids>Tannins ≥Steroids
Gubburu/763 MSL	Alkaloids>Lignins>Flavonoids>Phenols>Tannins≥Steroids
Etinala/949 MSL	Alkaloids>Lignins>Phenols>Flavonoids>Steroids≥Tannins
Type of secondary metabolites	Variation of secondary metabolites at different place with their concentration
Alkaloids	147>590>763>22>949>579
Phenols	949>763>22>147>590>579
Flavonoids	763>579>147>590>949>22
Tannins	22>949>147>590>579>763
Steroids	22>949>147>590>579>763
Lignins	949>590>22>147>763>579

Index : 22MSL= Ashwatpura : 147 MSL= Padubidare : 579 MSL= Banajalaya : 590 MSL= Navanagere : 763 MSL= Gubburu : 949 MSL= Etinala.

The alkaloids was dominant secondary metabolites which is followed by lignins, phenols, flavonoids, tannins and steroids at lower, higher coastal, higher middle and higher altitudes. There was a shifting between flavonoids and phenols at higher middle and lower higher altitudes respectively. The amount of tannin and steroids were same at all the altitude except at higher altitudes where steroid is higher than tannins. When the secondary metabolites are arranged on the basis of their quantity with reference to altitudes, there was no specific ascending or descending trends was not observed.

Z. rugosa

Quantitative estimation of phytochemicals: The percentage of phytochemicals at different altitudes /places of fruits of *Z. rugosa* are detailed in Table 4. The phytochemical screening of secondary metabolites revels those alkaloids, phenols and flavonoid showed the positive response whereas tannin, lignins, steroids and saponins showed negative response. The quantitative analysis of alkaloids, phenols and flavonoids reveals that the alkaloid was dominant in both two yearly average values. Further, alkaloid is followed by flavonoids and phenols in their concentration. The percent values of alkaloids ranged between 7.00 and 8.00 with an average of 7.70 per cent. The minimum of 7.00 and maximum of 8.00 % were recorded in the sample of 2010 and 2009 respectively. The number of investigators estimated secondary metabolites in the different parts of the medicinal plants (Mallikharjuna *et al.* 2007, Krishnamurthy and Asha 2011). Edeoga *et al.* (2005) recorded the percentage of alkaloids between 0.28 ± 0.12 and 5.63 ± 0.20 in *B. tinctoria* and *P. edulis* respectively. Krishnaiah *et al.* (2009) reported the percentage of alkaloids between 0.24 ± 0.03 and 0.52 ± 0.12 in *E. officinalis* and *A. indica* respectively. Mallikarjuna *et al.* (2007) observed the variation of quantity of alkaloids in different parts of the *S. potatorum* and they recorded the percentage of alkaloids between 1.30 and 2.20. The quantity of alkaloids may vary not only in the parts but also varied between fresh and preserved plant parts. Alkaloids are nitrogen-containing compounds widely distributed in different plant group. The alkaloids are the lead molecules of therapeutic importance and they are heterocyclic indole compounds which have proved to be having pharmacological properties such as hypotensive activity, anticonvulsant activity, antiprotozoal, antimicrobial and antimalarial activities. The biological properties of alkaloids were studied (Nobori *et al*., 1994). The percent value of phenol ranged between 0.39 and 0.95 with an average of 0.6 %. The minimum of 0.39 % and maximum of 0.95 % were recorded in the sample of 2009 and 2010 respectively. Doss (2009) recorded percentage of phenol

between 0.16 ± 0.10 and 12.85 ± 0.28 from different Indian medicinal plants. Krishnaiah *et al.* (2009) reported variation of percentage of phenol between 0.024 ± 0.13 and 0.719 ± 0.23 in the medicinal plant of Malaysia. Edeoga *et al.* (2005) observed variation of phenols in different medicinal plants of Nigeria. Mallikarjuna *et al.* (2007) reported variations of phenols among the different parts of the *S. potatorum*. Phenols constitute a large class of compounds in which a hydroxyl group (-OH group) is bound to an aromatic ring. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites and they possess biological properties such as atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. Natural antioxidant is due to the presence of rich phenolic compounds such as flavonoids, phenolic acid and tocophenol etc. The percent values of flavonoids ranged between 0.22 and 1.23 with an average of 0.74%. The minimum of 0.22 % and the maximum of 1.23% were recorded in the sample of 2009 and 2010 respectively. Flavonoids are group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and antioxidant enzymes and also act as anti-inflammatory agent. The flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. They also inhibit microbes which are resistant to antibiotics. Flavonoids may help in providing protection against some diseases such as oxidative stress, cellular damage. Oxidative stresses have been linked to cancer, ageing, atherosclerosis, inflammation, ischemic injury and neurodegenerative diseases (Krishnamurthy and Asha, 2011). The tannins, steroids and lignins were absent in the unripe fruit of *Z. rugosa*.

Separation of secondary metabolites by thin layer chromatography

The qualitative separation of secondary metabolites by thin layer chromatography revealed three distinct coloured spots for phenols, flavonoids and glycosides with different hRf values. The steroids have two and saponins have one coloured spots with specific Rf values (Table 5).

Alkaloids

Three quenching and fluorescing alkaloids, spots were reported from the fruit part of *A*. gomezians. Colour of the spots was green, blue and light green with the hRf values was 38, 60 and 88 respectively. Mallikharjuna *et al.* (2007) observed 20 quenching and fluorescing alkaloids from the various part of the plant (*Strychnos potatorum*). The roots and stem bark recorded highest number of alkaloids spots when compare to seeds. Again, it is interesting to note that the collected seeds recorded more number of spots when

compare to the market seeds. Therefore it may be attributed that the quality and quantity of alkaloids not only various with respect to different individual plants but also varies among the different parts of the same plant. Further, the methods of processing and storage may be important for quantitative and qualitative variation of alkaloids.

Phenols

The three phenol spots were reported in the fruit samples, colour of the spot is blue, dark blue and blue and the hRf value is 7, 10 and 20 respectively. The investigation of Mallikharjuna *et al.* **(**2007) on qualitative separation of phenols revealed the interesting results. Of the 11 coloured spots of phenols, the seeds contained maximum of 9 and 7 spots from the market and collected seeds respectively. In contract to alkaloids, phenols coloured spots were less in root and stem bark samples. The storage of seed may increase or derive new type of phenols.

Flavonoids

Three flavonoids spots were reported in the fruit samples. Colour of the spots is dark blue, yellow and yellow blue and hRf values are 53, 69 and 84 respectively. The thin layer chromatography of flavonoids of *Strychnos potatorum* revealed that the colour spots appeared in the roots, collected seeds and market seed were same, whereas stem bark had separate flavonoids spots with different colours and hRf values (Mallikharjuna *et al.,* 2007).

Glycosides

The three glycosides spots of light green, pink and light pink with hRf values of 31, 63 and 79 were observed in the fruit samples of *A*. gomezians. The qualitative separation of glycosides of *S. potatorum* (Mallikharjuna *et al.,* 2007) revealed 7 coloured spots with different hRf values. It is interesting to note that the glycosides of root and stem bark were same and collected seed and market seed were same. The coloured spots which were found in root and stem bark did not found in collected seed and market seed. At the same time coloured spots which were found in collected and marketed seed were also not found in root and stem bark.

Saponins

One saponins with intense yellow colour and hRf values of 75 was observed. The qualitative separation of saponins of *S. potatorum* revealed that the coloured spots of saponins were same for the different plant parts with same coloured and hRf values.

Steroids

It has been revealed that the presence of two sterols in the fruit samples. The colour of the spots was light green and greenish black with the hRf value of 8 and 66 respectively. The qualitative separation of sterols of *S. potatorum* (Mallikharjuna *et al.,* 2007) revealed four district spots with different colour and hRf values. The aerial part of the stem bark, seed and market seed contained maximum four spots, whereas, the root sample contained three spots. However the colour and hRf values were the same for the steroids of root, stem bark, collected seed and market seed respectively.

The qualitative separation of secondary metabolites by thin layer chromatography revealed three distinct coloured spots for phenols, flavonoids and glycosides with different hRf values. The steroids have two and saponins have one coloured spots with specific Rf values. The above observations are useful for further isolation and purification of natural compounds.

Conclusion:

Z. rugosa and *Artocarpus gomezianus* is underutilized edible medicinal and evergreen tree of Central Western Ghats is found between coastal and higher attitudes. The petroleum ether, chloroform, ethanol and aqueous extracts were screened for qualitative phytochemicals, preliminary metabolites and it is found that they were same. The quantitative estimation of secondary metabolites revealed that alkaloids were dominant followed by lignins, phenols, flavonoids, tannins, saponins and steroids except at higher altitude where tannin was low. The qualitative separation of secondary metabolites revealed three spots for alkaloids, phenols and favonoids, whereas two spots for saponins and one spots for steroids. The fruit of *Zizyphus rugosa* is a famine edible medicinal plant of Central Western Ghats. The extracts of petroleum ether, chloroform, ethanol and aqueous were screened for qualitative analysis. The qualitative analysis revealed that alkaloids, phenols and flavonoids are + ve response in all the extracts, whereas tannins, lignins, steroids and saponins showed –ve response. The quantitative estimation of secondary metabolites reveled that alkaloids were dominant, which is followed by phenols and flavonoids. Therefore, the data generated from these experiments have provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments however, there is need to further carry out advanced spectroscopic studies in order to elucidate the structure of these compounds. Furthermore, this data may be handy in probing of biochemistry of this plant in the future.

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Table 1: Preliminary screening of secondary metabolites (alkaloids, phenol, flavonoids, tannins, lignins steroids, saponins and glycosides) in various solvents both in hot and cold (PE - Petroleum ether: CL - Chloroform: ET - Ethanol: AQ - Aqueous) of fruits of *A. gomezianus* at different places

Test for secondary metabolites	Ashwatpura (22 meter)				Padubidare (147 meter)				Banajalaya (579 meter)				Navanagere (590 meter)				Gubburu (763 meter)				Etinala (949 meter)			
	PE	**CL**	**ET**	**AQ**	**PE**	**CL**	**ET**	**AQ**	**PE**	**CL**	**ET**	**AQ**	**PE**	**CL**	**ET**	**AQ**	**PE**	**CL**	**ET**	**AQ**	**PE**	**CL**	**EA**	**AQ**
ALKALOIDS																								
1. Wagner's test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
2. Dragenodorff's test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
3. Mayer's test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
PHENOL																								
1. Phenol test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
2. Ellagic acid test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
FLAVONOIDS																								
1. Shinoda's test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
2. Zinc-hydrochloride acid test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
3. Alkaline reagent test	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
4. Flavonoid test	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-

TANNINS																								
1. Ferric chloride test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
LIGNINS																								
1. Lignins test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
STEROIDS																								
1. Salkowski test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2. Liebermann-Burchardt's test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
GLYCOSIDES																								
1. Keller-Killiani test	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+
SAPONINS																								
1. Foam test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	+
2. Haemolysis test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	+

Table 2: Preliminary screening of secondary metabolites

Test for secondary metabolites	Hot Extraction			
	PE	**CL**	**ET**	**AQ**
Alkaloids				
Wagner's test	+	-	+	+
Dragenodorff's test	-	-	+	+
Mayer's test	+	+	+	+
Phenol				
Phenol test	+	+	+	+
Ellagic acid test	+	-	+	+
Flavonoids				
Shinoda's test	-	-	+	+
Zinc-hydrochloride acid test	-	-	+	+
Alkaline reagent test	+	+	-	-
Flavonoid test	+	+	-	-
Tannins				
Ferric chloride test	-	-	-	-
Lignins				
Lignins test	-	-	-	-
Steroids				
Salkowski test	-	-	-	-
Liebermann- Burchardt's test	-	-	-	-
Glycosides				
Keller-Killiani test	-	-	+	+
Saponins				
Foam test	-	-	-	-
Haemolysis test	-	-	-	-

Note : PE - Petroleum ether: CL - Chloroform: ET - Ethanol: AQ - Aqueous

Table 3: Percentage of secondary metabolites at different altitudes /places of fruits of *A. gomezianus*

Sl. No.	Factors/ Places	Ashwatpura (22 MSL)	Padubidare (147 MSL)	Banajalaya (579 MSL)	Navanagere (590 MSL)	Gubbure (763 MSL)	Etinala (949 MSL)
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	Alkaloids	11±1.00	37.00±3.00	7.7±0.38	26.67±3.40	15.33±2.21	10±2.00
2	Phenols	.98±0.20	0.88±0.06	0.59±0.19	0.78±0.13	1.13±0.09	1.50±0.11
3	Flavonoids	0.41±0.21	0.66±0.33	0.73±0.50	0.53±0.17	1.28±0.33	0.46±0.16
4	Tannins	0.23±0.05	0.20±0.02	0.16±0.02	0.18±0.00	0.16±0.02	0.23±0.02
5	Steroids	0.23±0.05	0.20±0.02	0.16±0.02	0.18±0.00	0.16±0.02	0.25±0.03
6	Lignins	5.28±2.00	3.00±0.75	1.76±0.37	5.33±2.55	2.91±0.78	5.61±2.87

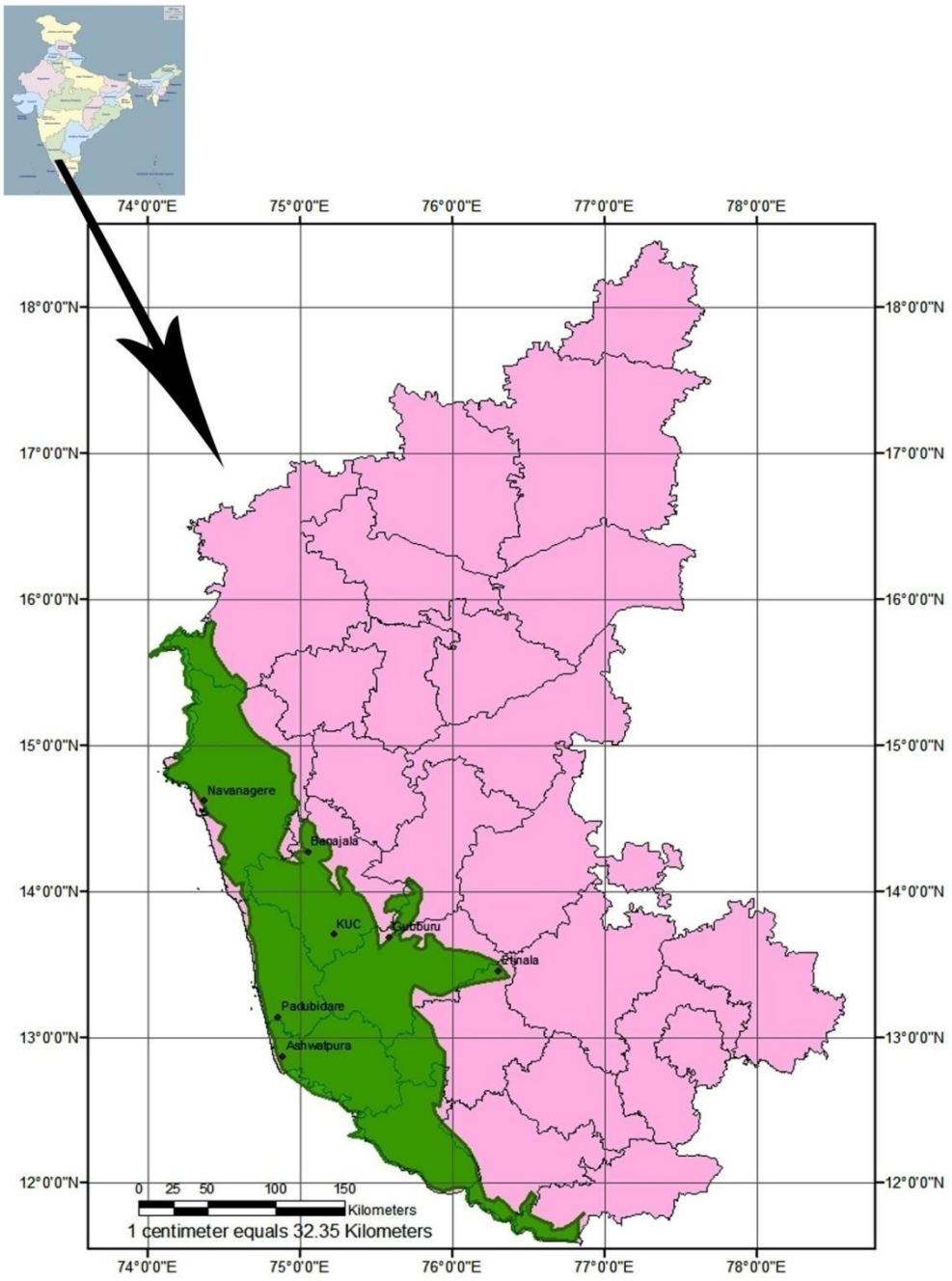
(Mean± SD: n=6)

Table 4: Quantitative estimation of secondary metabolites

Factors	I	II	III	Mean± SD
Alkaloids	7.90	7.50	7.70	7.70 ± 0.40
Phenol	0.53	0.55	0.69	0.60 ± 0.09
Flavanoids	0.73	0.72	0.72	0.75 ± 0.06

Table 5: Qualitative separation of alkaloids, phenols, flavonoids, glycosides, steroids and saponins by TLC (UV254nm) of *A. gomezianus* (Colour of the spot is observed by visual, distance traveled by solute and solvent are expressed in cm along with Rf and hRf values are appended)

Sl. No.	Colour spot	Distance traveled by solute (cm)	Distance traveled by solvent (cm)	Rf	hRf (Rf multiplication with 100)
	Alkaloids				
1	Green	2.3	6	0.38	38
2	Blue	4	6	0.6	60
3	Light green	5.3	6	0.88	88
	Phenols				
1	Blue	0.5	6.5	0.07	7
2	Dark Blue	1	6.5	1/6=0.6	10
3	Blue	1.5	6.5	1.5/6=0.88	20
	Flavonoids				
1	Dark blue	3.5	6.5	3.5/6.5=0.53	53
2	Yellow	4.5	6.5	4.5/6.5=0.69	69
3	Yellow blue	5.5	6.5	5.5/6.5=0.84	84
	Glycosides				
1	Light Green	2	6.3	2/6.3=0.31	31
2	Pink	4	6.3	4/6.3=0.63	63
3	Pink	5	6.3	5/6.3=0.79	79
	Steroids				
1	Light Green	0.5	6	0.5/6=0.08	0.08×100) = 8
2	Greenish black	4	6	4/6=0.66	0.66×100 = 66
	Saponins				
1	Intense yellow	4.5	6	4.5/6=0.75	0.75×100 = 75



Map 1.Sampling sites of Central Western Ghats

1.Ashwathpura,Dhakshina kannada 2.Padubidare,Udupi 3.Banajala,Shimoga, 4.Navanagere,Uttara kannada
5.Gubburu,Chikamagalur 6.Etinala,Hassa 7.Kuvempu Univesrity Campas

Map 1: Sampling sites of Central Western Ghats

Fig. 1: A Detail, of processing and preservation of *Artcoacrpus gomezianus*

Fig. 2: The habitat and fruits of *Zizyphus rugosa* Lam.

STEM CELLS AND ITS THERAPEUTIC APPLICATION

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Abstract:

A population of undifferentiated cells are called stem cells. They can divide through mitosis and produce more stem cells. There are two main broad types of stem cells: 1) Embryonic stem cells 2) Adult stem cells. In this study we saw therapeutic applications of stem cells in dental, cardiovascular, brain, cornea, cancer and hematopoiesis. Stem cells are also used to prevent and for treatment of various diseases. Despite the considerable advancements in stem cell biology, their usefulness is constrained by problems including the moral debates surrounding embryonic stem cells, tumour development, and rejection. Many of these restrictions are being overcome, though, and this could result in significant advancements in the treatment of disease.

Introduction:

A population of undifferentiated cells known as stem cells have the capacity to proliferate a great deal (self-renewal), often develop from a single cell (clonal), and differentiate into many types of cells and tissues (potent). There are numerous, varyingly potent sources of stem cells. Pluripotent cells are developed from the inner cell mass of the embryo by embryonic stem cells, and induced pluripotent cells are created by reprogramming somatic cells (Mojgan.B, *et al.,* 2021). From all three germ layers, pluripotent cells can develop into tissue (endoderm, mesoderm, and ectoderm). The mesenchymal stem cells that give rise to adipose tissue, bone, and cartilage are examples of multipotent stem cells that can develop into tissue from a single germ layer.

Stem cells are a population of undifferentiated cells that have the ability to self-renew, frequently develop from a single cell (clonal), and differentiate into a wide variety of cells and tissues (potent). There are many sources of stem cells, all of variable strength. Embryonic stem cells form pluripotent cells from the inner cell mass of the embryo, and somatic cell reprogramming produces induced pluripotent cells. Pluripotent cells have the capacity to differentiate into tissue from all three germ layers (endoderm, mesoderm, and ectoderm) (Derakhshan *et al.,* 2018). Multipotent stem cells, which can grow into tissue from a single germ layer, include the mesenchymal stem cells that give rise to adipose tissue, bone, and cartilage. Despite the considerable advancements in stem cell biology,

their usefulness is constrained by problems including the moral debates surrounding embryonic stem cells, tumour development, and rejection. Many of these restrictions are being overcome, though, and this could result in significant advancements in the treatment of disease. In this review, the topic of stem cells is introduced, along with the definition, history, and classification of these cells, as well as their use in regenerative medicine (Axel. S. *et al.,* 2015).

Stem Cells in Dental Application

Stem cell therapy is now-a days mostly used in various fields especially in dentistry as it provides better physiological structure and functions and also allows for quick and better healing. Adult stem cells and induced pluripotent stem cells (iPS) are mainly used for treatment of oral and maxillofacial defects. Oral derived stem cells are multipotent, which only differentiate into limited types of cells. There are various human dental stem cells like Dental pulp derived stem cells (DPSC), Stem cells from human exfoliated deciduous teeth (SHED), Periodontal ligament stem cells (PDLSC), Dental follicle stem cells (DFSC) and Stem cells from apical part of papilla (SCAP).

Dental stem cells are either mesenchymal stem cells which are derived from deciduous teeth, dental pulp, dental follicle and periodontal ligament or dental epithelium stem cells which are derived from incisors and molars which has potential for continuous growth. Dental stem cell therapy is mostly used to restore the anatomy and function of damaged tissue through regeneration. Continuous root formation, regeneration of pulpal tissues, reconstruction of periodontium, transplantation and replantation, root bio-engineering are main goals of regenerative dentistry.

Various applications of regenerative dentistry have been studied on diseased or damaged structures.

1) Regeneration of Pulp

For the regeneration of pulp, patients' own cells can be used because the risk of immune rejection gets reduced. Bleeding and clot formation in the root canals is due to over- instrumentation and the canal is irrigated with antimicrobials (Madiyal *et al.,* 2018). If scaffolding is used for regeneration it should contain growth factors, morphogenic protein, nutrients and antimicrobials. Three-dimensional cell printing are newer approaches that can be further researched.

2) Regeneration of Teeth.

Scaffolding that has to achieve odontogenesis should contain epithelial and mesenchymal stem cells or should have the capacity to differentiate into the epithelial and

mesenchymal germ layers as regeneration of tooth requires mesenchymal and epithelial layers interaction between them.

3) Regeneration of Periodontium.

Periodontium is a connective tissue composed of cementum, periodontal ligament, alveolar bone and gingival tissue. Periodontal plastic surgery requires tissue engineering which uses scaffolding and a matrix that grows over it (Madiyal *et al.,* 2018). To increase the production of cells to close the defect, various growth factors are used. Bone marrow stem cells, platelet rich plasma plays a great role in regeneration of periodontium.

Stem Cells in Cardiovascular application.

Cardiovascular, Coronary Heart disease and heart failure are leading causes worldwide. Heart diseases mostly involve damage to blood vessels. Stem cell transplantation is mostly used as a new treatment option for various diseases including heart disease. Two theories have been come up to explain the source of regenerated cardiomyocytes in adult mammals.

1. A progenitor or stem cells or mature cardiomyocytes re-enter the mitotic cell cycle (Pande. A, 2015). There are also many new theories coming up for the mechanism of regeneration for recovery. 1. Heart muscle cells are regenerated.
2. Regeneration of damaged heart tissue by stimulation of growth of new blood vessels.
3. Secretion of growth factors (Rouf *et al.,* 2016).

Types of cells for transplantation especially for heart diseases are Embryonic stem cells, Induced Pluripotent stem cells (iPSCs), Skeletal Myoblasts, Bone Marrow Derived stem cells and Cardiac progenitor cells.

Some Clinical applications of stem cells in the heart are explained.

1. Acute Myocardial Infarction.

In a trial, patients with acute myocardial infarction received intracoronary bone marrow infusions where Bone marrow-derived mononuclear cells (BMMNCs) and mesenchymal stem cells were used for study. BMMNC therapy showed the reduction in death number, recurrent myocardial infarction and stent thrombosis in patients with heart disease. In another study, infarct-related artery (IRA) infusion of CD34+ cells in patients with acute myocardial infarction, patients underwent IRA infusion of autologous bone marrow-derived CD34+ cells after coronary stenting. CD34+ cells are haematopoietic stem cells that have been shown to improve perfusion and function in myocardial and limb ischemia models by stimulating neovascularization endothelial lineage differentiation and through the secretion of proangiogenic factors (Pande. A, 2015).

Intramyocardial injection of adipose derived mesenchymal stem cells combined with ghrelin administration inhibited host cardiomyocyte apoptosis, reduced fibrosis and improved cardiac function.

2. Auricular Cartilage Regeneration.

Auricular cartilage regeneration is Tissue engineering cell based therapy using adipose derived stem cells (ASCs) and pluripotent stem cells. A study was done where ASCs were injected in the midportion of a surgically created auricular cartilage defect in rabbits. ASCs were observed to have regenerative effects on the auricular cartilage defects.

Stem Cells in Brain Application.

The Stem cells are undifferentiated cells which are capable of themselves renewing with the capacity to regenerate missing tissues and help to treat diseases. It nowadays has an important role in the renewal of traumatic brain injury (TBI). It is one of the main reasons for the number of deaths for the people living in industrialised countries. It is caused by its external mechanical force responsible for brain dysfunction, it has the effect which can be temporary damage, or else permanent dysfunction in many cases of TBI. Surgeries are often suggested for more serious injuries so that the monitoring can be done with ease and treat high intracranial pressure (ICP) to reduce. Elevated ICP performed by extraventricular drainage, by intraparenchymal catheter or the combined catheter. The substances like, hyperosmolar which is mannitol and the hypertonic saline solution is also the other way for the treatment route, the TBI is well classified into two
phases.

1. Primary Phase

This phase occurs at the time of exposure to external force which results in the damage, which causes mechanical breakdown of the brain tissue.

2. Secondary Phase

In this phase it has an onset about hours after the traumatic incident which represents the main cause of the worsening in the evolution of the Traumatic Brain Injury.There are many treatments for neurodegenerative diseases using the stem cells; it also plays a very important role in Cell based therapy mechanisms. Such as:

1. Anti-Inflammatory Regulation

It is important for the Occurrence of NP symptoms after the injury in the nerve, the inflammation during the injury releases a large number of chemical mediators like cytokines, chemokines, also lipid mediators, where the stem cells are used against these inflammation as a Anti -inflammation as these stem cells have cytokines which shows analgesic effects it has strong

immunosuppressive effects.

2. Weakening and Reversing Central Sensitization.

It is one of the most important mechanisms in the spinal as it is characterised by increased neuronal excitability which leads to the NP. After the injury there is release of excitatory amino acid in the spinal dorsal horn where the N-methyl-d-aspartate (NMDA) receptor is continuously activated for the purpose of maintaining the afferent nerve transmission to the sensory brain during injury.

3. Neuroprotection

The Glial-derived Neurotrophic factor (GDNE) is the important growth factor that successfully can reverse and can also normalise the NP which in turn plays an important factor for the neuroprotection. Here in this case the increase in VEGF caused due to Oxaliplatin which is almost similar to the cancerous neuralgia can be treated using stem cell therapy as these can play a very unique and balancing role in reducing the pain caused by the VEGF.

4. Mesenchymal Stem Cell (MSC)

MSC holds a very promising therapy effect as it is used for radiation. Injury by inhibiting inflammatory response & the regeneration of the injured tissues. Stem Cell in Cornea Application

The cornea is the transparent anterior part of the eye that is in a circular window which has a high refractive power which directs the light to the retina and also its optical function is an important protection shield which defends the interior structures of the eye from any kind of damage. Along with this the diabetes complications have nowadays recently become a prominent issues due to which there is a lot of complications include many disorders such as retinal, renal and neuronal which is related to the one of the most prominent issues which is also known as the Diabetic retinopathy (DR) these is defined as an vascular alteration in the retinal cells, which has the potential to cause vision loss and even forever blindness. The stem cell transplantation is considered to be a modern solution for the treatment of DR which has a promising effect on interference which can restore the damaged tissues as well as cells. The retinal stem cell transplantation is considered to be the most viable alternative therapy for patients with DR who want to regain their vision. In case of the adult their cornea stem cells reside in their limbal area, where the existence of limbal epithelial stem cells (LESCs) greatly progressed stromal and endothelial stem cells in the adult cornea. Recently the limbal localization of corneal epithelial stem cells are widely accepted. The limbal stem cells that reside in the basal layer of the limbal epithelium are interspersed with the early transient amplifying cells (TACs). Here the cell

differentiation mostly occurs as the cells migrate. These migrations are in two directions: first is toward the surface of the epithelium and the second is toward the centre of the cornea. The limbal epithelial stem cells have a quick proliferation response after the wounding which shows that the immediate wound healing response is the main function of TAC. The Limbal epithelial cells is well characterised according to its ability and its structures which is as follow:

1) The Morphology and Cell Size:

The resident stem cells in different adult tissues have undifferentiated phenotypes which are characterised by small cell size and high nucleus to cytoplasm ratio (N/C), where the nuclear size and the content of DNA is constant but the volume of cytoplasm is changed accordingly when there is new protein formation. In the studies it is observed that limbal basal cells have smaller diameter which is around 10 micrometre and it has larger N/C ratio which also have higher cell densities than central corneal or the limbal suprabasal cell layers.

2) The Plasticity:

The ectodermal origin of the corneal epithelium is differentiation of the LESCs in to different types of cell in the epithelial and neuronal lineages which is well demonstrated in the corneal cells that could be differentiated into hair follicle cells when it is placed over the hair forming embryonic dermis. Here there is differentiation that occurs in two steps in the corneal cells; the first one is that it shows limbal basal cell characteristics and then it is expressed as markers of epidermal differentiation and the formed hair. The second differentiation could be along the rod photoreceptor lineage which could be in vitro or in vivo so that the LESC can serve as a source for the treatment of the retinal degenerative diseases like age related macular degeneration or the retinitis pigmentosa.

3)Self-Renewal:

The stem cell is a quiescent which rarely undergoes cell division which results in the daughter cells. Here the transient amplifying cells (TACs) multiply to give rise to high numbers of differentiated cells which ensure the normal tissue homeostasis.

The corneal stem cells used for the treatment of ocular surface diseases which has become a widely used method for the improvement of the results of this intervention also properly exploit the recent research of corneal stromal and endothelial stem cell research. The development of the new therapeutic strategies comes into play so that the regenerative potential of cornea specific stromal precursor cells can provide the therapeutic tools that make it possible for the regeneration of corneal ulcers also the injuries with transparent avascular scar's. With the use of corneal stromal stem cells in the

tissue engineering for the components of artificial corneas is possible for endothelial replacement by the posterior deep lamellar keratoplasty from heterologous tissues which is nowadays a prominent surgical procedure.

Stem Cells in Cancer Application.

An important global health concern and the number one killer, cancer. By 2040, it is predicted that there will be more than 29 million new instances of cancer worldwide, accounting for around 1 in 6 fatalities. The lack of a definitive cancer treatment has been caused by a number of causes, including the failure of conventional treatments, drug resistance delayed diagnosis and the absence of a robust immune system in infected patients. Most notably, the primary causes of cancer death are metastasis following treatment and tumour recurrence following resection. Therefore, therapeutic approaches that can treat or stop tumours from metastasizing and recurring could represent a significant advance in the field of cancer therapy. The Cancer Genome Atlas (TCGA) and other organisations' pan-cancer initiatives have demonstrated that malignancies arising from various tissues have comparable genetic fingerprints. A basal-like molecular profile is seen in some breast and bladder malignancies, and it is defined by basal cell cytokeratin expression and p63 activation. Additionally, high-grade serous ovarian cancer and basal-like breast cancer share a number of biological characteristics, including actionable targets. Origin of Cancer Stem Cells (CSCs) as Stem Cell Malignancies The assumption that cancer is a stem cell malignancy and the introduction of CSCs as being in charge of metastasis and tumour recurrence are being supported by mounting evidence. These malignancies' suggested processes include cell-cell fusion, horizontal gene transfer, genetic instability, and the cell microenvironment. In light of this, a number of hypotheses about the origin of CSCs have been put forth, some of which include disrupted tissue-resident stem cells, such as specific adult stem cells and their precursors, dedifferentiated somatic cells, and improperly activated residual embryonic cells in adult tissues (Bharati, M *et al.,* 2021).

1) Particular Stem/Progenitor Cells for Tissues

Adult stem cells, sometimes referred to as tissue-specific stem/progenitor cells, are an uncommon cell type with the ability to regenerate themselves.

As well as multi-lineage differentiation skills. These cells are found in a variety of tissues, including the intestines, breasts, lungs, ovaries, and prostate, and they serve as either a repair or maintenance mechanism for those tissues. In a "classical paradigm," it has been presumptive that each niche space of the adult tissues will invariably produce one new stem cell and one daughter cell as a result of the asymmetric mitotic division.

However, in a "neutral competition model," each daughter cell, and even fully differentiated cells, are presumptively assumed.

2) Reprogramming Genes to Produce Stem/Progenitor Cells

By converting differentiated somatic cells into the pluripotent state by inducing four transcription factors (TFs)—Pou5f1, Sox2, c-Myc, and KLF4—Takahashi and Yamanaka achieved a significant breakthrough in 2006. Since the majority of these TFs are oncogenes, it has been postulated that genetic and epigenetic changes in differentiated somatic cells will activate dedifferentiation processes in adult cells and cause them to become CSCs. In this case, the formation of a state resembling pluripotency is caused by the steady downregulation of genes involved in differentiation in somatic cells and the overexpression of stemness-related genes. Human mammary epithelial cells that resemble basal tissue and differentiated airway epithelial cells have both shown this transition. Similar to this, NF-B is activated in intestinal cancers.(Miao *et al.,* 2019).

3) Cancer Vaccine Using Pluripotent Stem Cells (PSCs)

The use of PSCs as a universal preventative cancer vaccine is one of the new prospective applications of PSCs in cancer research that has been highlighted recently. Although PSCs and CSCs share several stemness characteristics and can both constitute a hazard to the body, this concept may initially seem incongruous (Kim *et al.,* 2019)

The ability to grow genetically stable PSCs on a large scale in vitro makes it possible to produce huge quantities of CSC-specific antigens. On the one hand, the conditioned medium of PSCs per se can offer a setting akin to the embryonic setting that can support cancer treatment through differentiation therapy, and on the other hand, PSCs can be viewed as a valuable source to have significant numbers of CSCs-specific antigens [Mojgan barati *et al.,* 2021]. To date, preventive vaccinations against colon, lung, and ovarian cancer have been created using inactivated PSCs in animal models. Through a variety of methods, such as the attraction of immunomodulatory MSCs, myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs) , cancer cells can inhibit the activation of immune cells when in the immunosuppressive environment of tumours.

Stem Cell in Regeneration

Hematopoietic Stem Cell Factors:

Two crucial characteristics that hematopoietic stem cells (HSCs) have are self-renewal and differentiation. Hematopoiesis preserves these characteristics of HSCs. Long-term and short-term HSCs are produced as a result of this technique, and they are now often used in the treatment of several haematological illnesses. HSCs are used clinically to replace damaged bone marrow after chemotherapy or to replace bone marrow in

individuals with aplastic anaemia, congenital neutropenia, sickle cell anaemia, or thalassemia. HSCs' capacity for self-renewal guarantees sustained hematopoiesis after transplantation. However, because they lose their capacity for self-renewal after a few passages, HSCs must be infused in vast quantities in order to reach their target site and meet the needs. As a result, a deeper understanding of ex vivo HSC growth is required.

1) Hematopoiesis

Self-renewability and multipotency were found to be the two main properties of HSCs by in vivo evidence and colony-forming experiments. The canonical lineage model of hematopoiesis places the self-renewability of HSCs at the top of the hierarchy (Zoya M *et al.,* 2022). The first analysis of the mouse HSC cell cycle dynamics showed that 99% of these cells divide every two months, typically staying in the G0 phase.

The results of subsequent in vivo research showed that among all blood cells, latent HSCs have the best ability for self-renewal. This subset of dormant HSCs develops through asymmetric division, keeping the capacity for long-term self-renewal and giving rise to an actively dividing progenitor subset that upholds lineage commitment. Due to asymmetric cell division, ex vivo growth of HSCs has created significant heterogeneity within the HSC pool.

2) Determinants of Dermal Regeneration.

Applications of regenerative medicine have received a lot of attention in an effort to improve replacemental medical techniques for the improvement of wounded skin tissues. Regenerative therapies include a variety of technology techniques, including cell reprogramming, gene targeting, stem cell therapy, soluble medicines, and tissue engineering. One fundamental idea behind these applications is to use engineering methods to provide the right physicochemical and biochemical variables in order to assist a natural wound-healing cascade (Azar. N. D *et al.,* 2019). Growth factors and cytokines are among the bioactive substances that are engaged in various stages of tissue repair and are required to support skin regeneration. Many different cell types release cytokines, which are extracellular signalling proteins that influence activity of different cells, such as immunological cells. Interleukins, lymphokines, interferons, and tumour necrosis factors are a few of them. The study of cytokines in wound healing is difficult because analysis of individual cytokine responses in the human body typically oversimplifies the phenomenon. Additionally, as cytokine responses depend on time and concentration in the wound bed, altering the healing process by controlling the cytokine milieu is a significant problem. Growth factors are signalling proteins that are released at the site of a wound and are necessary for cell-to-cell communication among immune cells, smooth muscle cells,

fibroblasts, myofibroblasts, keratinocytes, and endothelial cells. In order to preserve the biological functioning of transplanted cells used to replace an organ, they can promote angiogenesis.

Conclusion:

Stem cells have various applications in the areas of scientific research & cell therapy. Stem cell therapy has been a boon in the medical field as it has a very promising role & potential in therapeutic application. It also plays an irreplaceable and evident role in the development, regeneration and also the homeostasis. Stem cell in dental treatment is based on 3 elements-restore, retain & revitalise. Here in this study, cells like mesenchymal and epithelial stem cells derived from various dental sources are used for dental treatment mainly for regeneration. For cardiovascular disease, stem cell therapy is a novel & promising way. Cardiac progenitor stem cells, Bone marrow derived stem cells, iPSCs and skeletal myoblasts are some of stem cells used for cardiac repair. It is very known for repairing the damaged cells, exerting anti-inflammatory responses and the production of its immunoregulatory effects. Also the transplantation of retinal tissue is an effective and the most appealing technique for the replacement or repairing of the damaged retinal pigment epithelium of the cell. It also improves the transplantation with success and potentiates. The stem cell is an important function for the therapy of diabetic retinopathy and a variety of diseases.

Various ways and routes have been studied & reported to provide significant benefits in clinical practice. But stem cells are not fully understood for any of the applications. There are many issues related to the use of stem cells.

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