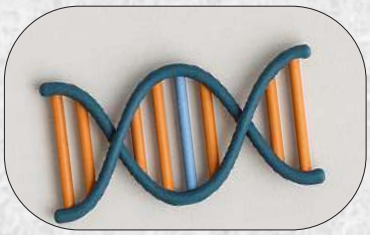
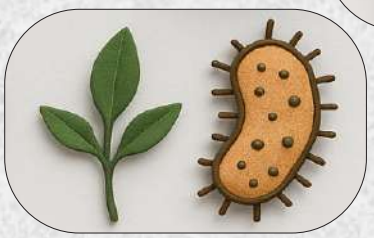
An International Edition

ISBN: 978-93-49938-17-5

Interdisciplinary Perspectives in Pharmaceutical, Chemical and Biological Sciences





Editors

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Published By

Nature Light Publications, Pune

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First Edition: October, 2025

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Published by:
Nature Light Publications, Pune
309 West 11, Manjari VSI Road, Manjari Bk.,
Haveli, Pune- 412 307.
Website: www.naturelightpublications.com
Email: naturelightpublications@gmail.com
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Preface

The book "Interdisciplinary Perspectives in Pharmaceutical, Chemical and Biological Sciences" is a scholarly endeavor that bridges multiple scientific disciplines to foster innovation, sustainability, and holistic understanding in modern research. It brings together contemporary studies from diverse yet interconnected fields—ranging from nanotechnology and green chemistry to pharmacology, aeronautics, and biodiversity conservation—reflecting the essence of interdisciplinary collaboration in solving real-world challenges.

In an era marked by rapid technological advancements and complex global issues, the integration of pharmaceutical, chemical, and biological sciences has become indispensable. The chapters in this volume collectively explore the synergy of these fields through innovative research and practical applications. The discourse begins with frontier topics such as Nanotechnology Using Computer Science in Aeronautics, which underscores the transformative power of computational intelligence in material design and performance enhancement. Subsequent studies, including Manilkara sapota and Its Phytochemical Profile and Crystallography and Structural Determination of Inorganic Compounds, highlight the structural and chemical foundations that underpin drug development and analytical chemistry.

Equally significant are the biomedical and pharmaceutical dimensions presented in chapters on Antimicrobial Resistance and Plasmid Curing in Nosocomial Infectious Diseases, Isoxazole-Substituted Chalcones: Emerging Medicinal Applications, and Modern Approaches in Drug Discovery and Design—each contributing to the ongoing quest for novel therapeutic strategies. The integration of Eco-friendly Green Synthesis and Metal-Organic Frameworks (MOFs) further emphasizes sustainable innovation, aligning chemistry with environmental stewardship.

Bridging traditional and modern paradigms, the chapter on Herbo-metallic Preparations provides an ethnopharmacological perspective, while

topics such as Industrial Effluent Impact on Germination, Plant-Based Bioadhesives, and Biodiversity Conservation reinforce the role of science in ecological balance and sustainable development. Additionally, insights into Regulatory Affairs, Quality Control, and GMP underline the importance of compliance, safety, and global standards in pharmaceutical practices.

This compendium serves as a valuable resource for researchers, academicians, and students seeking to explore interdisciplinary linkages that define the future of scientific inquiry. By blending theoretical insights with applied research, the book aspires to inspire innovation, promote sustainable practices, and encourage collaborative thinking across scientific boundaries.

We extend our sincere gratitude to all contributing authors and reviewers for their valuable efforts in shaping this compilation. Their contributions illuminate the path toward an integrative understanding of science—where chemistry, biology, and pharmaceutical sciences converge to advance human knowledge and societal well-being.

— Editors

Interdisciplinary Perspectives in Pharmaceutical, Chemical and Biological Sciences

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Nanotechnology Using Computer Science in Aeronautics

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DOI: 10.5281/zenodo.17541689

Abstract

This thesis explores the valuation of nanotechnology firms by analyzing the value relevance of non-financial variables in equity valuation. Nanotechnology companies often exhibit high growth potential despite limited immediate revenues, making traditional financial metrics insufficient. Prior research suggests that intangible assets are primary value drivers for these firms.

This study focuses on the application of the Discounted Cash Flow (DCF) method, using the Capital Asset Pricing Model (CAPM) to determine discount rates and assess company-specific risks. Brunswik's lens model is employed to quantify how analysts use non-financial data in asset valuation. Through correlation and regression analysis, the study identifies the significance of individual information factors in evaluating nanotechnology firms.

The predicted equity values are compared against actual market capitalizations to evaluate accuracy. Results indicate that non-financial variables, such as technological innovation, intellectual capital, and strategic partnerships, are critical in valuing nanotechnology institutions. The findings highlight analysts' ability to integrate non-financial information effectively and recommend methods to enhance valuation practices in the sector. Additionally, the study briefly discusses the integration of nanotechnology in aeronautics and computer science, including advancements in sensor technology and nanomaterials.

Keywords: Nanotechnology, Equity Valuation, Non-financial Variables, DCF Method, CAPM, Brunswik's Lens Model, Aeronautics, Sensors, Nano Fibers

Introduction

Nanoscience primarily involves the synthesis, characterization, exploration, and application of nanostructured materials. These nanomaterials are defined by having at least one dimension in the nanometer range. One nanometer is approximately equivalent to the length of 10 hydrogen atoms or 5 silicon atoms arranged in a single line. Research interest in materials with grain sizes ranging

from a few to several hundred nanometers has significantly increased in recent years.

At the nanoscale, certain physical and chemical properties of materials can differ greatly from those of their bulk counterparts. For example, the theoretical strength of nanomaterials can be achieved, or quantum effects may become prominent. Nanocrystals may exhibit lower melting points—sometimes differing by as much as 1000°C—and reduced lattice constants due to the larger fraction of surface atoms or ions, making surface energy a key factor in thermal stability.

Thus, many material properties must be reconsidered, as the surface-to-volume ratio increases significantly at the nanoscale, which has a substantial impact on material performance. Traditionally, properties such as elastic modulus have been measured using macroscopic methods on bulk specimens. However, as fabrication techniques evolve and devices shrink to the nanoscale, traditional bulk property measurements are no longer sufficient to predict behavior.

Although nanotechnology is a relatively new research area, nanomaterials have been used for centuries. For instance, ancient Chinese artisans utilized gold nanoparticles as inorganic dyes to impart red color to ceramic porcelain over a thousand years ago.

Purpose of This Nanotech Report

This report aims to support the Environmental Protection Agency (EPA) in evaluating the potential application of nano-enabled technologies for the remediation of radioactive contamination. Specifically, the report explores technologies that are enabled by nanoscale systems. It serves as a guide for identifying and assessing emerging nanotechnology applications and their implications—both health-related and ecological—for sites contaminated with radionuclides.

Application of Nanotechnology

Nanotechnology is often described in terms of the extremely small size of its components—ranging from atomic dimensions to approximately 100 nanometers. However, its essence lies beyond mere miniaturization. It involves rearranging atoms and molecules, leading to novel material properties and unexpected behaviors. This transition marks a shift from fixed behaviors at the atomic scale to dynamic, tunable properties at the nanoscale.

Researchers from a wide range of disciplines—including colloid science, electronics, chemistry, physics, and genetics—are actively investigating nanotechnology. Its potential applications span numerous fields, particularly in

medicine and engineering.
In medicine, for example, nanotechnology may improve implantable tissue scaffolds, enable tissue regeneration, or even lead to the development of artificial organs.

Examples of Nanotech Applications

- **IBM** has incorporated nanoscale layering in disk drives to harness the giant magnetoresistive effect, leading to higher-density data storage.
- **Gilead Sciences** uses lipid spheres (liposomes), approximately 100 nm in diameter, to encapsulate anticancer drugs for treating AIDS-related Kaposi's sarcoma.
- **Carbon Nanotechnologies**, co-founded by Richard E. Smalley (co-discoverer of buckyballs), is developing flexible carbon nanotubes through more efficient manufacturing processes.
- **NanoPhase Technologies** incorporates nanocrystalline particles into other materials to produce tough ceramics, transparent sunscreens, and catalysts for environmental applications.

Design And Assembly

The design and assembly of artificial nanostructures, the discovery of novel physical effects, and the development of cutting-edge nanodevices represent promising frontiers in nanotechnology. One breakthrough is the discovery of "left-handed" or metamaterials, which exhibit unconventional properties such as inverse refraction, inverse Doppler, and inverse Cherenkov effects.
In nanomaterials science, structural engineering will take precedence over impurity engineering. Materials are no longer raw inputs; they are fabricated directly into functional nanostructures. Nanotechnology's potential is most evident in the creation of nano-devices like electronic gnats, rather than in large-scale industrial applications.
The nanoscale also blurs the line between living and non-living matter. Biological components such as proteins, membranes, and nucleic acids are naturally occurring nanostructures formed through self-assembly. This analogy opens exciting possibilities for nanodevice fabrication inspired by biology—referred to as biomimicry. Examples include artificial pearl growth in mussels and the arrangement of 2D nanostructures via ion bombardment on semiconductor surfaces.

Key Features of Modern Nanotechnology

- Artificial manipulation of nano-objects and manual or automated assembly of nanodevices using a bottom-up approach.
- Intentional interference in chemical self-assembly processes with precise control at the molecular level.
- Design and fabrication of submicron-scale nanodevices integrated into micro, meso, and macro systems.

However, nanotechnology faces several challenges:

- **Size Limits:** Reducing particle size does not always improve material properties. For example, in oxide ceramics, an optimal particle size of 10–20 nm achieves the best balance of hardness and durability.
- **Thermal Instability:** Smaller particles are more prone to thermal instability and phase transitions, reducing long-term durability.
- **Radiation Sensitivity:** Cosmic rays and background radiation can displace atoms in nanostructures, degrading device performance.
- **Thermal Noise:** Thermal vibrations limit the precision of nanodevices like scanning probe microscopes.
- **Contamination Sensitivity:** Even trace impurities can disrupt self-assembly processes. High-purity reagents and cleanroom conditions are essential.

In the future, technical decisions, innovative solutions, and groundbreaking inventions will likely be realized
within specially designed and assembled artificial nanostructures, developed through advanced materials
science. This emerging discipline emphasizes the creation of tailored nanostructures to meet specific functional
requirements. As illustrated in Figure 1, the core concept of materials science highlights the intrinsic
interconnection between composition, structure, properties, processing technologies, and their resulting
applications.

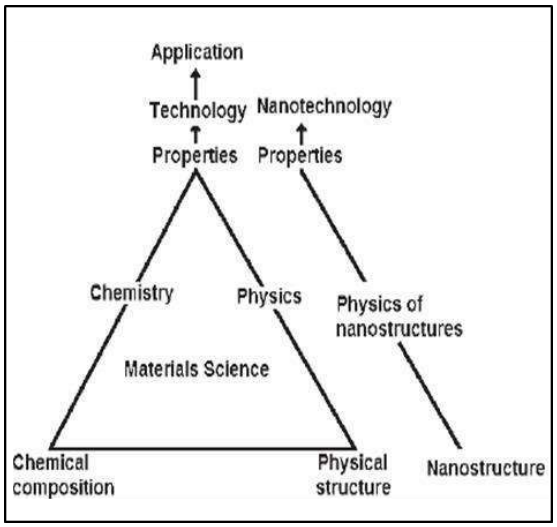

Fig. 1 Fundamental triad of materials science.

Material Potential and Nanotechnology Frontiers

1. Material is not merely a dull bar, blank, block, or ingot—it represents an immense concept, akin to the universe itself. It is the very medium in which new physical laws may be discovered. Currently, there are around 100 pure natural elements listed in the Periodic Table. Based on these, it is theoretically possible to form approximately 10,000 binary (XY), 1,000,000 ternary (XYZ), and over 100,000,000 quaternary compounds, considering only their chemical compositions. This abundance increases dramatically when the physical structure, including nanostructures, is also taken into account.
2. Despite these possibilities, only about 500,000 compounds are currently documented in modern crystallographic databases. This highlights a vast, unexplored frontier of novel compounds with potentially unique properties—offering an exciting and challenging avenue for future nanotechnology research. At present, nanotechnology (NT) remains in its infancy.
3. Ambitious global projects—such as biochips and nanobio-robots for medical applications, or smart dust for space exploration—have served as significant motivators for the intense development of NT. These initiatives could dramatically influence the evolution of human civilization. Countries like the USA, those in the European Community (EC), Japan, Russia, and others have allocated substantial funding for nanotechnology projects. The prospects for NT at the dawn of the 21st century appear highly optimistic. Although the harsh realities of technological and market challenges may temper these

somewhat idealistic visions, the development of nanotechnology is ultimately inevitable—and likely destined for success.

Discussion

1. This study addresses only a limited set of non-financial factors considered when valuing a nanotechnology firm. With a sample size of just 17 evaluated companies, the findings cannot be regarded as fully representative of the entire nanotechnology sector.
2. Additional limitations stem from the experimental setting itself. While controlled experiments offer insights, they cannot fully replicate real-world investment conditions. For example, this study could not account for the qualitative assessment of a firm's management team, which often plays a pivotal role in investment decisions. In real-life scenarios, investors often rely on face-to-face impressions and personal rapport with a company's leadership. These interpersonal dynamics—vital for assessing long-term commitment and alignment of goals—were absent in this analysis.
3. Therefore, the study's outcomes are heavily dependent on the experience and judgment of the participating analysts. Fortunately, the participants were all seasoned investment analysts, whose expertise and commitment contributed to the reliability of the valuation predictions used in this research.
4. Private equity investors typically do not rely on a single valuation estimate. Instead, they calculate multiple discount rates to generate various case scenarios for better-informed decisions. It is also important to note that the participants' ex-post valuations were conducted during October and November 2008. Given the global financial crisis that began in 2008, analysts were likely more cautious in their decision-making than they might have been in 2003, which was the assumed baseline year for company valuation.
5. This study aimed to reflect the primary valuation methods used in practice. It does not attempt to encompass every possible valuation approach or identify a universally optimal method for valuing nanotechnology companies. Nonetheless, the application of Brunswik's lens model in this study provides valuable insights into the relationship between subjective judgment and objective market realities. It highlights how non-financial variables can significantly influence valuation outcomes and should be considered in both theoretical and practical investment contexts.

NASA: Computer Technology Innovations

Carbon Nanotube SPM (Scanning Probe Microscope) Tips

NASA has explored advanced nanotechnology-based tools, such as Carbon Nanotube (CNT) SPM tips, to push the boundaries of precision in computer technology and electronics fabrication. These ultra-sharp and highly conductive tips have transformative potential in nanoscale manipulation and device development. Key features and applications include:

- **Alignment with Moore's Law**

CNT-based tips support continued miniaturization of electronic components, in line with Moore's Law, which predicts the doubling of transistor density approximately every two years.

- **Molecular Manipulation with Sub-Ångström Precision**

Carbon nanotube tips allow for manipulation of individual atoms and molecules with sub-angstrom accuracy, enabling unprecedented control at the nanoscale.

- **Nanoscale Patterning on Silicon Surfaces**

These tools can engrave ultra-fine patterns on silicon substrates, a critical step in the fabrication of nanoelectronic circuits and memory devices.

- **Applications in Advanced Electronics**

CNT SPM tips are utilized in semiconductor device prototyping, high-resolution lithography, and precision diagnostics—contributing to the evolution of next-generation nanoelectronics and quantum devices.

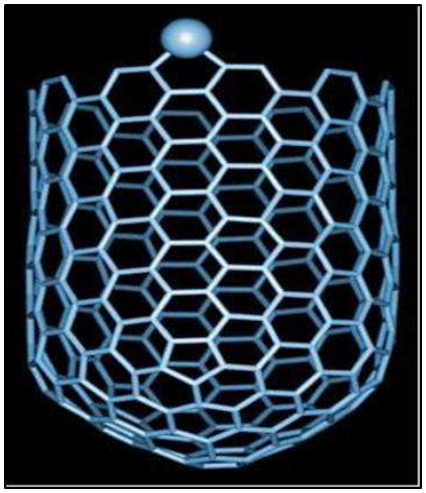

Fig 2. Carbon Nanotube

Materials

Nanotechnology has the potential to significantly enhance the efficiency and effectiveness of material usage across various industries. For instance,

nanotechnologies have improved the functionality of catalytic converters, reducing the required quantity of platinum group metals (PGMs) by up to 95%. This reduction not only lowers the demand for these rare and expensive materials but also lessens the ecological impact associated with mining PGMs, which are typically found in very low concentrations.

Despite these benefits, it's important to note that the manufacturing of precise nanomaterials can be material- and energy-intensive. However, the increased functionality of nanomaterials may enable the replacement of toxic materials while maintaining essential properties such as electrical conductivity, mechanical strength, and thermal transfer.

A notable example is the development of lead-free conductive adhesives using self-assembled monolayer molecules based on nanotechnology. These adhesives could potentially replace lead-based solder, which is widely used in the electronics industry. The use of nanotech-based adhesives not only reduces lead pollution, but also simplifies the manufacturing process by eliminating several steps—such as the application of acid flux, and the need for detergent and water cleaning.

Moreover, nanotechnology plays a significant role in the development of Organic Light Emitting Diodes (OLEDs). OLEDs serve as environmentally friendly alternatives to traditional Cathode Ray Tubes (CRTs), which contain lead, and conventional Flat Panel Displays, which often require mercury. OLED technology not only eliminates the need for these hazardous substances but also offers additional advantages such as lower energy consumption and reduced material usage throughout the product's life cycle.

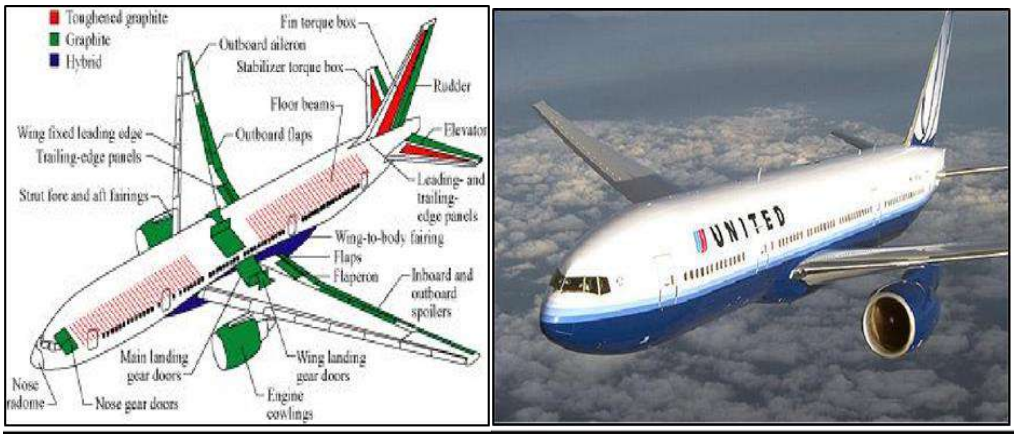

Figure 3: Advanced composite materials used in aeronautics.

Advanced Composite Materials

Traditionally, aircraft structures relied heavily on aluminum, a metal that, while durable, made airplanes heavier and increased fuel consumption. In the 1950s, fiberglass was introduced in aviation, first appearing in the Boeing 707 passenger jet, where it comprised only 2% of the aircraft's structure.

Today, the aviation industry has undergone a significant transformation. Approximately one-third of modern commercial aircraft structures are now made from advanced composite materials, which are known for being stronger and lighter than traditional metals.

Benefits Of Composite Materials in Aviation Include:

- Making aircrafts approximately 20% lighter,
- Resulting in greater fuel efficiency,
- Enhancing aerodynamic performance and structural strength.

Advanced composites often incorporate nanowires arranged in tape or fabric form, which are then placed into a mold under controlled heat and pressure. During this process:

- A resin matrix flows over the nano-fibers,
- Heat is removed, allowing the material to solidify into a strong composite,
- The resulting material can be molded into various shapes.

In certain applications, fibers are tightly wound to further increase strength, making the final product suitable for aeronautical and aerospace components.

In general, Nano sensors can be classified in two main categories:

- Sensors that are used to measure Nano scale properties (this category comprises most of the current market) and
- Sensors that are themselves Nano scale or have Nano scale components. The second category can eventually result in lower material cost as well as reduced weight and power consumption of sensors, leading to greater applicability and enhanced functionality.

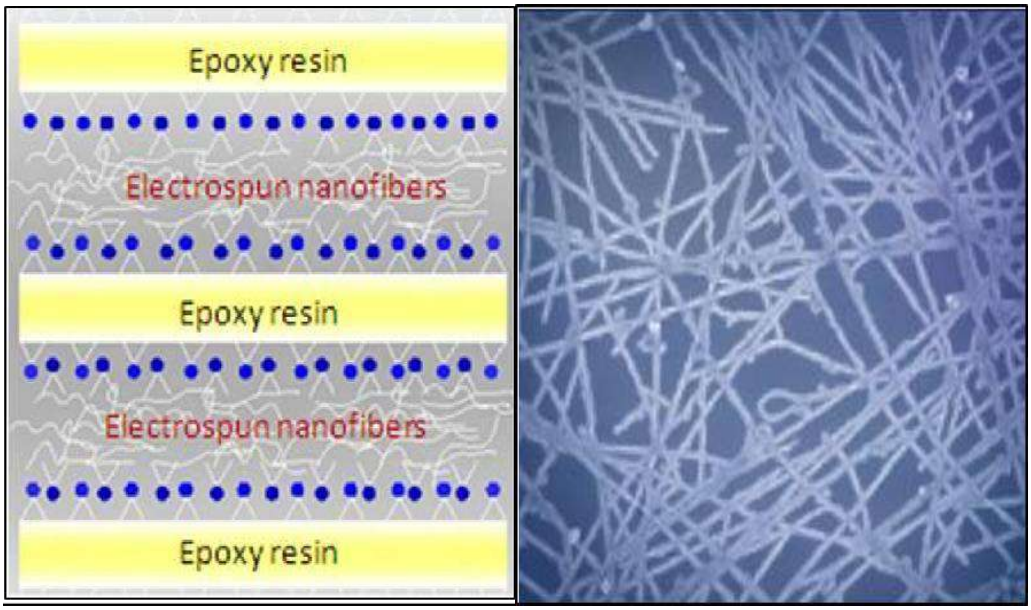

Fig 4. Epoxy Resin and Electrospun Nanofibers Structure.

Fuel Additives

Nanomaterials also demonstrate significant potential as fuel additives and automotive catalysts, as well as catalysts for utility boilers and other energy-generating systems. A notable example is the use of cerium oxide nanoparticles in the United Kingdom, where they are employed as additives in on- and off-road diesel fuels to help reduce harmful emissions.

Manufacturers report that the incorporation of these nanoparticles leads to a 5–10% reduction in fuel consumption, accompanied by a decrease in vehicle emissions, highlighting both environmental and economic benefits.

Sensors

The development and application of sensors based on nanoscale science and technology is expanding rapidly. This growth is fueled by advancements in the microelectronics industry and the increasing availability of nanoscale fabrication techniques.

As illustrated in Figure 5, Carbon Nanotubes (CNTs) form the core of a new chemical sensor platform. These sensors are:

- Easily manufactured
- Reusable
- Capable of accurately detecting a wide range of gases and volatile organic compounds (VOCs)

Such sensors are integrated into battery-powered handheld devices and have the potential to support a wide range of applications, including:

- Monitoring systems for human spaceflight
- Industrial chemical detection
- Medical diagnostics

Conclusions

The primary objective of this thesis was to explore value estimation methods currently used in practice and assess their applicability to nanotechnology firms. Specifically, it sought to model the relationship between predicted company values and the actual equity values of nanotech enterprises.

Nanotechnology was selected due to its nature as an emerging industry, where future developments are highly uncertain. This allowed for the examination of whether valuation techniques could provide meaningful insights into future value. Under the guiding hypothesis that nanotechnology valuation is more of an art than a science, the research aimed to identify and analyze variables typically overlooked in conventional company valuations. To investigate this, a study was conducted involving the evaluation of seventeen nanotech companies.

The findings from this experiment contribute to a deeper understanding of the unique challenges and considerations involved in valuing companies operating at the forefront of nanotechnology innovation.

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Manilkara sapota and Its Phytochemical Profile

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Article DOI Link: https://zenodo.org/uploads/17542190
DOI: 10.5281/zenodo.17542190

Abstract

Plants remain an indispensable source of food, nutrition, and traditional medicine, with *Manilkara zapota* (Sapotaceae) widely utilized for its therapeutic properties. The present study assessed the phytochemical composition of *M. zapota* fruits from two distinct sites of southern Tamil Nadu, India, through qualitative screening, quantitative estimation, and Gas Chromatography–Mass Spectroscopy (GC–MS) analysis. Among the solvents tested, ethanol yielded the highest diversity of metabolites, including alkaloids, flavonoids, phenols, saponins, amino acids, and proteins, while petroleum ether and chloroform extracts contained fewer constituents. Quantitative assays revealed that Site II fruits possessed significantly higher levels of phenols, flavonoids, and triterpenoids compared to Site I, suggesting environmental factors play a role in metabolite accumulation. GC–MS profiling further confirmed this variation, with 12 compounds identified in Site I and 25 in Site II. Key bioactive constituents such as n-hexadecanoic acid, β-amyrin, urs-12-en-3-ol acetate, camphene, and limonene are known for their antioxidant, antimicrobial, anti-inflammatory, and

cytoprotective activities. The findings emphasize the richness of M. zapota in secondary metabolites and validate its ethnomedicinal applications. Moreover, the influence of both solvent extraction and growth environment on phytochemical yield highlights the importance of standardization in plant-based drug research. Overall, this study provides scientific evidence supporting the pharmacological potential of *M. zapota* fruits and establishes a basis for future therapeutic explorations.

Keywords: *Manilkara zapota*, Sapotaceae, phytochemicals, secondary metabolites, GC–MS, pharmacological activity

Introduction

Plants are vital resources for food and medicine, providing essential nutrients and bioactive compounds that support human health. Approximately 80% of people in developing countries rely on traditional medicine as their primary healthcare system (Tran et al., 2020). Compared to synthetic drugs, plant-derived remedies often show greater therapeutic potential with fewer side effects. They are eco-friendly, biodegradable, renewable, and serve as valuable leads in modern drug discovery (Pandey et al., 2011; Forni et al., 2019).

Medicinal activity in plants is attributed to their diverse phytochemical constituents such as alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids (Okwu, 2001). These metabolites contribute to health promotion, economic benefits, and societal development. However, challenges such as insufficient scientific validation and limited funding hinder their wider application (Kamboj, 2000). Historically, many important drugs—including aspirin, digoxin, morphine, and quinine—originated from plants (Gilani & Rahman, 2005).

The Sapotaceae family (order: Ericales) comprises about 800 species of evergreen trees and shrubs distributed across tropical regions. Many members provide edible fruits, latex, and other economically important products. Among them, Manilkara zapota (commonly known as sapodilla, chikoo, or sapota) is widely cultivated in India, Mexico, and Southeast Asia (Fig .1). Traditionally, it has been used for wound healing, inflammation, fever, and gastrointestinal disorders. The fruit is rich in vitamins, fiber, and antioxidants, while its bark, latex, and seeds are employed in various therapeutic and cosmetic applications. Reported pharmacological activities include diuretic, analgesic, antispasmodic, antioxidant, antimicrobial, and anticancer effects. Its seed oil is valued in skincare and hair care formulations, while fruit pulp is beneficial for digestive

and respiratory health.

Fig .1 Manilkara zapota (L.) P. Royen

Plants such as *M. zapota* derive their medicinal value from secondary metabolites produced in response to environmental stresses. Unlike primary metabolites, these compounds are not directly involved in growth or reproduction but play vital roles in defense, pollinator attraction, and ecological adaptation (Korkina et al., 2018). They are classified into major groups such as terpenes, phenolics, alkaloids, and glycosides (Kumar & Pandey, 2013). Polyphenols, especially flavonoids, are abundant and exhibit antioxidant, anti-inflammatory, and antimicrobial activities (Nijveldt et al., 2001; Scalbert et al., 2000). Terpenes, the main constituents of essential oils, contribute to ecological interactions and pharmaceutical applications (Omar et al., 2016).

To identify and characterize these bioactive metabolites, Gas Chromatography–Mass Spectroscopy (GC-MS) is one of the most widely used analytical techniques. GC-MS enables both qualitative and quantitative analysis by separating, detecting, and identifying compounds based on their mass-to-charge ratios (Thomas et al., 2013). It has been applied extensively in phytochemical studies for structural elucidation, isotopic analysis, and compound quantification (Maher et al., 2015). Thus, GC-MS serves as a powerful tool for validating the phytochemical profile and therapeutic potential of *M. zapota*.

In this study, the phytochemical constituents of *M. zapota* fruit extracts collected from two different sites were analyzed using GC-MS, with the objective of identifying and comparing bioactive compounds that may contribute to its medicinal properties.

Methodology

Phytochemical Screening

Collection of Plant Material

Fresh fruits of *Manilkara zapota* L. were collected in February 2025. Two sampling sites were selected:

- Sample I: *M. zapota* plants growing at Murasancode in Kanyakumari District
- Sample II: *M. zapota* plants growing at Kudunulam in Tirunelveli District

Preparation of Plant Extracts (Parekh and Chanda, 2007)

Fresh fruits were collected, washed thoroughly, and air-dried in shade until completely free of moisture. The dried plant material was macerated using a mixer grinder and stored in airtight containers under refrigeration.

Ten grams of powdered fruit were extracted with 250 mL of petroleum ether, ethyl acetate, chloroform, and ethanol separately using a Soxhlet extractor for 8 hours at temperatures below the solvent boiling points. Extracts were filtered through Whatman No. 1 filter paper and concentrated under vacuum at 40 °C using a rotary evaporator. Residues were stored in a freezer until further use.

Qualitative Phytochemical Screening

Each solvent extract was analyzed for different phytochemical constituents using the following methods:

- **Test for Reducing Sugars (Sadhasivam and Manickam, 1996)**

1 mL of filtrate was boiled with 1 mL each of Fehling's solutions A and B. A red precipitate indicated the presence of sugars.

- **Test for Alkaloids (Adegoke et al., 2010)**

0.4 g of extract was mixed with 8 mL of 1% HCl, warmed, and filtered. Filtrates were tested with (a) Mayer's reagent and (b) Dragendorff's reagent. Yellow precipitate (Mayer's) and red precipitate (Dragendorff's) indicated alkaloids.

- **Ninhydrin Test for Amino Acids (Yasuma and Ichikawa, 2000)**

Few drops of Ninhydrin reagent were added to extract and boiled for 2–3 minutes. A bluish-black color indicated amino acids.

- **Ferric Chloride Test for Flavonoids (Raman, 2006)**

Extracts treated with $FeCl_3$ showed blackish-red color, indicating flavonoids.

- **Test for Steroids (Kokate, 1994)**

5 mL extract was dissolved in chloroform and layered with concentrated H_2SO_4. A reddish-brown ring with green fluorescence indicated steroids.

- **Test for Tannins (Trease and Evans, 1989)**

Addition of $FeCl_3$ to extracts produced blue-black, green, or blue-green precipitates, confirming tannins.

- **Test for Terpenoids (Evans, 1997)**

5 mL extract was mixed with chloroform and layered with concentrated H_2SO_4. A

reddish-brown interface indicated terpenoids.

- **Ferric Chloride Test for Phenols (Mace, 1965)**

Extract (50 mg) in distilled water treated with $FeCl_3$ showed dark green color, indicating phenols.

- **Foam Test for Saponins (Kumar et al., 2009)**

Extract shaken with water produced persistent foam for 10 min, confirming saponins.

- **Borntrager's Test for Anthraquinones (Sofowora, 1993; Harborne, 1984)**

Extract treated with chloroform and ammonia produced a bright pink aqueous layer, indicating anthraquinones.

- **Biuret Test for Proteins (Gahan et al., 1984)**

Filtrate treated with $CuSO_4$ and KOH produced a pink color, confirming proteins.

- **Catechin Test (Rinaldo et al., 2010)**

Matchstick dipped in aqueous extract, dried, moistened with HCl, and warmed turned pink/red, indicating catechins.

- **Sugar Test (Sadhasivam and Manickam, 1996)**

Test solution with anthrone and concentrated H_2SO_4 heated to produce green–purple color confirmed sugars.

Quantitative Determination of Non-Enzymatic Antioxidants

- **Total Flavonoids (Naima Saeed et al., 2012)**

Determined using aluminium chloride method with catechin standard. Absorbance measured at 510 nm.

- **Total Phenols (Naima Saeed et al., 2012)**

Measured using Folin–Ciocalteu's reagent and sodium carbonate. Absorbance read at 750 nm.

- **Total Terpenoids (Ghorai et al., 2012)**

Extracts treated with chloroform, H_2SO_4, and methanol. Absorbance measured at 538 nm.

- **GC–MS Analysis (Bagavathi and Ramasamy, 2012)**

The ethanolic extract of M. zapota was analysed using a Perkin-Elmer GC Clarus 500 system equipped with an AOC-201 autosampler and a mass spectrometer. An Elite 5MS fused silica capillary column (30 m × 0.25 mm ID × 0.25 μm df) was employed for the analysis. Helium (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min, and the injection volume was 2 μL. The injector temperature was maintained at 200 °C, while the ion source temperature was also set at 200 °C. The oven was programmed initially at 110 °C with a 2 min

isothermal hold, followed by a temperature ramp of 10 °C/min up to 200 °C, and then 5 °C/min up to 280 °C, where it was held for 9 min. Mass detection was carried out at 70 eV with a scan range of 45–450 Da and a scan interval of 0.5 s. Data acquisition and processing were performed using Turbo Mass Ver-5.2 software, and the relative abundance of compounds was calculated by peak area normalization.

Results

Phytochemical Screening

Bioactive plant products are gaining increasing attention worldwide, and several pharmacological effects are being investigated for their phytochemical constituents. Manilkara zapota, a member of the Sapotaceae family comprising nearly 65 genera and about 800 species, is recognized for its nutritional and therapeutic properties. Sapota fruit is rich in glucose and calories, serving as a good source of energy. In addition, antioxidants such as ascorbic acid, polyphenols, and flavonoids contribute to skin health by delaying premature ageing, while minerals including calcium, phosphorus, copper, selenium, magnesium, and iron strengthen bones, alleviate joint discomfort, and reduce the risk of osteoporosis.

Qualitative Analysis of Secondary Metabolites

The secondary metabolites of *M. zapota* fruits grown at selected sites demonstrated variation in their availability across different solvent extracts. Petroleum ether and chloroform extracts showed similar profiles, with petroleum ether extracts confirming the presence of steroids and reducing sugars, while chloroform extracts revealed steroids, reducing sugars, and sugars. Ethanolic extracts exhibited a wide range of metabolites, including steroids, triterpenoids, reducing sugars, phenolic groups, proteins, alkaloids, flavonoids, tannins, saponins, amino acids, and sugars.

The aqueous extract of fruits from site I plants contained triterpenoids, reducing sugars, phenolic groups, flavonoids, tannins, and sugars. A similar pattern was observed in fruits from site II. Ethyl acetate extracts from site I and site II revealed the presence of steroids, reducing sugars, tannins, saponins, and sugars.

Conversely, certain metabolites were absent in different extracts. Steroids, proteins, alkaloids, catechins, anthraquinones, and amino acids were not detected in the aqueous extracts from both sites. Petroleum ether extracts lacked triterpenoids, phenolic groups, proteins, alkaloids, flavonoids, tannins, anthraquinones, saponins, amino acids, and sugars. Chloroform extracts showed

the absence of triterpenoids, phenolic groups, proteins, alkaloids, flavonoids, catechins, tannins, anthraquinones, saponins, and amino acids. Ethanolic extracts did not contain catechins or anthraquinones, while ethyl acetate extracts from both sites lacked triterpenoids, phenolic groups, proteins, alkaloids, flavonoids, catechins, anthraquinones, and amino acids.

Quantitative Analysis of Secondary Metabolites

The quantitative analysis of aqueous and ethanolic fruit extracts of *M. zapota* from the selected sites was carried out for total phenols, flavonoids, and triterpenoids. The ethanol extracts consistently showed higher levels of these secondary metabolites compared to aqueous extracts.

In the ethanolic extract of fruits from site II plants exhibited significantly elevated metabolite concentrations compared to fruits from plants of site I. Phenol content was 90.1 ± 0.017 mg/100 g dry wt. in the site II plant fruit extract, while only 37 ± 0.01 mg/100 g dry wt. was observed in the fruits from plants of site I. Similarly, flavonoid levels were 106 ± 0.012 mg/100 g dry wt.in the extract of fruits from site II, nearly double that of extract of fruits from site I plants (45.3 ± 0.021 mg/100 g dry wt.). Triterpenoid content was 61.9 ± 0.03 mg/100 g dry wt. at site II, slightly higher than site I (57.8 ± 0.016 mg/100 g dry wt.).

In aqueous extracts, phenolic content was 22.65 ± 0.043 mg/100 g dry wt. at site I and 27.85 ± 0.051 mg/100 g dry wt. at site II. Flavonoid concentration was 25.7 ± 0.01 mg/100 g dry wt. at site I, increasing to 39.3 ± 0.031 mg/100 g dry wt. at site II. Triterpenoids were 44.7 ± 0.003 mg/100 g dry wt. at site I and 49.9 ± 0.02 mg/100 g dry wt. at site II. Analysis of variance revealed statistical significance with a P value of 0.0201 at the 0.05% level.

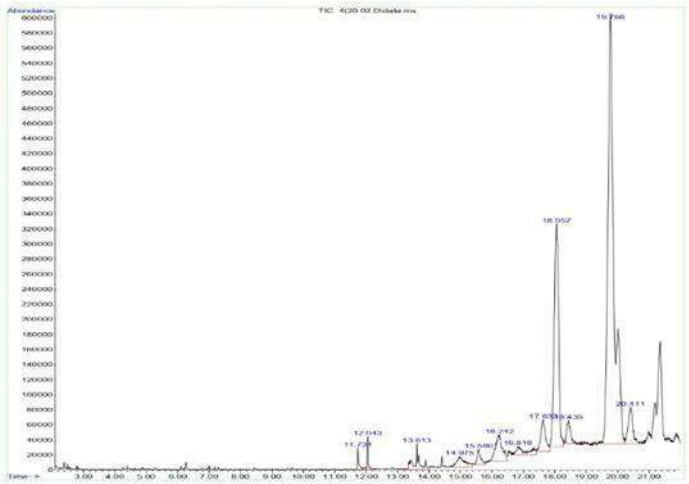
GC–MS Analysis

The ethanolic fruit extract of *M. zapota* was analyzed using gas chromatography–mass spectrometry (GC–MS). The extract from site I revealed the presence of 12 distinct compounds (Fig .2). Structural identification was carried out by comparing mass spectra with reference data in Turbo Mass Ver-5.2 software. The identified compounds included n-Hexadecanoic acid, Hexadecanoic acid ethyl ester, 2(1H)-Naphthalenone octahydro-4a-methyl-7-(1-methylethyl)-(4a.alpha.,7beta.,8a.beta.), Pyrene hexadecahydro-, Tetratetracontane, 1,8-Dioxa-5-thiaoctane, 8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl-, 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine, 2H-3,9a-Methano-1-benzoxepin octahydro-2,2,5a,9-tetramethyl- [3R-

(3alpha.,5a.alpha.,9.alpha.,9a.alpha.)], β-Amyrin, 2-Ethylacridine, Urs-12-en-3-ol acetate (3β) [or α-Amyrin acetate], and Lanosta-8,24-dien-3-ol acetate (3β).

Among these, Urs-12-en-3-ol acetate (3β) or α-Amyrin acetate exhibited the highest peak area (56.45%) with a retention time (Rt) of 19.768 minutes, indicating its dominance in the extract. In contrast, n-Hexadecanoic acid had the lowest peak area (0.59%) at an Rt of 11.73 minutes.

Fig. 2 GC-MS Profile of M. zapota fruits from site I plants



In contrast, the ethanolic fruit extract of plants from site II revealed a more diverse phytochemical profile, with 25 compounds eluted at different retention times (Fig .3). Compound identification was confirmed using the NIST library. The detected compounds included 1-Octanol, 2-butyl; Camphene; Tridecane; Hexadecane; 1,2-Pentadiene; (E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene; trans,cis-2,6-Nonadien-1-ol; 3-Ethyl-3-methylheptane; Bicyclo[4.3.0]nonane, 3-methylene; 2-Propyn-1-amine, N-methyl; 7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl; 1,9-Nonanediol, dimethanesulfonate; Methyl 2,3-di-O-acetyl-4,6-di-O-methyl-α-D-mannopyranoside; n-Hexadecanoic acid; 2H-1-Benzopyran-2-one, 5,7-dimethoxy; 9,17-Octadecadienal,(Z); 7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy-; [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester; 1,2-Dihydroanthra[1,2-d]thiazole-2,6,11-trione; Silicic acid, diethyl bis(trimethylsilyl) ester; 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene; 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine; 1,2-Bis(trimethylsilyl)benzene; Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl; and 2-Ethylacridine.

The compound 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene (21st

compound) exhibited the highest peak area (27.19%) at an Rt of 16.516 minutes. In contrast, Bicyclo[4.3.0] nonane, 3-methylene (9th compound) displayed the lowest peak area (0.22%) with an Rt of 8.526 minutes.

Fig. 3 GC-MS Profile of M. zapota fruits from site II plants

Discussions

The phytochemical screening of *Manilkara zapota* fruits extracted with different solvents revealed notable variations. Petroleum ether extracts contained only steroids and reducing sugars, while chloroform extracts showed steroids, reducing sugars, and sugars. Ethyl acetate extracts included tannins, and ethanolic extracts exhibited the richest phytochemical profile, containing steroids, triterpenoids, alkaloids, flavonoids, phenols, saponins, amino acids, and proteins. Similar findings of glycosides, phenolics, and terpenoids were reported by Mahajan and Badgujar (2008). The therapeutic activities of these compounds, including antioxidant, anti-inflammatory, antibacterial, and protective roles, are well documented (Okwu, 2001; Waqas-Ahmed et al., 2016; Williams et al., 2004).

Quantitative analysis showed higher concentrations of phenols, flavonoids, and triterpenoids in fruits from Site II compared to Site I, likely due to environmental stress factors (Akula & Ravishankar, 2011). Previous reports also confirm site-dependent variation in phytochemical content (Sebaa & Cherifi, 2021; Singh et al., 2016). Members of the Sapotaceae family are rich in secondary metabolites such as catechins, quercetin derivatives, and phenolic acids, which contribute to diverse pharmacological activities (Baky et al., 2016).

GC–MS analysis confirmed this variability, with Site I extracts yielding 12 compounds, while Site II extracts yielded 25 compounds. Key identified compounds included n-hexadecanoic acid, β-amyrin, urs-12-en-3-ol acetate, camphene, limonene, and tridecane, many of which possess antimicrobial, antioxidant, anti-inflammatory, and cytoprotective properties (Aparna et al., 2012; Pinto et al., 2008; Okoye et al., 2014; Benelli et al., 2018). The greater diversity of compounds at Site II supports the role of abiotic stress in enhancing metabolite production (Muthulakshmi et al., 2012; Kumar et al., 2014).

Overall, the present study confirms that *M. zapota* fruits are a rich source of bioactive phytochemicals, with variations in secondary metabolite content influenced by extraction solvent and environmental conditions. These findings highlight the pharmacological potential of M. zapota and justify further research into the therapeutic applications of its active compounds.

Conclusion

The present investigation highlights the phytochemical richness and pharmacological potential of *Manilkara zapota* fruits. Ethanolic extracts demonstrated the greatest diversity of secondary metabolites, while quantitative analysis revealed significant site-dependent variations, with fruits from Site II containing higher concentrations of phenols, flavonoids, and triterpenoids. GC–MS analysis further confirmed the variability in compound diversity and identified several bioactive metabolites with well-established therapeutic properties.

These results reinforce the role of *M. zapota* as a valuable medicinal plant within the Sapotaceae family and provide a scientific basis for its traditional applications. Moreover, the observed variation in phytochemical composition emphasizes the influence of environmental conditions on metabolite accumulation. Collectively, the findings suggest that *M. zapota* holds strong potential for future pharmacological, nutraceutical, and cosmetic applications, warranting further studies for bioactivity validation and drug development.

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Crystallography and Structural Determination of Inorganic Compounds

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Article DOI Link: https://zenodo.org/uploads/17542265
DOI: 10.5281/zenodo.17542265

Abstract

Inorganic compounds form the foundation of countless materials that shape our world, from catalysts and ceramics to superconductors and pigments. Understanding their intricate structures is key to unlocking their unique properties and potential applications. Crystallography and structural determination techniques offer powerful tools to explore the atomic arrangements within these compounds, revealing insights that drive innovation in chemistry, materials science, and beyond. In this book chapter, delve into the fundamental principles of inorganic crystallography, explore cutting-edge methods for structural analysis, and uncover how these techniques illuminate the secrets hidden within the crystalline world.

Keywords: Crystallography, intricate structures, atomic level, inorganic chemistry.

Introduction to Inorganic Compounds

Inorganic compounds form the foundation of countless materials and substances that shape our world, from minerals and metals to catalysts and advanced ceramics. Unlike their organic counterparts, which are primarily based on carbon-hydrogen bonds, inorganic compounds encompass a diverse array of elements and bonding arrangements, resulting in a vast landscape of chemical behavior and

properties. Understanding the structure of these compounds at the atomic level is crucial for unlocking their potential applications in fields such as materials science [1], Inorganic chemistry [2-5], and electronics [6].

Crystallography, the science of determining the arrangement of atoms in crystalline solids, plays a pivotal role in the study of inorganic compounds. Through techniques like X-ray diffraction, researchers can precisely map out the three-dimensional structure of a compound, revealing insights into its bonding, stability, and function. This structural determination not only aids in the identification and classification of new compounds but also guides the design of novel materials with tailored properties.

We will delve into the fundamental concepts of inorganic compounds, explore the principles and methods of crystallography, and examine how structural determination serves as a key tool in advancing our understanding and utilization of these essential substances.

The Importance of Crystallography in Chemistry

Crystallography plays a pivotal role in the field of chemistry, particularly when it comes to understanding the intricate details of inorganic compounds. By revealing the precise arrangement of atoms within a crystal, crystallography provides invaluable insights into the structure, bonding, and properties of materials. This technique allows chemists to visualize the three-dimensional architecture of molecules, which is essential for predicting reactivity, stability, and functionality (Table.1)

Table 1. Timeline of Crystallography

Sr. No.	Name of the Scientist	Year	Discovery	Reference
1	Max von Laue	1912	1st X-ray diffraction experiment	8
2	Davisson–Germer	1927	1st Electron diffraction	9
3	George Paget Thomson and Alexander Reid	1927	Discovery of two main branches of crystallography, X-ray crystallography and Electron diffraction.	10

Information taken from Wikipedia (7)

Inorganic compounds, often characterized by complex lattice structures and

diverse bonding patterns, can be challenging to study using conventional methods. Crystallography overcomes these challenges by producing detailed electron density maps that highlight atomic positions with remarkable accuracy. This level of structural determination not only aids in confirming the identity of synthesized compounds but also facilitates the design of new materials with tailored properties [2-5].

Moreover, crystallographic data serve as a foundation for computational modeling and theoretical studies, bridging the gap between experimental observations and chemical theory [11-14]. Whether its developing catalysts, designing advanced materials, or exploring coordination complexes, the insights gained through crystallography are indispensable. In essence, crystallography is the key that unlocks a deeper understanding of inorganic chemistry, driving innovation and discovery across the discipline [15].

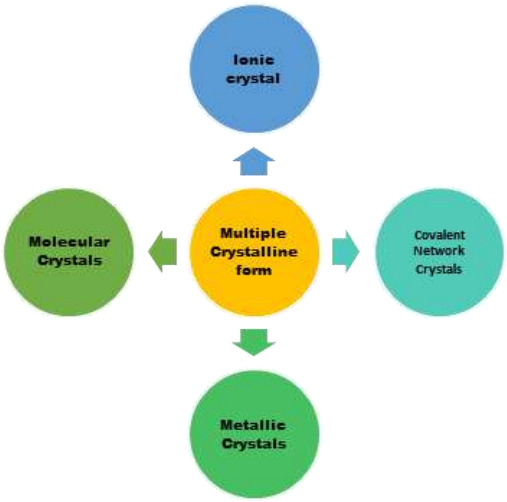
Basics of Crystal Structures and Symmetry

Understanding the basics of crystal structures and symmetry is fundamental to unlocking the secrets of inorganic compounds. At its core, a crystal structure describes the orderly and repeating arrangement of atoms, ions, or molecules in a three-dimensional space. This regular pattern extends infinitely in all directions, giving rise to the solid's unique physical properties such as hardness, melting point, and optical behavior [16].

One of the key concepts in crystallography is symmetry, which refers to the invariance of a crystal structure under certain operations, including rotations, reflections, inversions, and translations [17]. These symmetry operations are not just mathematical abstractions; they dictate how atoms are positioned relative to one another and influence the classification of crystals into different crystal systems and space groups [18]. There are seven crystal systems cubic, tetragonal, orthorhombic, hexagonal, trigonal, monoclinic, and triclinic each defined by specific constraints on the lengths and angles of their unit cells, the smallest repeating unit in the crystal lattice [19]. The symmetry elements present in these systems help scientists categorize the crystal and predict its behavior [20].

By mastering these foundational principles, researchers can accurately determine the arrangement of atoms within an inorganic compound, paving the way for deeper insights into its chemical properties and potential applications. This knowledge is essential not only for academic study but also for practical fields like materials science [1], nanotechnology [21], and pharmaceuticals [22], where the structure-property relationship is paramount.

Common Types of Inorganic Crystals



In the fascinating world of inorganic chemistry, crystals play a pivotal role in understanding the properties and behaviors of compounds [17-19]. Inorganic crystals are solid materials whose atoms, ions, or molecules are arranged in highly ordered, repeating patterns extending in all three spatial dimensions. Recognizing the common types of inorganic crystals is essential for anyone studying crystallography or involved in structural determination, as these categories provide insight into bonding, symmetry, and physical characteristics.

1. Ionic Crystals

Ionic crystals are composed of positively and negatively charged ions held together by strong electrostatic forces known as ionic bonds. Classic examples include sodium chloride (NaCl) and magnesium oxide (MgO). These crystals typically exhibit high melting points, are brittle, and conduct electricity when molten or dissolved in water. Their lattice structures maximize the attraction between oppositely charged ions while minimizing repulsion between like charges.

2. Covalent Network Crystals

In covalent network crystals, atoms are bonded by a continuous network of covalent bonds extending throughout the material. Diamond and quartz (SiO2) are prime examples. These crystals are extremely hard, have high melting points, and are generally poor conductors of electricity. The rigid three-dimensional bonding leads to remarkable mechanical strength and chemical stability.

3. Metallic Crystals

Metallic crystals consist of metal atoms arranged in a lattice where electrons are delocalized, forming a sea of electrons that allows metals to conduct heat and electricity efficiently. Common metallic crystal structures include body-centered cubic (BCC), face-centered cubic (FCC), and hexagonal close-packed (HCP). These metals are typically malleable, ductile, and have varying degrees of hardness depending on the specific metal and its crystal structure.

4. Molecular Crystals

Molecular crystals are formed by molecules held together by relatively weak intermolecular forces such as van der Waals forces, hydrogen bonding, or dipole interactions. Examples include ice (solid H20), dry ice (solid CO2), and sulfur (S2). These crystals generally have lower melting points and are softer compared to ionic or covalent network crystals, reflecting the weaker forces between molecules.

Understanding these common types of inorganic crystals provides a foundational framework for exploring their diverse structural features using techniques such as X-ray crystallography, neutron diffraction, and electron microscopy. This knowledge is crucial for unlocking the secrets of inorganic compounds and advancing applications in materials science, chemistry, and nanotechnology. Salunke et al. and coworker reported Single Crystal X-ray structure and Interaction of the ligands with metal ions [2-5] (Table 2 and figure. 1 to figure. 9.)

Table 2. Single Crystal X-ray structure and Interaction of the ligands with metal ions.

Sr. No.	Crystal type
1	Ionic Compound
2	Alkali metal complex
3	Transition metal complex
4	Bidentate organic ligand

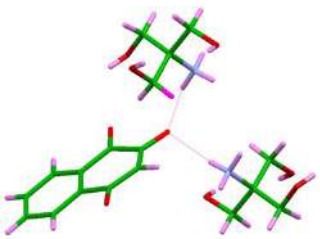


Fig.1. Hydrogen bonding interaction of ionic compound

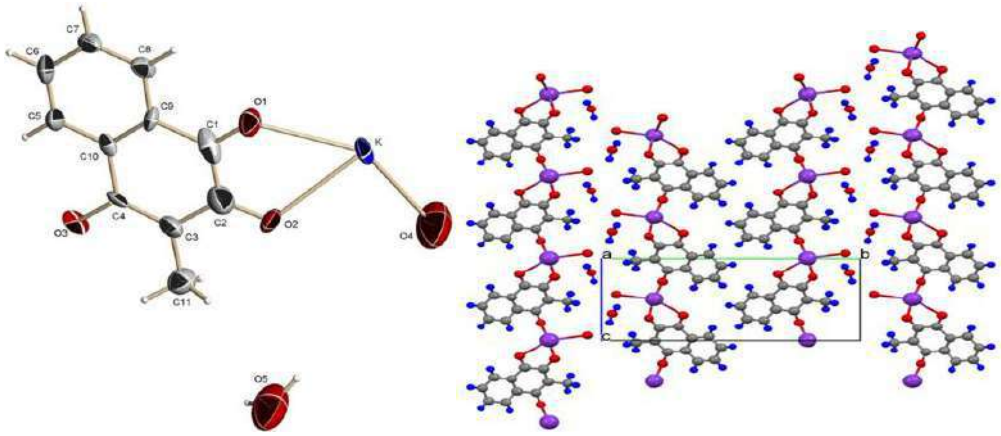


Fig. 2. ORTEP plot of alkali metal complex and Hydrogen bonding within complex through

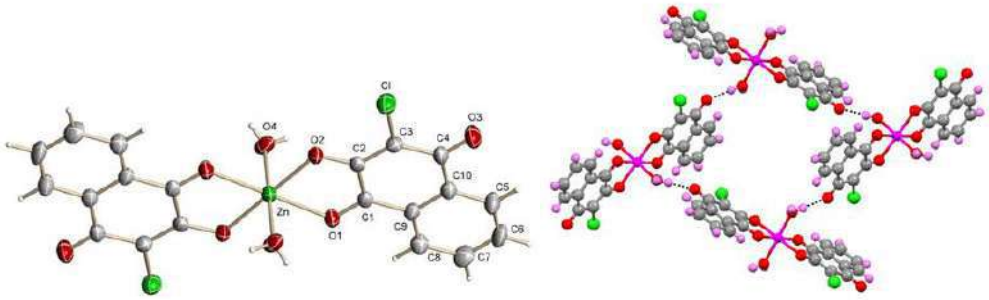


Fig. 3. ORTEP diagram of transition metal Complex (Zinc) and Hydrogen bonding through O(4)H(2)---O (3) of Complex

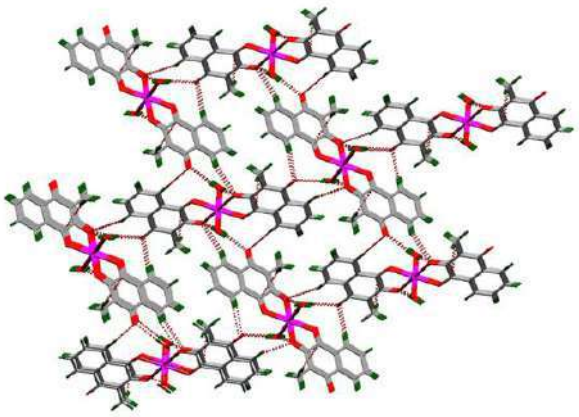


Fig. 4. C-H···O and O-H···O interaction of metal complex

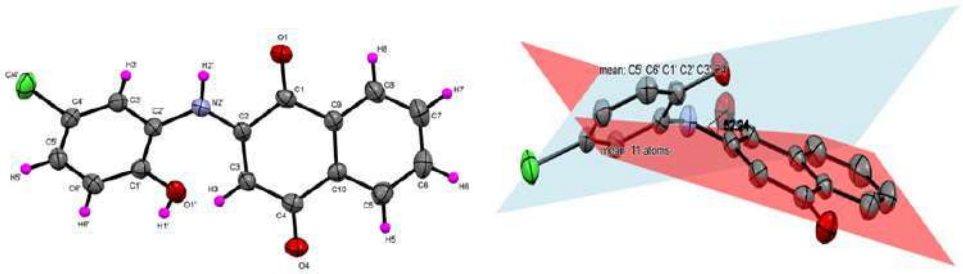


Fig. 5. ORTEP diagram of bidentate Organic ligand

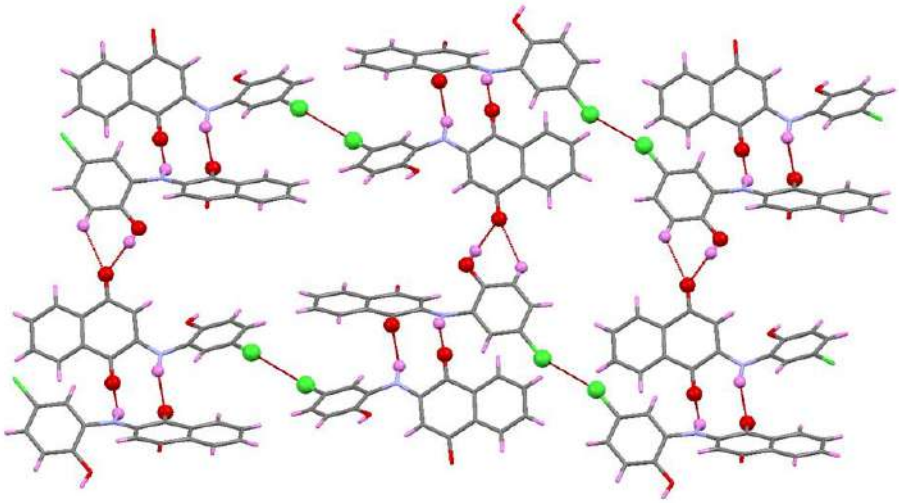


Fig. 6. O-H-O hydrogen-bonded interactions

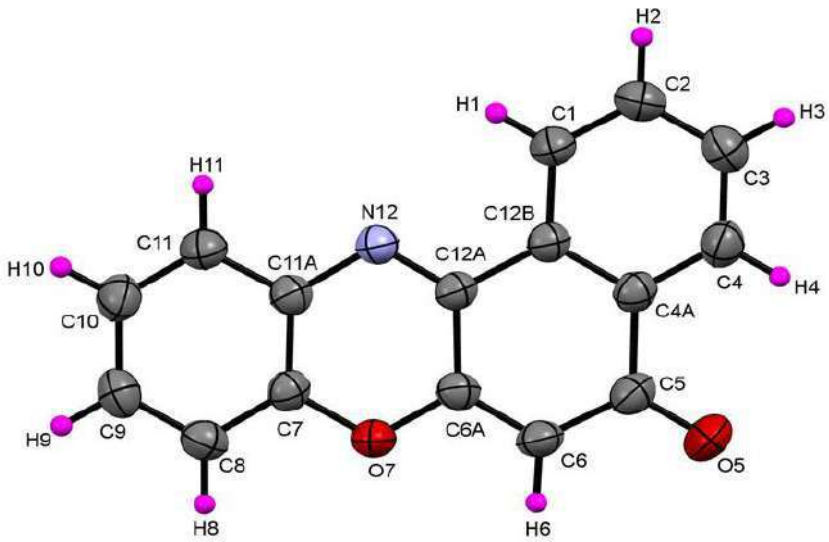


Fig. 7. ORTEP diagram of bidentate organic molecule

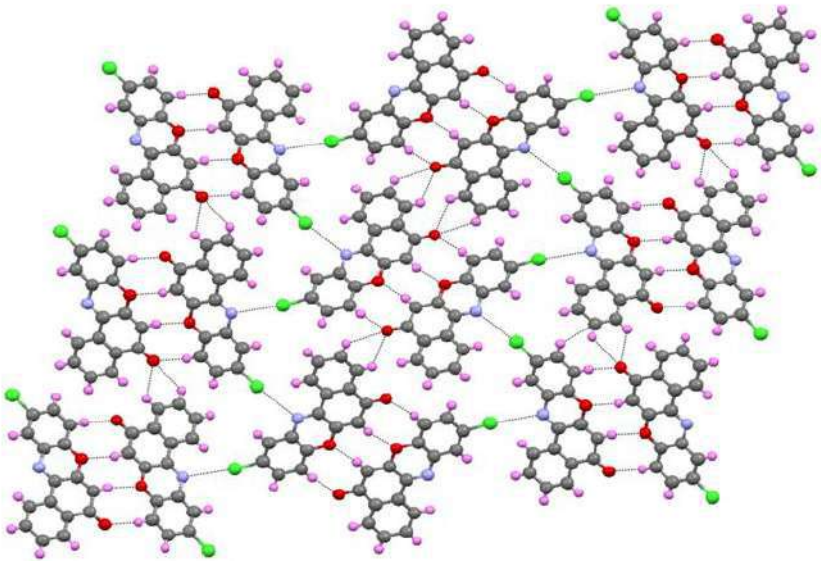


Fig. 8. Molecular packing between donor atom

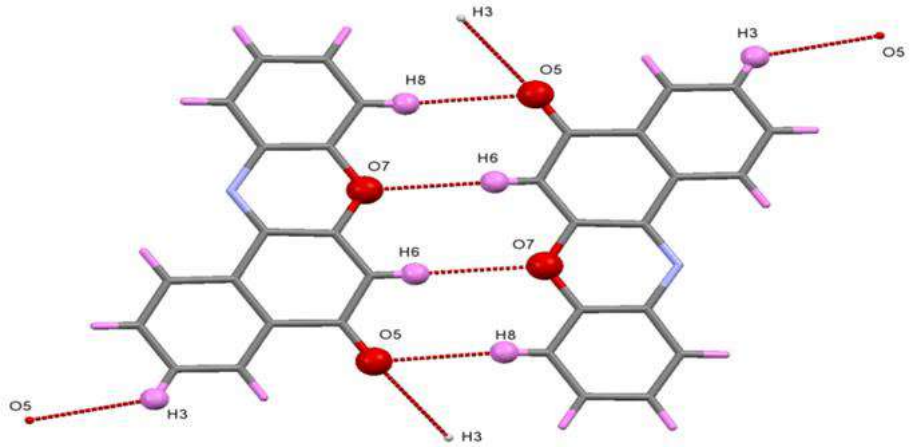


Fig. 9. Dimers formation through C-H···O interaction of organic molecule.

Techniques for Crystallization of Inorganic Compounds

Crystallization is a fundamental step in the structural determination of inorganic compounds, as it allows researchers to obtain high-quality crystals suitable for X-ray diffraction analysis. Achieving optimal crystallization requires careful selection of techniques tailored to the unique properties of the compound under study. Common methods include slow evaporation, where a saturated solution of the compound is allowed to evaporate gradually, promoting orderly crystal growth; slow cooling, which involves reducing the temperature of a hot, saturated solution to encourage crystal formation; and vapor diffusion, where a solvent that

poorly dissolves the compound diffuses into the solution, causing the compound to precipitate as crystals. Additionally, techniques such as hydrothermal synthesis are employed for compounds that require elevated temperatures and pressures to crystallize [23]. Fine-tuning parameters like solvent choice, concentration, temperature gradients, and supersaturation levels is crucial to obtaining well-formed crystals. Mastering these crystallization techniques is essential for unlocking detailed insights into the atomic arrangement and bonding characteristics of inorganic materials.

Overview of X-ray Crystallography

X-ray crystallography is a fundamental technique used to determine the atomic and molecular structure of a crystal. By directing X-rays at a crystallized sample, the rays are diffracted in specific patterns that can be measured and analyzed. These diffraction patterns provide detailed information about the arrangement of atoms within the crystal lattice, allowing scientists to construct a three-dimensional model of the compounds structure with remarkable precision.

The process begins with obtaining a high-quality crystal of the inorganic compound, which is essential for producing clear diffraction patterns. Once the crystal is exposed to X-rays, detectors capture the angles and intensities of the diffracted beams. Advanced computational methods then interpret this data to reveal the positions of atoms, bond lengths, and angles, offering insights into the compounds chemical bonding and electronic properties.

X-ray crystallography is invaluable in the field of inorganic chemistry because it enables researchers to confirm the identity of synthesized compounds, explore novel materials, and understand how structural variations influence physical and chemical behaviors. By unlocking the precise architecture of inorganic compounds, this technique serves as a cornerstone for advancements in materials science, catalysis, and nanotechnology.

Other Structural Determination Methods (Neutron, Electron Diffraction)

While X-ray crystallography remains the most widely used technique for determining the structures of inorganic compounds, other complementary methods such as neutron and electron diffraction offer unique advantages that can be crucial in certain cases [24].

1. Neutron Diffraction

Employs neutrons instead of X-rays to probe the atomic arrangement within a crystal. Because neutrons interact with atomic nuclei rather than the electron cloud, this technique is particularly sensitive to light atoms such as hydrogen,

which are often difficult to locate accurately using X-ray diffraction. Additionally, neutron diffraction can provide detailed information about magnetic structures, making it invaluable in the study of magnetic materials and complex inorganic frameworks. The ability to distinguish isotopes and detect subtle differences in atomic positions allows researchers to gain deeper insights into bonding and dynamics within inorganic compounds.

2. Electron Diffraction

Utilizes a beam of electrons, which have a much shorter wavelength than X-rays, enabling the examination of very small crystals or even individual nanoparticles that may be unsuitable for traditional X-ray crystallography. This method is especially powerful in cases where sample size is limited or when working with materials that are challenging to crystallize in large, well-ordered forms. Electron diffraction is often used in conjunction with transmission electron microscopy (TEM), providing both structural and morphological information at the nanoscale. Moreover, advances in electron diffraction techniques have significantly improved resolution and data quality, broadening their applicability to complex inorganic systems.

By integrating data from neutron and electron diffraction with X-ray crystallography, researchers can achieve a more comprehensive understanding of inorganic structures, overcoming limitations inherent to any single method. These complementary approaches expand the toolkit available for structural determination, enabling scientists to unlock the secrets of inorganic compounds with greater precision and depth.

Preparing Samples for Crystallographic Analysis

Preparing samples for crystallographic analysis is a critical step that can significantly influence the accuracy and quality of the structural data obtained. Whether you are working with single crystals or powdered inorganic compounds, meticulous sample preparation ensures that the crystals are suitable for diffraction experiments and that the resulting data reflects the true atomic arrangement.

For single-crystal crystallography, obtaining well-formed, defect-free crystals of adequate size is paramount. Crystals should ideally be transparent, free from cracks or inclusions, and large enough typically between 0.1 to 0.5 millimeters in each dimension to provide strong, interpretable diffraction patterns. Selecting the right crystal often involves careful growth optimization, including controlling temperature, solvent conditions, and supersaturation levels to encourage the formation of high-quality crystals.

In the case of powder X-ray diffraction (PXRD), samples must be finely ground to achieve a homogeneous, random orientation of crystallites. The powder should be pressed gently into sample holders or packed into capillaries to minimize preferred orientation and reduce the presence of air gaps, which can affect diffraction intensity. Proper sample mounting helps produce reproducible and high-resolution diffraction patterns essential for phase identification and structural refinement.

Additionally, minimizing contamination and moisture exposure is crucial, as impurities or hydration can alter the crystal structure or introduce unwanted scattering. In some cases, samples may require cooling or the use of inert atmospheres during preparation and analysis to preserve their integrity.

Overall, investing time and care in sample preparation lays the foundation for successful crystallographic studies, enabling precise determination of inorganic compound structures that can lead to deeper insights into their properties and applications.

Data Collection and Processing

Accurate data collection and meticulous processing are the cornerstones of successful crystallography and structural determination of inorganic compounds. This stage begins with selecting a high-quality single crystal, which is essential for obtaining clear diffraction patterns. Using advanced X-ray diffractometers, the crystal is exposed to X-rays, producing diffraction spots that contain critical information about the atomic arrangement within the compound.

Once the raw diffraction data are collected, they undergo rigorous processing to convert the diffraction patterns into interpretable intensity data. This involves indexing the diffraction spots to determine the unit cell parameters, integrating the intensities, and applying necessary corrections for factors such as absorption and background noise. Sophisticated software tools assist in refining data quality, ensuring that systematic errors are minimized.

Proper data processing lays the groundwork for accurate structural solution and refinement. Without precise and reliable data, subsequent steps in crystallographic analysis can lead to ambiguous or incorrect structural models. Therefore, investing time and care into data collection and processing not only enhances the reliability of the structural determination but also deepens our understanding of the intricate architectures of inorganic compounds.

Interpreting Crystal Structures

Interpreting crystal structures is a crucial step in understanding the properties and behavior of inorganic compounds. Once the crystallographic data has been collected and processed, the real work begins: analyzing the arrangement of atoms within the crystal lattice. This involves examining parameters such as bond lengths, bond angles, coordination environments, and symmetry elements to gain insights into the compounds chemical bonding and overall stability.

By carefully studying the three-dimensional arrangement revealed through techniques like X-ray diffraction, chemists can identify key structural motifs and connect them to physical and chemical properties. For example, subtle variations in bond distances can indicate different oxidation states or the presence of unusual bonding interactions. Additionally, understanding how molecules pack within the crystal can shed light on properties like conductivity, magnetism, or catalytic activity.

Interpreting crystal structures also requires familiarity with crystallographic software and visualization tools that allow researchers to manipulate and explore the atomic framework in detail. These tools help highlight features such as hydrogen bonding networks, polyhedral connectivity, and void spaces, which are often critical for explaining reactivity or material functionality.

Ultimately, mastering the interpretation of crystal structures empowers chemists to design new inorganic materials with tailored properties, predict reactivity trends, and contribute to advancements in fields ranging from materials science to catalysis and pharmacology.

Structural Determination of Key Inorganic Compounds

In this chapter, we delve into real-world examples that highlight the critical role of crystallography in uncovering the structures of important inorganic compounds, we gain insights into the methodologies and challenges involved in structural determination, as well as the implications these findings have on material properties and applications.

One notable example is the determination of the crystal structure of zeolites microporous aluminosilicate minerals widely used in catalysis and ion exchange. Through X-ray diffraction techniques, researchers were able to elucidate the intricate frameworks of these compounds, revealing the size and connectivity of their pores. This structural knowledge directly informs their effectiveness in industrial processes, enabling tailored design of catalysts with enhanced selectivity and stability.

Another compelling case study involves transition metal complexes, such as coordination compounds containing iron, cobalt, or nickel centers. Crystallographic analysis has been pivotal in understanding their geometric arrangements and electronic environments, which dictate magnetic, catalytic, and electronic properties. For instance, the precise determination of bond lengths and angles in a cobalt complex helped clarify its spin state and reactivity, guiding further synthesis of related compounds with optimized characteristics.

Additionally, the structural determination of metal-organic frameworks (MOFs) showcases how combining inorganic nodes with organic linkers leads to highly porous, tunable materials. Crystallography not only verifies the intended architecture but also uncovers defects or variations that can impact gas storage capabilities. These insights have propelled advancements in areas such as carbon capture and drug delivery.

Through these case studies and others, it becomes evident that crystallographic techniques are indispensable tools in inorganic chemistry. They not only confirm molecular structures but also open avenues for innovation by linking atomic-level arrangements to macroscopic functionalities. Understanding these examples equips researchers and students alike with a deeper appreciation of the power and versatility of structural determination in advancing the field of inorganic compounds.

Challenges and Limitations in Inorganic Crystallography

Inorganic crystallography plays a pivotal role in understanding the atomic and molecular structures of inorganic compounds, providing insights that drive advancements in chemistry, materials science, and related fields. However, despite significant technological progress, researchers still face a range of challenges and limitations when working with inorganic crystals.

One of the primary obstacles is the quality of the crystals themselves. Obtaining single crystals of sufficient size and purity can be difficult, especially for complex or novel inorganic compounds. Poor crystal quality often leads to weak or diffuse diffraction patterns, making data analysis and accurate structural determination more complicated.

Another limitation arises from the inherent disorder and complexity present in many inorganic materials. Structural defects, twinning, and mixed occupancy can obscure diffraction data, require advanced modeling techniques and sometimes lead to ambiguous or incomplete structural solutions. Additionally, certain elements with low atomic numbers, such as lithium or oxygen, scatter X-rays weakly, making them harder to locate precisely within the crystal lattice.

Moreover, environmental factors such as temperature, pressure, and humidity can influence crystal stability, potentially causing phase transitions or degradation during measurement. These factors necessitate careful experimental design and sometimes the use of specialized equipment like low-temperature stages or high-pressure cells.

Lastly, while X-ray crystallography remains the gold standard for structural determination, it is not always sufficient by itself. Complementary techniques such as neutron diffraction, electron microscopy, or spectroscopy are often required to fully characterize complex inorganic systems, adding layers of complexity and resource requirements to the research process.

Understanding these challenges is crucial for practitioners in the field, as it informs better experimental planning, data interpretation, and ultimately, the successful unlocking of the secrets held within inorganic compounds.

Advances in Crystallographic Techniques and Technologies

The field of crystallography has witnessed remarkable advancements in recent years, revolutionizing how inorganic compounds are analyzed and understood. Cutting-edge technologies such as synchrotron radiation sources and cryo-electron microscopy have significantly enhanced the resolution and speed of structural determination. Synchrotron facilities provide intense, tunable X-ray beams that allow researchers to probe crystals with unprecedented precision, enabling the elucidation of complex inorganic structures that were previously difficult or impossible to resolve.

Moreover, developments in detector technology, including fast pixel detectors, have dramatically reduced data collection times while improving accuracy. Coupled with sophisticated software algorithms for data processing and refinement, these innovations facilitate more reliable and automated analysis, minimizing human error and expediting the path from crystal to structure.

Another exciting advancement is the integration of artificial intelligence and machine learning in crystallography. These tools assist in pattern recognition, phase determination, and prediction of crystal structures, accelerating research workflows and opening new possibilities for discovering novel inorganic materials.

Together, these technological breakthroughs not only deepen our fundamental understanding of inorganic compounds but also pave the way for new applications in materials science, catalysis, and nanotechnology. Staying abreast of these advances is essential for researchers aiming to unlock the full potential

of crystallographic methods in structural determination.

Applications of Structural Information in Material Science and Chemistry

Understanding the structural information of inorganic compounds is fundamental to advancing both material science and chemistry. By determining the precise arrangement of atoms within a crystal, researchers can predict and tailor the physical, chemical, and electronic properties of materials. For instance, knowledge of crystal structures enables the design of catalysts with enhanced activity and selectivity, development of novel superconductors, and optimization of battery materials for improved energy storage. Structural insights also aid in elucidating reaction mechanisms and guiding the synthesis of new compounds with desired functionalities. In material science, this information supports the creation of stronger alloys, corrosion-resistant coatings, and innovative semiconductors. Overall, the detailed structural data obtained through crystallography not only deepens our fundamental understanding but also drives the innovation of advanced materials with applications spanning electronics, medicine, energy, and beyond.

Future Directions in Inorganic Compound Analysis

As the field of inorganic compound analysis continues to evolve, future directions are poised to revolutionize our understanding of crystal structures and material properties. Advances in computational techniques, such as machine learning and artificial intelligence, are increasingly being integrated with traditional crystallographic methods to predict and model complex inorganic structures with unprecedented accuracy. These tools enable researchers to analyze vast datasets, identify subtle structural patterns, and even propose novel compounds with tailored functionalities.

Additionally, improvements in experimental technologies like high-resolution synchrotron radiation, neutron diffraction, and electron microscopy are providing deeper insights into atomic arrangements and dynamic processes at the nanoscale. The combination of these cutting-edge techniques allows for real-time monitoring of structural changes under various environmental conditions, such as temperature, pressure, and chemical exposure.

Looking ahead, the integration of in situ and operando techniques will become more widespread, facilitating the study of inorganic compounds in their working states, which is critical for applications in catalysis, energy storage, and electronics. Furthermore, the development of open-access crystallographic

databases and collaborative platforms promises to accelerate discovery by fostering data sharing and interdisciplinary research.

Together, these future directions hold great potential to unlock new functionalities in inorganic materials, driving innovation across chemistry, materials science, and related fields. Embracing these emerging trends will empower scientists to unravel the complexities of inorganic compounds more efficiently and with greater precision than ever before.

In conclusion, unlocking the secrets of inorganic compounds through crystallography and structural determination offers invaluable insights that drive advancements across chemistry, materials science, and beyond. By mastering the techniques and principles outlined in this book, researchers alike can deepen their understanding of molecular structures and their properties. The knowledge gained here will empower you to explore the intricate world of inorganic compounds with confidence and precision. Embrace these tools and methods, and open the door to discoveries that could shape the future of science.

Abbreviations

ORTEP: Oak Ridge Thermal Ellipsoid Plot Program

BCC: body-centered cubic

FCC: face-centered cubic

HCP: hexagonal close-packed

TEM: transmission electron microscopy

PXRD: powder X-ray diffraction

MOFs: metal-organic frameworks

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Antimicrobial Resistance and Plasmid Curing in Nosocomial Infectious Diseases

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Article DOI Link: https://zenodo.org/uploads/17542445
DOI: 10.5281/zenodo.17542445

Abstract

Nosocomial bacterial infections are associated with high morbidity and mortality, posing a huge burden to healthcare systems worldwide. The ongoing COVID-19 pandemic, with the raised hospitalization of patients and the increased use of antimicrobial agents, boosted the emergence of difficult-to-treat multidrug-resistant (MDR) bacteria in hospital settings. Therefore, current available antibiotic treatments often have limited or no efficacy against nosocomial bacterial infections, and novel therapeutic approaches need to be considered. In this review, we analyze current antibacterial alternatives under investigation, focusing on metal-based complexes, antimicrobial peptides, and antisense antimicrobial therapeutics. The association of new compounds with older, commercially available antibiotics and the repurposing of existing drugs are also revised in this work.

Keywords: Nosocomial infections, multidrug-resistant bacteria, novel antimicrobial agents, antimicrobial peptides

Introduction

Antimicrobial resistance (AMR) is an evolutionary response of bacteria, viruses, and fungi to withstand antimicrobial drugs introduced into their environment. In this systematic review we will focus on the resistance in bacteria only and the reader is advised to go through other reviews on viral and fungal resistance. In

2020, the COVID-19 pandemic has clearly demonstrated how fragile our world is and how infections do not respect borders. However, we have long been living in a silent pandemic of AMR. Each year, infections caused by resistant bacteria cause 68,000 deaths in the EU/EEA and the United States combined, and are contributing to US €55 billion economic loss in the United States and to €1.6 billion in the EU/EEA annually. Although the AMR problem is not evenly distributed across the globe, there is no single country on Earth that can safely state that it will not be affected by the AMR spread. The first warning on a potential catastrophe came from Fleming in his speech on accepting the Nobel prize for discovery of penicillin, the first industrially produced antibiotic. Although he did not foresee the global spread of antibiotic resistance, he was the first one to recognize the danger of the resistance for everyone who relies on these drugs. In 2019, the Center for Disease Control and Prevention (CDC) released a report stating that the post-antibiotic era has started.

Mechanisms of Antimicrobial Resistance in Nosocomial Pathogens

Antimicrobial resistance (AMR) in nosocomial pathogens is a multifaceted problem driven by various mechanisms that allow bacteria to evade the effects of antibiotics. These mechanisms can be broadly categorized into intrinsic resistance, acquired resistance through mutations, and acquired resistance through horizontal gene transfer (HGT). Understanding these mechanisms is crucial for developing effective strategies to combat the spread of drug-resistant infections in healthcare settings.

Intrinsic resistance refers to the natural insensitivity of certain bacterial species to specific antibiotics due to their inherent biological characteristics. For instance, some bacteria possess cell wall structures that are impermeable to certain drugs, or they naturally produce enzymes that inactivate particular classes of antibiotics. An example is the outer membrane of Gram-negative bacteria, which acts as a barrier against many antibiotics that are effective against Gram-positive bacteria. Similarly, efflux pumps, which are membrane-bound proteins that actively pump antibiotics out of the bacterial cell, can be intrinsically present in some species, contributing to their natural resistance.

Acquired resistance, on the other hand, develops when bacteria that were once susceptible to an antibiotic become resistant. One primary way this occurs is through spontaneous mutations in existing chromosomal genes. These mutations can alter the antibiotic's target site, reducing its binding affinity, or modify regulatory genes that control the expression of resistance mechanisms. For example, mutations in genes encoding ribosomal proteins can lead to resistance

to aminoglycosides or macrolides, while mutations in DNA gyrase or topoisomerase IV can confer resistance to fluoroquinolones. These genetic changes are often selected for under antibiotic pressure, allowing resistant strains to proliferate.

However, the most significant driver of the rapid spread of AMR, particularly in nosocomial settings, is horizontal gene transfer (HGT). HGT allows bacteria to acquire new genetic material, including antibiotic resistance genes (ARGs), from other bacteria. This process can occur through three main mechanisms: transformation, transduction, and conjugation. Transformation involves the uptake of free DNA from the environment. Transduction is the transfer of genetic material via bacteriophages (viruses that infect bacteria). Conjugation, often mediated by plasmids, is a direct cell-to-cell transfer of genetic material and is considered the most important mechanism for the dissemination of ARGs among diverse bacterial populations.

Plasmids are extrachromosomal DNA molecules that can replicate independently of the bacterial chromosome. They frequently carry multiple ARGs, as well as genes encoding virulence factors, and can be readily transferred between bacteria, even across different species. This makes plasmids highly efficient vehicles for the rapid spread of multidrug resistance. The selective pressure exerted by widespread antibiotic use in hospitals further accelerates the acquisition and dissemination of these resistance-conferring plasmids. Common examples of plasmid-mediated resistance include extended-spectrum beta-lactamases (ESBLs) and carbapenemases (e.g., KPC, NDM), which confer resistance to broad-spectrum beta-lactam antibiotics, and the mcr gene, which mediates colistin resistance. The presence of these highly mobile genetic elements in nosocomial pathogens poses a severe threat to patient care, leading to infections that are difficult, if not impossible, to treat with conventional antibiotics.

The Role of Plasmids in the Spread of Antimicrobial Resistance

Plasmids are extrachromosomal, self-replicating DNA molecules found in bacteria, distinct from the bacterial chromosome. They play a pivotal role in bacterial evolution and adaptation, particularly in the context of antimicrobial resistance (AMR). The significance of plasmids in the rapid dissemination of AMR among nosocomial pathogens cannot be overstated, as they serve as highly efficient vectors for the horizontal transfer of antibiotic resistance genes (ARGs).

One of the primary reasons for the critical role of plasmids in AMR spread is their ability to carry multiple ARGs simultaneously. These genes often confer resistance to different classes of antibiotics, leading to multidrug-resistant (MDR)

phenotypes. For instance, a single plasmid can harbor genes for beta-lactamases (conferring resistance to penicillin and cephalosporins), aminoglycoside-modifying enzymes, and efflux pumps, making the host bacterium resistant to a wide array of commonly used antibiotics. This co-localization of ARGs on plasmids allows for the co-selection of multiple resistances even when only one antibiotic is present, further accelerating the development of MDR strains.

Furthermore, plasmids are highly mobile genetic elements. They can be transferred between bacteria through a process called conjugation, which involves direct cell-to-cell contact. During conjugation, a copy of the plasmid is transferred from a donor bacterium to a recipient bacterium, even if they belong to different species or genera. This promiscuous transfer capability is particularly problematic in healthcare settings, where diverse bacterial populations coexist and antibiotic selective pressure is high. For example, resistance plasmids can move from commensal bacteria to pathogenic species, or between different pathogenic species, quickly spreading new resistance mechanisms throughout a hospital environment.

Beyond conjugation, plasmids can also be acquired through transformation (uptake of naked DNA from the environment) and transduction (phage-mediated transfer). While conjugation is often the most efficient mechanism for plasmid transfer, the combination of these HGT methods ensures that ARGs carried on plasmids can rapidly disseminate within and between bacterial communities. This dynamic exchange of genetic material allows bacteria to quickly adapt to new environmental challenges, such as the presence of antibiotics, without undergoing lengthy evolutionary processes.

The stability and persistence of plasmids within bacterial populations also contribute to their role in AMR. Many resistance plasmids have sophisticated mechanisms to ensure their stable inheritance during cell division, such as active partitioning systems and post-segregational killing systems (toxin-antitoxin systems). These mechanisms prevent the loss of plasmids from bacterial cells, even in the absence of antibiotic selection pressure, thereby maintaining the pool of ARGs within the bacterial population. This stability means that once a resistance plasmid is established in a bacterial lineage, it can persist and continue to spread, even if antibiotic use is reduced.

In nosocomial infections, the prevalence of plasmid-mediated resistance is particularly alarming. Hospital environments are rich in diverse bacterial species, including both commensals and pathogens, and are characterized by intense antibiotic use. This creates an ideal breeding ground for the emergence and

spread of MDR plasmids. Pathogens like Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa frequently acquire resistance plasmids encoding carbapenemases (e.g., KPC, NDM) and extended-spectrum beta-lactamases (ESBLs), leading to infections that are extremely difficult to treat and contributing significantly to patient morbidity and mortality. The continuous evolution and diversification of these resistance plasmids pose a constant challenge to public health and infection control efforts.

Plasmid Curing: A Strategy to Combat Antimicrobial Resistance

Given the critical role of plasmids in the dissemination of antimicrobial resistance (AMR), strategies aimed at eliminating or 'curing' these extrachromosomal elements from bacterial populations have emerged as a promising approach to combat drug-resistant infections. Plasmid curing refers to the process of selectively removing plasmids from bacterial cells, thereby rendering the bacteria susceptible to antibiotics once again or reducing their virulence. This approach offers a distinct advantage over traditional antibiotic development, as it targets the resistance mechanism itself rather than developing new drugs that bacteria may eventually overcome.

One of the primary motivations behind plasmid curing is to reverse the multidrug-resistant (MDR) phenotype of pathogenic bacteria. By eliminating resistance plasmids, bacteria lose their ability to inactivate antibiotics, modify drug targets, or efflux antimicrobial compounds. This re-sensitization to existing antibiotics can significantly expand the therapeutic options available for treating infections caused by previously untreatable or difficult-to-treat strains. In essence, plasmid curing aims to 'turn back the clock' on bacterial resistance, making older, more affordable, and often less toxic antibiotics effective once more.

Moreover, plasmid curing can help to reduce the overall burden of antibiotic resistance genes (ARGs) in the environment and within healthcare settings. Plasmids are not only transferred between bacteria but can also be shed into the environment, where their ARGs can be picked up by other susceptible bacteria. By reducing the prevalence of resistance plasmids in bacterial populations, plasmid curing strategies can contribute to a broader effort to mitigate the spread of AMR, potentially slowing down the emergence of new resistant strains.

Another benefit of plasmid curing is its potential to reduce bacterial fitness costs associated with carrying resistance plasmids. While plasmids confer a survival advantage in the presence of antibiotics, maintaining and replicating these extrachromosomal elements can impose a metabolic burden on the host

bacterium. The loss of resistance plasmids can therefore lead to an increase in bacterial fitness, potentially making cured bacteria less competitive in the absence of antibiotic pressure. This could, in theory, lead to the displacement of resistant strains by more fit, susceptible strains over time, although this ecological outcome is complex and depends on various factors.

Plasmid curing is particularly attractive for combating nosocomial infections, where the rapid spread of MDR pathogens is a major concern. Hospitals are hotspots for the evolution and transmission of resistance plasmids due to high antibiotic usage and close patient contact. Implementing effective plasmid curing strategies in such environments could help to de-escalate resistance levels, improve patient outcomes, and reduce healthcare costs associated with treating complex, drug-resistant infections. However, the development of practical and efficient plasmid curing agents and methods remains a significant challenge, requiring a deep understanding of plasmid biology and bacterial physiology.

Methods for Plasmid Curing

The elimination of plasmids from bacterial cells, known as plasmid curing, can be achieved through various methods, broadly categorized into physical, chemical, and biological approaches. The effectiveness of these methods often depends on the specific plasmid, bacterial host, and the conditions under which curing is attempted. The goal of any curing method is to selectively inhibit plasmid replication or segregation without significantly harming the host bacterium.

Physical Methods: These methods typically involve subjecting bacterial cultures to environmental stressors that interfere with plasmid maintenance. Elevated temperatures, for instance, can be used to cure temperature-sensitive plasmids or to disrupt the replication machinery of other plasmids. Prolonged incubation at temperatures slightly above the optimal growth temperature for the host bacterium can lead to a higher rate of plasmid loss. Similarly, exposure to ultraviolet (UV) radiation or other forms of electromagnetic radiation can induce DNA damage, which may preferentially affect plasmids due to their smaller size and different replication strategies compared to the bacterial chromosome. However, physical methods often lack specificity and can be detrimental to the host cell, potentially inducing chromosomal mutations or cell death.

Chemical Methods: A wide array of chemical agents has been explored for their plasmid-curing properties. These chemicals typically interfere with DNA replication, transcription, or protein synthesis, or they intercalate into the DNA

structure, thereby disrupting plasmid maintenance. Examples include acridine dyes (e.g., acriflavine, ethidium bromide), which intercalate into DNA and can inhibit plasmid replication or segregation. Sodium dodecyl sulfate (SDS), a detergent, can also be used to destabilize bacterial membranes and interfere with plasmid partitioning. Other chemical agents, such as novobiocin, mitomycin C, and certain antibiotics (e.g., rifampicin, chloramphenicol at sub-inhibitory concentrations), have also shown plasmid-curing activity by targeting specific cellular processes. The challenge with chemical methods lies in finding agents that are highly effective at curing plasmids without exerting excessive toxicity on the host bacterium or selecting for new resistance mechanisms.

Biological Methods: These approaches leverage biological processes or agents to selectively eliminate plasmids. One promising biological strategy involves the use of anti-plasmid systems, such as CRISPR-Cas technology. CRISPR-Cas systems can be engineered to target specific sequences on resistance plasmids, leading to their degradation and subsequent curing. This method offers high specificity and can be tailored to remove particular resistance plasmids. Another biological approach involves the use of bacteriophages that specifically target plasmid-carrying bacteria or interfere with plasmid replication. Additionally, the introduction of incompatible plasmids can lead to the displacement of existing plasmids, as bacteria often cannot stably maintain two plasmids belonging to the same incompatibility group. Natural products and plant extracts have also been investigated for their ability to cure plasmids, offering a potentially less toxic and more sustainable alternative. These biological methods are often considered more targeted and potentially safer than physical or chemical approaches, but their development and application can be complex.

Combination Approaches: Increasingly, researchers are exploring combination therapies that utilize multiple curing agents or combine curing agents with traditional antibiotics. The rationale behind this is that a multi-pronged approach may enhance curing efficiency, reduce the likelihood of resistance development to the curing agent, and synergistically re-sensitize bacteria to antibiotics. For instance, a chemical agent that inhibits plasmid replication could be combined with an antibiotic to which the bacteria were previously resistant, aiming to both eliminate the plasmid and kill the now-susceptible bacteria. The development of effective and safe plasmid-curing agents remains an active area of research, with a focus on high specificity, low host toxicity, and broad applicability across diverse bacterial pathogens.

Future Perspectives in Plasmid Curing

Despite the significant potential of plasmid curing as a strategy to combat antimicrobial resistance (AMR), several challenges must be addressed to translate this concept into effective clinical applications. Overcoming these hurdles will require continued research and innovative approaches.

One of the primary challenges is the specificity and efficiency of curing agents. Many traditional chemical and physical curing methods lack specificity, often causing damage to the host bacterial chromosome or inducing stress responses that can lead to unintended consequences, such as the selection of compensatory mutations. Ideal curing agents should selectively target plasmids without harming the bacterial host, ensuring that the cured bacteria remain viable but susceptible. The development of highly specific biological tools, such as engineered CRISPR-Cas systems, offers a promising avenue for targeted plasmid elimination, but their delivery and efficacy in complex biological environments, such as the human body, still need extensive research and optimization.

Another significant challenge is the reversibility and stability of the cured state. Even if a plasmid is successfully removed, there is a risk that the bacteria could re-acquire resistance genes through horizontal gene transfer (HGT) from other resistant bacteria in the environment. This is particularly relevant in healthcare settings where resistant strains are prevalent. Therefore, effective plasmid curing strategies may need to be combined with stringent infection control measures and stewardship programs to prevent re-acquisition of resistance. Furthermore, the metabolic burden associated with carrying plasmids can sometimes be compensated for by bacteria, meaning that the fitness advantage of cured bacteria might not always be sufficient to outcompete resistant strains in all ecological niches.

The delivery of curing agents to the site of infection and into bacterial cells presents another practical obstacle. Many potential curing agents may not readily penetrate bacterial membranes or reach sufficient concentrations within infected tissues. Developing effective delivery systems, such as nanoparticles or bacteriophage-based vectors, will be crucial for the clinical application of plasmid curing. The pharmacokinetics and pharmacodynamics of these agents also need to be thoroughly investigated to ensure their safety and efficacy in vivo.

Regulatory and commercialization challenges are also considerable. The development of any new antimicrobial strategy, including plasmid curing agents, is a lengthy and expensive process. Demonstrating safety and efficacy in clinical trials, navigating regulatory approvals, and ensuring commercial viability will

require substantial investment and collaboration between academia, industry, and regulatory bodies.

Looking to the future, several promising avenues are being explored. CRISPR-Cas based systems are at the forefront of targeted plasmid curing, offering unprecedented precision in removing specific resistance genes or entire plasmids. Further research into optimizing these systems for in vivo application, including efficient delivery and minimizing off-target effects, is critical. Phage therapy, either using phages that specifically target plasmid-carrying bacteria or engineering phages to deliver curing agents, also holds great promise. Combination therapies, where plasmid curing agents are used in conjunction with conventional antibiotics or other anti-virulence strategies, could offer synergistic effects, enhancing bacterial susceptibility and improving treatment outcomes.

Ultimately, the successful implementation of plasmid curing as a viable strategy against AMR will likely involve a multi-faceted approach, integrating novel curing agents with improved delivery systems, robust infection control, and judicious antibiotic use. While challenges remain, the potential to disarm multidrug-resistant pathogens and restore the efficacy of existing antibiotics makes plasmid curing a compelling area of research in the ongoing fight against nosocomial infectious diseases.

Conclusion

Antimicrobial resistance (AMR) in nosocomial infectious diseases represents one of the most pressing global health crises of our time. The rapid emergence and spread of multidrug-resistant (MDR) pathogens within healthcare settings threaten to undermine the efficacy of modern medicine, leading to prolonged hospital stays, increased morbidity and mortality, and substantial economic burdens. A key driver of this alarming trend is the pervasive role of plasmids, which serve as highly efficient vehicles for the horizontal transfer of antibiotic resistance genes (ARGs) among diverse bacterial populations.

Plasmids facilitate the rapid acquisition of resistance mechanisms, enabling bacteria to evade the action of a wide array of antibiotics. Their ability to carry multiple ARGs, coupled with their promiscuous transfer through conjugation, transformation, and transduction, has created a complex web of resistance that is difficult to untangle. The stability and persistence of these resistance plasmids further ensure that once established, ARGs remain within bacterial communities, perpetuating the cycle of resistance even in the absence of direct antibiotic pressure.

In response to this escalating threat, plasmid curing has emerged as a promising and innovative strategy. By selectively eliminating resistance plasmids from bacterial cells, this approach aims to re-sensitize MDR pathogens to existing antibiotics, thereby restoring therapeutic options and potentially reducing the overall burden of ARGs. The concept of plasmid curing offers a paradigm shift from solely developing new antibiotics to actively disarming resistant bacteria, making them vulnerable once more.

Various methods, including physical, chemical, and biological approaches, have been explored for plasmid curing. While traditional physical and chemical methods often suffer from a lack of specificity and potential host toxicity, advanced biological tools, particularly CRISPR-Cas based systems, offer unprecedented precision in targeting and removing specific resistance plasmids. These newer approaches hold the potential to overcome many of the limitations of earlier methods, paving the way for more effective and safer curing strategies.

However, the journey from concept to clinical reality for plasmid curing is fraught with challenges. Issues such as ensuring high specificity and efficiency of curing agents, preventing the re-acquisition of resistance, developing effective delivery systems, and navigating regulatory hurdles all require significant attention. Despite these obstacles, the future of plasmid curing appears bright, with ongoing research focused on refining CRISPR-Cas technologies, exploring phage-mediated strategies, and developing synergistic combination therapies. Integrating these novel curing approaches with stringent infection control practices and judicious antibiotic stewardship will be paramount in our collective effort to mitigate the impact of AMR.

Ultimately, combating antimicrobial resistance in nosocomial infections demands a multi-pronged, adaptive strategy. Plasmid curing, by directly addressing the genetic basis of resistance, represents a vital component of this strategy. Continued investment in research and development in this area offers a beacon of hope in the ongoing battle against drug-resistant superbugs, aiming to safeguard the effectiveness of antibiotics for future generations and preserve the foundations of modern healthcare.

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Isoxazole-Substituted Chalcones: Emerging Medicinal Applications

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Article DOI Link: https://zenodo.org/uploads/17542617
DOI: 10.5281/zenodo.17542617

Abstract

Chalcone–isoxazole hybrids have emerged as a promising class of bioactive molecules with diverse pharmacological applications, particularly in antimicrobial and anticancer therapy. The structural fusion of the chalcone framework with an isoxazole moiety enhances biological potency and broadens therapeutic potential. Several derivatives (compounds 25–28) demonstrated potent anti-tuberculosis activity against Mycobacterium tuberculosis with low cytotoxicity, with compound 27 identified as a lead candidate. Structure–activity relationship (SAR) studies highlighted the role of nonpolar substituents, such as halogens and alkyl groups, in improving antimicrobial efficacy. Similarly, incorporation of thiazole–isoxazole motifs significantly strengthened antiproliferative activity. Notably, compound 38 exhibited outstanding cytotoxicity across multiple cancer cell lines, including MCF-7, A549, Colo-205, and A2780, with submicromolar IC_{50} values. Although most derivatives displayed moderate to good cytotoxicity, their multifunctional properties—including antibacterial, antioxidant, and antiproliferative effects—underscore their versatility as scaffolds for drug discovery. Future efforts focusing on substituent optimization and hybrid design strategies may lead to the development of broad-spectrum therapeutic agents with enhanced efficacy.

Keywords: Chalcone, Isoxazole, Antibacterial, Antioxidant, and Antiproliferative activity.

Introduction

Chalcones are a class of natural compounds characterized by the 1,3-diphenyl-2-propen-1-one framework and are classified within the flavonoid family. Structurally, they are open-chain flavonoids in which two aromatic rings, commonly referred to as ring A and ring B, are connected via a three-carbon α,β-

unsaturated carbonyl system (Figure 1). This enone linkage (–CO–CH=CH–) acts as a highly electrophilic bridge between the rings, giving chalcones a planar or nearly linear configuration. The conjugated double bond system along with a fully delocalized π-electron network over both aromatic rings imparts unique chemical and biological properties, making chalcones versatile scaffolds for medicinal chemistry. A wide variety of substituents can be introduced onto the aromatic rings, allowing fine-tuning of physicochemical properties and bioactivity, which has made chalcones an attractive framework for drug discovery and synthetic modifications [1].

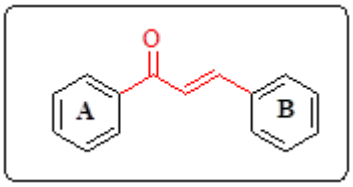

Figure 1: General structure of chalcone

The conformational behavior of chalcones has been extensively studied to understand their structural stability and reactivity. Two major analyses of the enone linkage have shown that rotation around the α,β-unsaturated carbonyl bond can lead to different conformers, with the E-isomer being thermodynamically favored due to its lower steric hindrance and greater planarity. Consequently, almost all naturally occurring and synthetically isolated chalcones exist in the E-configuration. This stable conformation contributes to the extensive conjugation and delocalization of π-electrons, which in turn influences both the chemical reactivity and the interaction of chalcones with biological targets. The planar nature of chalcones is also crucial for their ability to participate in π–π stacking interactions, hydrogen bonding, and other molecular interactions that underlie their diverse pharmacological activities [2-3].

Chalcones are widely distributed in nature, from ferns to higher plants, and many possess polyhydroxylated aromatic rings. In plants, they are enzymatically converted to (2S)-flavanones by chalcone isomerase, reflecting their close structural and biosynthetic relationship. This explains their frequent co-occurrence with flavanones. Figure 2 illustrates plant sources of chalcones, while Figure 3 shows examples of natural chalcones [4-11].

Humulus lupulu (Hop)

Carthamus tinctorius (Safflower)

Nymphaea caerulea (Water lily)

Angelica keiskei (Ashitaba)

Piper methysticum (Kava)

Caesalpinia sappan (Sappan wood)

Rhus verniciflua (Varnish tree)

Syzygium samarangense (Wax apple)

Semecarpus anacardium

Figure 2: Some natural sources of chalcones

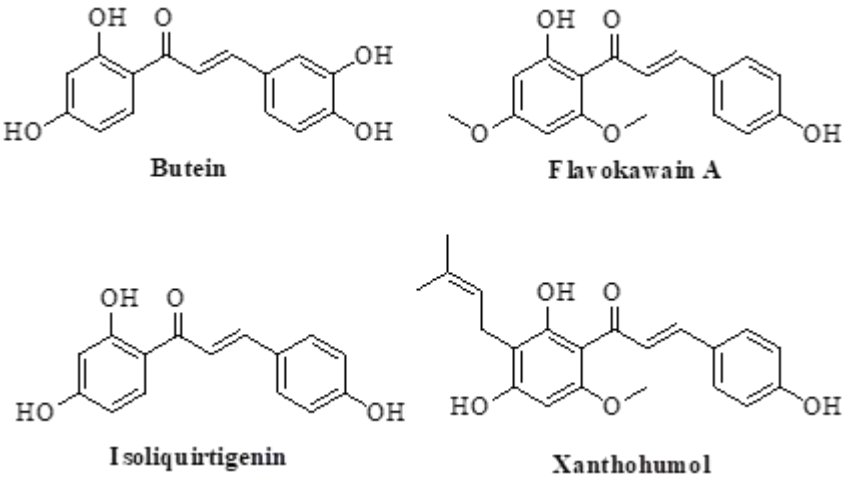

Figure 3: Some examples of natural chalcones

Isoxazole is a five-membered aromatic heterocycle with three carbon atoms, one nitrogen, and one oxygen in a 1,2-oxazole arrangement. Its delocalized π-electrons confer chemical stability and unique reactivity. Isoxazole derivatives are of great interest due to their versatile chemistry and broad biological activities c In medicinal chemistry, isoxazole is a key scaffold for drug design, with derivatives exhibiting antimicrobial, anti-inflammatory, anticancer, antiviral, and analgesic activities, making them important candidates in therapeutic development. Isoxazole–chalcone derivatives represent a unique class of hybrid molecules that integrate the biologically active chalcone scaffold with the versatile isoxazole heterocycle. The combination of these two pharmacophores often enhances the overall bioactivity compared to the individual components. Recent studies have demonstrated that these compounds exhibit significant

antimicrobial, anti-tuberculosis, anticancer, antioxidant, and anti-inflammatory activities, highlighting their multifunctional potential [12-14].

The therapeutic versatility of isoxazole–chalcone hybrids is further supported by structure–activity relationship (SAR) analyses, which have identified critical substituents and functional groups that enhance potency and selectivity. For instance, halogen and alkyl substituents on the aromatic rings often improve antimicrobial and anti-tuberculosis activity, while electron-donating or withdrawing groups can modulate anticancer and antioxidant properties. Several lead compounds have shown low cytotoxicity alongside strong bioactivity, making them promising candidates for further drug development. The ability to fine-tune their chemical structure, combined with their broad spectrum of biological effects, positions isoxazole–chalcone derivatives as highly valuable scaffolds in medicinal chemistry for the design of novel therapeutic agents targeting multiple diseases [15-17]. This book chapter explores the latest medicinal applications and structure-activity relationships of isoxazole-substituted chalcones.

Medicinal Applications of Isoxazole-Substituted Chalcone Derivatives

Wan et al. synthesized a new series of isoxazole–chalcone derivatives and investigated their anticancer potential by testing them against a panel of human lung cancer cell lines, including H1792, H157, A549, and Calu-1. The in vitro assays revealed that several of these compounds displayed promising antiproliferative effects, with half-maximal inhibitory concentration (IC_{50}) values ranging from 1.35 to 19.63 μM. Notably, compounds 1 and 2 (Figure 4) stood out for their strong cytotoxic activity across all four cancer cell lines, suggesting that structural incorporation of an isoxazole moiety into the chalcone framework confers significant anticancer potential. These findings highlight the value of such hybrid scaffolds as lead structures for the development of novel chemotherapeutic agents, particularly for targeting lung cancer.

Further mechanistic studies provided insights into how these compounds exert their anticancer effects. Both compounds 1 and 2 were found to induce apoptosis in A549 lung carcinoma cells via an exogenous apoptotic pathway, specifically mediated by the activation of death receptor 5 (DR5). The involvement of DR5 indicates that these molecules can trigger programmed cell death by engaging the extrinsic pathway, which plays a critical role in overcoming the resistance of cancer cells to conventional chemotherapeutics. This dual demonstration of potent cytotoxicity and apoptosis induction suggests that isoxazole–chalcone derivatives, particularly compounds 1 and 2, may serve as promising candidates

for further preclinical evaluation as targeted anticancer agents [18].

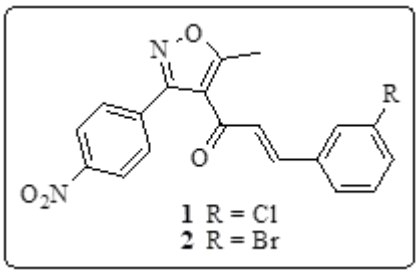

Figure 4: Isoxazole-substituted chalcone derivatives 1-2

Niu et al. reported the design and synthesis of a novel series of chalcone derivatives bearing isoxazole groups, which were subsequently evaluated for their effects on melanin synthesis using both mushroom tyrosinase and mouse B16 melanoma cells. The results demonstrated that compounds 3–7 (Figure 5) exhibited remarkably strong activity, with half-maximal effective concentration (EC_{50}) values ranging between 1.3 and 8.1 μM. These values were significantly lower—and therefore more potent- than that of the reference compound 8-methoxypsoralen, which displayed an EC_{50} of 14.8 μM. Further structure–activity relationship analysis revealed that the nature and position of substituents on the benzene ring played a key role in determining biological activity. Among the tested substituents, the order of potency was observed as follows: 4-Cl > 3,4-F > 3,4-Cl > 4-F > 4-Cl,3-F, suggesting that halogen substitution at specific positions greatly enhanced the melanogenesis-stimulating potential of these chalcone derivatives.

In addition to the mushroom tyrosinase assay, the effects of these compounds were assessed in B16 melanoma cells, where all tested derivatives demonstrated superior melanin-producing activity compared with 8-methoxypsoralen. Notably, compound 4 stood out for its exceptional potency, producing a remarkable 463% increase in melanin content, which was nearly three times greater than the activity of 8-methoxypsoralen (115%). These findings strongly indicate that chalcone derivatives containing isoxazole moieties not only exhibit enhanced bioactivity in comparison with established references but also hold significant potential as promising lead molecules for the development of therapeutic agents targeting melanin-related disorders [19].

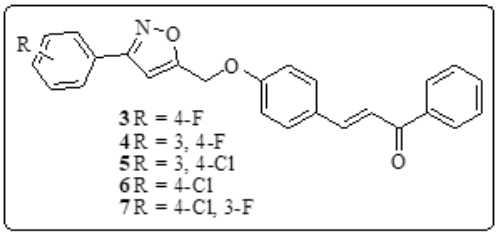

Figure 5: Isoxazole-substituted chalcone derivatives 3-7

Yin et al. reported the development of a novel isoxazole-substituted chalcone derivative, designated as compound 8 (Figure 6), and carried out a series of pharmacological evaluations to investigate its biological potential. Experimental studies revealed that compound 8 demonstrated a significantly stronger inhibitory effect on tyrosinase activity in mouse B16 melanoma cells compared to the reference compound, 8-methoxypsoralen. At a concentration of 50 μM, compound 8 inhibited tyrosinase activity by 135.7%, whereas 8-methoxypsoralen at the same concentration showed only 120.1% inhibition. This suggests that structural modification of chalcone with an isoxazole moiety confers a marked improvement in activity, positioning compound 8 as a more effective regulator of melanogenic enzymes.

Beyond tyrosinase inhibition, compound 8 was also observed to stimulate melanin synthesis in B16 melanoma cells at a notable level. Specifically, treatment with 50 μM of compound 8 resulted in a melanin production rate of 199.8%, which was considerably higher than the 127.9% observed with 8-methoxypsoralen under identical conditions. Mechanistic investigations using Western blot analysis provided further insight, demonstrating that compound 38 enhances melanogenesis by modulating the Akt and GSK3β signaling pathways. These findings strongly indicate that compound 8 not only exerts dual activity—tyrosinase inhibition and melanin production—but also possesses mechanistic pathways that favor pigmentation. Taken together, this evidence highlights the potential of isoxazole-substituted chalcone derivative 8 as a promising lead candidate for the development of novel therapeutic agents aimed at treating pigmentation disorders such as vitiligo [20].

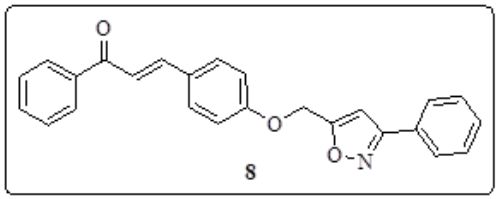

Figure 6: Isoxazole-substituted chalcone derivative 8

Sunitha et al. reported the synthesis of a novel series of diisoxazole-like chalcone derivatives and systematically evaluated their antibacterial and antifungal properties. The study focused on assessing the efficacy of compounds 9-12 (Figure 7), which were tested at concentrations of 75 and 100 µg/mL against a broad panel of pathogenic microorganisms. These included eight bacterial strains—*Micrococcus luteus (M. luteus)*, methicillin-resistant *Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), Bacillus cereus (B. cereus), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumoniae (K. pneumoniae), Escherichia coli (E. coli)*, and *Proteus vulgaris (P. vulgaris)*—as well as three clinically important fungi, namely *Microsporum canis, Microsporum gypseum,* and *Epidermophyton floccosum*. Compounds demonstrated strong inhibitory activity across both bacterial and fungal strains, highlighting their potential as broad-spectrum antimicrobial agents. The dual effectiveness against both Gram-positive and Gram-negative bacteria, in addition to antifungal activity, emphasizes the versatility of these diisoxazole-like chalcones in combating microbial infections.

Further analysis of the structure–activity relationship (SAR) provided insights into the influence of substituents on antimicrobial activity. The study revealed that the presence of electron-withdrawing groups at the R1 position and electron-donating groups at the R2 position significantly enhanced biological activity. This substitution pattern appeared to strengthen the compounds' ability to interact with microbial targets, thereby improving their potency. Such findings not only validate the rational design of these derivatives but also provide a valuable framework for the future optimization of chalcone-based antimicrobial agents. Taken together, the work of Sunitha et al. underscores the medicinal potential of diisoxazole-like chalcones and identifies specific structural features that could be exploited for the development of next-generation antibacterial and antifungal drugs [21].

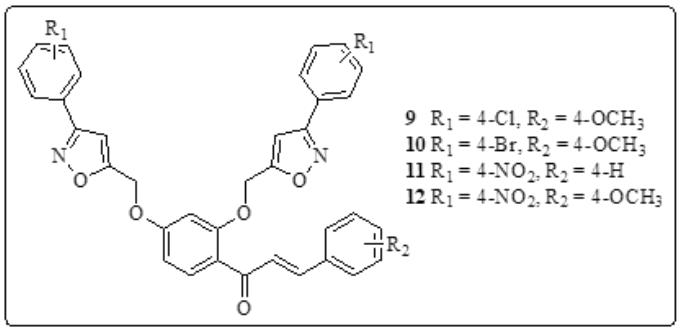

Figure 7: Isoxazole-substituted chalcone derivatives 9-12

Sunitha et al. synthesized a novel series of monoisoxazole-like chalcone derivatives and investigated their antibacterial potential against a broad spectrum of microorganisms. Among the synthesized compounds, derivatives 13–16 (Figure 8) were particularly noteworthy, showing strong antibacterial activity when tested at concentrations of 75 and 100 µg/mL. Their efficacy was demonstrated against both Gram-positive and Gram-negative bacterial strains, including *Micrococcus luteus (M. luteus), Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), Bacillus cereus (B. cereus), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumoniae (K. pneumoniae), Escherichia coli (E. coli)*, and *Proteus vulgaris (P. vulgaris)*. The wide range of activity demonstrated by these compounds underscores their potential as promising antibacterial agents capable of targeting diverse bacterial pathogens, including clinically relevant strains that are often resistant to existing drugs.

In addition to antimicrobial screening, the study also provided insights into the structure–activity relationship (SAR) of the synthesized compounds. It was observed that the incorporation of strong electron-withdrawing substituents on the phenyl ring attached to the isoxazole moiety significantly enhanced antibacterial potency. This finding suggests that electronic effects of substituents play a critical role in influencing the biological activity of these chalcone derivatives, possibly by increasing their interaction with bacterial enzymatic or membrane targets. Such SAR observations not only validate the design strategy employed by Sunitha et al. but also highlight important chemical modifications that could guide future optimization of monoisoxazole-like chalcones. Overall, these results establish this class of compounds as valuable scaffolds for the development of novel antibacterial agents with improved efficacy and broad-spectrum activity [22].

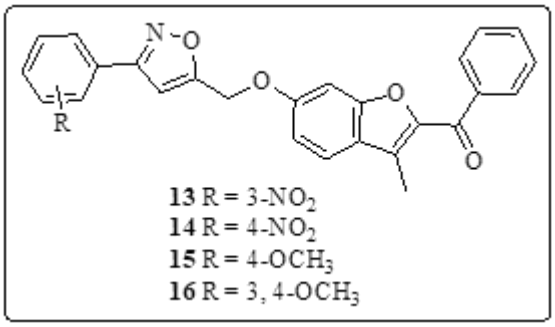

Figure 8: Isoxazole-substituted chalcone derivatives 13-16

Shaik et al. reported the rational design and synthesis of chalcone derivatives containing an isoxazole ring, aiming to explore their multifunctional biological potential. The synthesized compounds were systematically evaluated for their antibacterial, antioxidant, and anticancer activities. Among them, compounds 17–19 (Figure 9) displayed particularly strong antibacterial effects, with minimum inhibitory concentration (MIC) values ranging from 1 to 16 μg/mL against tested bacterial strains. Interestingly, the study highlighted that the introduction of electron-donating substituents on the phenyl moiety significantly enhanced antibacterial potency, suggesting a strong structure–activity relationship (SAR). This observation provides valuable guidance for further structural modifications in order to optimize chalcone derivatives for broad-spectrum antibacterial applications.

In addition to antibacterial effects, several compounds demonstrated noteworthy antioxidant and anticancer properties. Compounds 20–22, along with compound 18, exhibited strong radical-scavenging activity, with IC_{50} values between 5 and 8 μg/mL, indicating their potential role in combating oxidative stress–related disorders. Furthermore, compounds 19 and 23–24 showed remarkable cytotoxicity against DU-145 prostate cancer cells, with IC_{50} values in the range of 5–8 μg/mL. These findings suggest that isoxazole-containing chalcone derivatives act as multifunctional agents, capable of exerting antibacterial, antioxidant, and anticancer effects. Collectively, this study underscores their therapeutic promise and highlights them as attractive lead molecules for further development in the search for new drugs with diverse pharmacological activities [23].

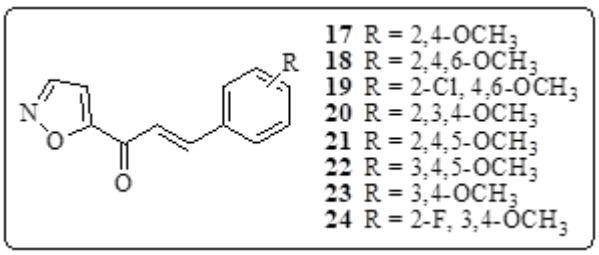

Figure 9: Isoxazole-substituted chalcone derivatives 17-24

Sahoo et al. successfully designed and synthesized a novel series of 5-phenyl-3-isoxazolecarboxylic acid methyl ester–chalcone hybrids and evaluated their antimicrobial activity, with a particular focus on Mycobacterium tuberculosis H37Rv (Mtb H37Rv). The synthetic strategy began with the preparation of chalcone acids through a conventional aldol condensation reaction. In this step,

4-formylbenzoic acid was condensed with various substituted acetophenones in the presence of potassium hydroxide as a base, using methanol as the reaction medium. These intermediate chalcone acids were subsequently coupled with methyl 5-(3-aminophenyl) isoxazole-3-carboxylate to form the desired hybrid molecules. The coupling reaction was facilitated by the use of 4-dimethylaminopyridine (DMAP) as a base and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) as a coupling agent. Through this synthetic pathway, a series of target molecules—namely, isoxazole methyl ester–chalcone derivatives—were obtained for further biological evaluation.

The biological screening of these hybrids compounds revealed promising antimicrobial activity. Compounds 25-28 (Figure 10) displayed significant in vitro inhibitory effects against Mtb H37Rv, with a minimum inhibitory concentration (MIC) value of 0.12 μg/mL, demonstrating potent antimycobacterial properties. Importantly, cytotoxicity assays conducted on Vero cells indicated that these compounds exhibited no measurable toxicity at concentrations up to 10 μg/mL (CC_{50} > 10 μg/mL), with selectivity indices (SI) exceeding 320, suggesting a favorable safety profile. Among the series, compound 27 emerged as the most potent candidate, showing remarkable anti-drug-resistant activity with MIC values ranging between 0.03 and 0.5 μg/mL against resistant strains of Mtb H37Rv. These results underscore the therapeutic promise of isoxazole–chalcone hybrids, particularly compound 27, as potential lead molecules for the development of new antimycobacterial agents capable of overcoming drug resistance [24].

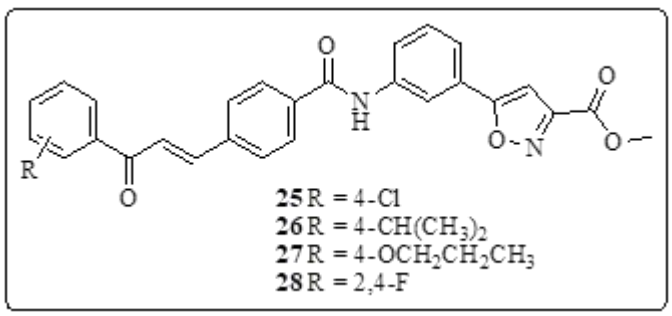

Figure 10: Isoxazole-substituted chalcone derivatives 25-28

Chaithanya et al. reported the design and synthesis of novel chalcone-incorporated thiazole–isoxazole derivatives (29-38) (Figure 11) and evaluated their potential as anticancer agents. All the synthesized derivatives were subjected to preliminary cytotoxicity screening against four human cancer cell lines, including MCF-7 (breast cancer), A549 (lung

cancer), Colo-205 (colon cancer), and A2780 (ovarian cancer), using the MTT assay. The majority of the tested compounds demonstrated significant anticancer activity, showing enhanced cytotoxic effects in comparison to the standard reference drug, etoposide. These results indicate that incorporation of thiazole–isoxazole motifs into the chalcone scaffold can effectively enhance antiproliferative properties.

Notably, compound 38 emerged as the most promising candidate, showing IC_{50} values of 0.33 ± 0.085 μM for MCF-7, 0.12 ± 0.064 μM for A549, 0.77 ± 0.075 μM for Colo-205, and 0.93 ± 0.082 μM for A2780 cells. These findings highlight the potential of chalcone–thiazole–isoxazole hybrids as highly effective anticancer agents, suggesting that further structural optimization and mechanistic studies could lead to the development of novel therapeutic candidates with broad-spectrum anticancer activity [25].

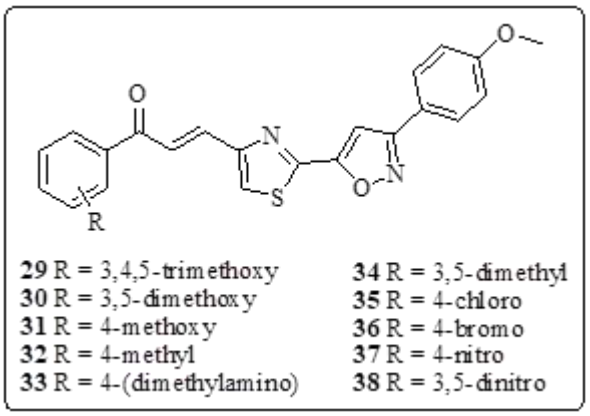

Figure 11: Isoxazole-substituted chalcone derivatives 29-38

Conclusion

Chalcone–isoxazole hybrids have emerged as an important class of bioactive molecules with diverse therapeutic applications, particularly in the areas of antimicrobial and anticancer drug discovery. The structural integration of chalcone and isoxazole motifs provides a synergistic framework that enhances biological potency and widens the spectrum of activity. Compounds such as 25–28 demonstrated remarkable anti-tuberculosis effects against Mycobacterium tuberculosis with low cytotoxicity, highlighting their potential as scaffolds for developing new anti-infective agents. The structure–activity relationship (SAR) findings, particularly the role of nonpolar substituents like halogens and alkyl groups, offer valuable insights for designing more effective derivatives.

Beyond antimicrobial activity, the exploration of chalcone–isoxazole hybrids in cancer therapy has yielded promising results. The incorporation of thiazole–isoxazole motifs significantly enhanced antiproliferative potential, as evidenced by compound 38, which exhibited submicromolar IC_{50} values across multiple cancer cell lines. While many derivatives displayed moderate to good cytotoxicity, the exceptional activity of certain candidates underscores the importance of rational hybrid design in improving therapeutic efficacy. The multifunctional properties of these compounds, spanning antibacterial, antioxidant, and anticancer effects, further strengthen their relevance as versatile drug candidates.

Overall, chalcone–isoxazole hybrids represent a versatile and powerful platform for medicinal chemistry research. Their broad-spectrum bioactivity and favorable safety profiles provide a strong foundation for further preclinical and clinical exploration. Future research should focus on fine-tuning substituent patterns, developing hybrid frameworks with additional pharmacophores, and exploring synergistic combination therapies. Such efforts will not only improve their therapeutic performance but also pave the way for the discovery of next-generation drugs capable of addressing global health challenges such as tuberculosis, drug-resistant infections, and cancer.

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Eco-friendly Green Synthesis,Characterization,Biological Activity of Silver & Iron Nanoparticles from Various Plants and Spices Extract

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Article DOI Link: https://zenodo.org/uploads/17542800
DOI: 10.5281/zenodo.17542800

Abstract

In the recent years the green and eco-friendly method of synthesis for metal nanoparticles is an an emerging field in nanotechnology and nanoscience. The importance of nanoparticles in society and industries is due to the remarkable change in the physical and chemical properties of the materials in nanodimensions. This paper aims to present a brief overview of different biosynthesis routes of silver nanoparticles (NPs) & Iron nanoparticles, their applications and influence of the method used on the size and morphology of these nanoparticles. A detailed and comprehensive study of available biological methods, also referred to as a bottom-up approach, as well as techniques reported, have been provided with an eye for details and comparison between the techniques involving fungi, bacteria, algae and plant extracts. Plant-derived bioreductants such as leaf, stem or root extracts of various plants are seen as suitable solutions to green synthesis of silver NPs & Iron NPs, implementing an easy, non-toxic, clean and environmentally friendly approach. Furthermore, reports on the antimicrobial activities with the zone of inhibition for various pathogens have also been included.

Keywords: Nanotechnology, Silver, Iron Nanoparticles, Biologi-cal Methods, Antimicrobial Activity

Introduction

Matter can be comprehensively isolated into two classes in view of the size: Macroscopic and Mesoscopic. Naturally visible matter is noticeable to the unaided eye while Mesoscopic particles, for example, microscopic organisms and cells that have aspects on the request for micron(s), can be seen with optical magnifying lens. Falling into the hole between the minute and the mesoscopic is

one more class of issue, the nanoscopic particles. The size of nanoparticles is contrasted with that of other "little" particles in Figure 1 underneath, where the bacterium is colossal in correlation [1].

Nanostructures generally range from 1-100 nm in aspect. These particles have high surface to volume proportion and a high part of surface iotas. At nano level they have explicit physicochemical properties like optical property, attractive property, synergistic property and so on [2]. With the rise of new physical and compound strategies for the combination of nanoparticles, the worries for natural defilement have been expanded. The engineered methodology creates unsafe results that could influence the climate straightforwardly. In this manner there is an incredible prerequisite for green science that incorporates techniques which are climate amicable. In this strategy for green amalgamation there is no prerequisite for high strain, energy,

temperature or poisonous synthetic compounds. Consequently, these days numerous analysts are redirecting themselves from utilizing engineered strategies. They are attempting to turn themselves towards organic frameworks for the most part plants for nanoparticle amalgamation as it is financially savvy and can be effectively increased to be utilized for enormous scope production.[3].

Organic frameworks, for example, plants microorganisms produce inorganic materials and the majority of these are available in nanoscale aspects. The cell removes from these natural living beings can be utilized to blend nanoparticles of various size and substance creations. Biosynthesis of metal nanoparticles separated from various parts (for the most part leaf) of the plant is the best cycle of combination at an entirely reasonable expense. During the union bioreduction of metal particles happens. Concurring to the analysts the polyol parts present in the plant remove are answerable for the decrease of iron particles while water solvent heterocyclic parts balance out the nanoparticles framed. Fitting forerunners, for example, Ferric Chloride can be utilized for the decrease of plant separates. [4]. Here we report the amalgamation of nanoparticles, lessening Ferric particles present in the fluid arrangement of Ferric chloride by the assistance of various plant extricates. Through intricate screening process including around 45 plants, we chose 10 most appropriate plants as the possible contender for the combination of iron nanoparticles.

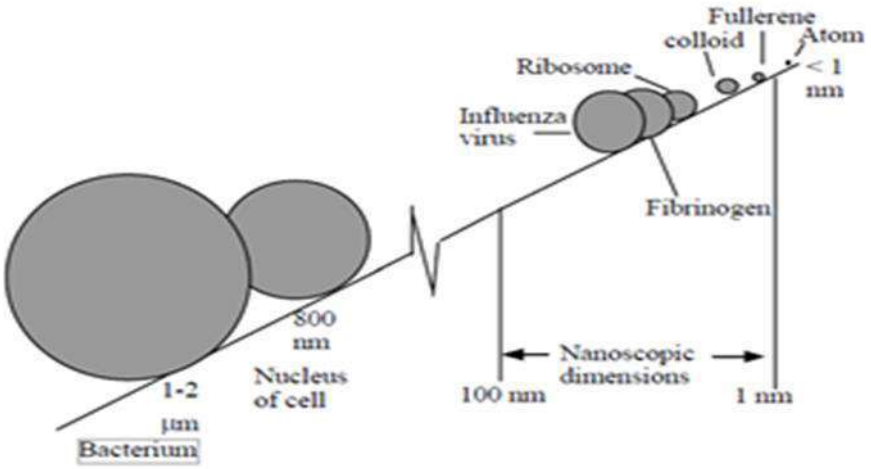

Figure1. Comparison of nano range with other sizes

The circle of nanotechnology has been at the center of attention in ongoing years, as the astounding development of numerous significant enterprises, like synthetic compounds, hardware, horticulture, medication and the space business, has been reformed because of its impact on the above-expressed ventures [5]. The development of metallic nanoparticles is a functioning region for analysts for scholastic purposes as well as in the improvement of nanotechnology. Metallic nanoparticles stand out as they are seen to have strange physical and compound properties, which essentially vary from their properties when taken in mass sums [6]. Any adjustment of their size would cause an immediate change in the reactant, electronic and optical properties of the nanoparticles [7]. For example, metallic silver as silver nanoparticles has upgraded synthetic and actual properties as contrasted and typical silver metal [8]. In addition, they show better antibacterial [9], antifungal [10] and antiviral [11] properties in correlation with metallic silver and different silver mixtures. Uses of silver nanoparticles (AgNPs) incorporate, yet are not restricted to, hardware [12], biosensing, photonics, optoelectronics, detecting, drugs [13], materials, water treatment [14], DNA sequencing [15] and surface-upgraded Raman dissipating (SERS) [16]. Ag nanoparticles go about as an antimicrobial specialist [17] and are being utilized for the treatment as well as the counteraction of HIV [18]. AgNPs have arranged application, like colours, photo graphics, wound treatment and conductive/auto static composites [19]. Such a wide assortment of uses has driven scientists to plan better and more conservative ways for the creation of AgNPs for a huge scope. The plan of exploratory strategies for the creation of nanoparticles with

various substance sythesis, sizes, shapes and dispersity is an significant feature of nanotechnology [20].

Throughout the most recent couple of years, the imperative meaning of manufacturing clean, non-poisonous and harmless to the ecosystem solvents and synthetic substances has catalyzed the biosyn- postulation of nanoparticles. Organic cycles fixated on microscopic organisms, parasite, bio-determined synthetic substances and plant removes are definitely concentrated due to their eco-accommodating nature and morphological control [21]. Natural sources accessible in nature, including microorganisms, green growth, yeast, parasites, lower plants and higher angiosperm plant items, can be utilized for the amalgamation of nanoparticles. These surrounding natural frameworks give fantastic instances of nano-phasic materials with profoundly enhanced attributes. The assembling of inorganic materials might happen in twoways, either extracellular or intracellular [22]. In current research areas of nanotechnology, creating solid exploratory guidelines for the amalgamation of nanoparticles over a scope of substance structure, size, and synchronized, non-poisonous, clean and eco-accommodating monodispersity is a huge test. Albeit many papers have been accounted for over the most recent couple of years, a more prominent number of comprehensive distributions are required so the world may find the uses of the biosynthesis of different metal nanoparticles. The utilization of harmless to the ecosystem materials, for example, plant remove, microbes, parasite furthermore compounds [23] for the combination of silver nanoparticles offers many advantages of similarity with drug furthermore other biomedical applications, attributable to the utilization of nontoxic synthetic substances for the blend methodology. Substance combinations of nanoparticles include the presence of some poisonous synthetic compounds assimilated on a superficial level that might have a lamentable impact whenever utilized in the field of drugs. In contrast, green union has an edge over synthetic and actual techniques for union as it is modest, eco-accommodating also can be increased to bigger scope blend easily.

This technique doesn't need the utilization of high strain, energy, temperature and harmful synthetics as contrasted and synthetic union. Blend of nanoparticles utilizing natural means, particularly plants, is biocompatible as they emit practical biomolecules which effectively decrease metal particles. Additionally, natural specialists, for example, plants engaged with the diminishing system likewise go about as covering specialists and are eco-accommodating [24]. Thus, we give diagrams of different reports on the natural method for nanoparticle union with wanted attributes, with an eye for subtleties to permit powerful

correlation and significant determination.

Synthesis of Silver Nanoparticles

The union of silver NPs can be completed by a few strategies including compound (e.g., substance decrease, microemulsion strategies, pyrolysis, UV-started photo- decrease, photoinduced decrease, electrochemical engineered technique, illumination strategies, microwave assisted combination, polymers and polysaccharides, Tollens strategy), physical (e.g., vanishing buildup, laser removal, circular segment release technique, direct metal faltering into the fluid medium) and organic techniques (e.g., utilization of green growth, organisms, microorganisms and plants as bioreductant) [25].

The synthetic and actual cycles for the most part include dangerous synthetic substances, high energy necessities and other severe circumstances. The sizes and morphology of silver nanoparticles blended from these two strategies are very factor contingent upon the circumstances and strategies applied. As opposed to the substance and actual strategies, the organic technique, otherwise called the base up approach, has had the option to biosynthesis silver nanoparticles with better sizes and morphologies. The majority of the NPs created were accounted for to have a dominantly circular shape. Different advantages of the utilization of the green methodology are the utilization of natural reductants, low to zero energy prerequisites and better attributes of the metallic silver nanoparticles, with the upside of disposal of the requirement for harmful synthetic substances to be utilized as surfactant or stabilizers since different proteins present in the plant extricates go about as decreasing as well as covering specialists for silver NPs [26]. The following is a correlation between different bio-based strategies to examine and rehearse the most appropriate organic methodology for the biosynthesis of silver NPs to address the future difficulties of interest and supply of metallic silver NPs.

Preparation of Plant Extract and the Precursor

For the synthesis of iron nanoparticles, 0.001 M Ferric Chloride was prepared by using triple distilled water. Plant extracts were prepared by taking approximately 25gms leaves/seeds/buds. These were of thoroughly washed with sterile distilled water, dried and finely crushed with the help of mortar and pestle by adding 5-10 ml of deionized water gradually. The mixture was poured in a flask and heated for 5-10 minutes at 700C before finally decanting it. The mixture was then filtered using Whatman No. 1 filter paper. Wherever necessary the plant mixture was centrifuged at 5000 rpm for 5 minutes and the supernatant was collected as

the plant extract and used for further process. Clean and aseptic condition was maintained throughout the process.

Synthesis of Iron Nanoparticles

During the synthesis of Iron Nanoparticles both the precursor and the reducing agentwere mixed in a clean sterilized flask in 1:1 proportion. For the reduction of Fe ions, 5ml of plant extract was mixed to 5 ml of 0.001 M aqueous of FeCl3 solution with constant stirring at 50-600

Organic Methods

Bacteria

Highly stable silver NPs with an average size of 40 nm were prepared by reduction of silver ions using culture superannuate of Bacillus licheniformis. Similar bacteria were reported to be able to synthesize well dispersed silver NPs with an average diameter of 50 nm. Microwave irradiation was used to support uniform heating in the case of extracellular biosynthesis of silver NPs by bioreductant culture supernatant of B. subtilis. The silver metal NPs produced by this method were reported to be monodispersed, within the size range of 5-20 nm. Various researchers reported the ability of Pseudomonas stutzeri AG259 to biosynthesize intracellularly silver NPs of varying compositions, with a size range of 35-46 nm, or up to 200 nm in the case of high concentrations of silver ions of varying geometrical structures. Shahverdi AR et al. successfully demonstrated the rapid bioreduction abilities of culture supernatants of Entero-bacter cloacae, Escherichia coli and Klebsiella pneumonia to reduce silver ions into metal silver NPs within five minutes of exposure [27].

The effects of visible-light irradiation on the biosynthesis of silver NPs using culture supernatant of Klebsiella pneumonia were studied by Mokhtari N et al. The size range of such NPs was calculated to be 1-6 nm. The mechanism of bioreduction of Diamminesilver to biosynthesize metallic silver NPs using Aeromonas sp. SH10 and Corynebacterium sp. SH09 was suggested by Mouxing FU et al. Spherical silver particles were observed when strains of Lactobacillus were used to reduce silver ions with an average size of between 25-50 nm. In the case of agglomeration of silver NPs, the average size of the agglomerated metal particles was observed to be 100 nm. Enzymatic process was attributed as the reason for the stability of the biosynthesized silver NPs [28]. Table 1 provides sizes of silver NPs' biosynthesis by reducing silver ions by bioreductant bacteria.

Table 1. Biosynthesis of Silver NPs using Bacteria

Organism	Mode	Characteristics of Silver NPs			Characterization Instrument	Microbial activity against / Applications*
		Size (nm)	Shape	Others		
Pseudomonas stutzeri AG259	–	Up to 200	Equilateral triangles, hexagons	Agglomerations	TEM, quantitative EDX, electron diffraction	–
Plectonema boryanum (strain UTEX 485)	extracellular intracellular	1 – 15	Spherical	25°C	TEM, TEM-SAED, TEM-EDS, XPS	–
		1 – 40	Spherical	60°C		
		5 – 200	Spherical , octahedral crystal platelets	100°C		
Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae	extracellular	50 – 100	Predominantly spherical	–	SEM, UV-visible spectroscopy	–

Fungi

A few analysts, including Ahmad et al., Macdonald et al., Ahmad et al., Kumar et al. what's more Korbekandi et al., have shown incredible interest in the capability of Fusarium oxyspo- rum to incorporate silver NPs to lay out new ways to deliver them in a harmless to the ecosystem and costeffective manner. Ahmad An et al. analyzed the given strain to deliver 5 - 50 nm silver NPs extracellularly and referenced the high strength of these silver NPs due to proteins in the strain. Macdonald IDG et al. Showed distinct fascination with this subject and attempted to comprehend the collaboration of these proteins including cytochrome c (Cc) with silver NPs. Crafted by Ahmad An et al. Also, Kumar SA et al. give further knowledge into the bioreduction of silver particles by utilizing bioreductant F. oxysporum and portray the enzymatic cycle and the subsequent solidness of silver NPs. The morphology of the biosynthe- estimated NPs and the impacts of pH on the covering proteins were outlined by Kumar SA et al. Korbekandi H et al. detailed the morphology of silver NPs arranged utilizing Fusarium oxysporum to be practically round, with a size scope of 25 - 50 nm and 100 nm on account of person furthermore agglomerates separately, by Scanning Electron Magnifying lens (SEM) micrographs. The creators express the biosynthesis of silver NPs by Fusarium oxysporum to be intracellular instead of extracellular. The bio

reduction of silver ions and its stability was further explained to be the result of an enzymatic process [29].

The potentials of Fusarium acuminatum Ell. and Ev. (USM-3793) cell extracts were exploited to obtain metallic silver NPs with an average diameter of 13 nm. The NPs were synthesized quite rapidly, i.e., within 15-20 minutes of reaction, by the cell extracts of the mentioned algae and remained within the size range of 5 – 40 nm. Vigneshwaran N et al. reported the use of Phanerochaete chrysosporium to reduce silver ions acquiring predominantly pyramidal-shaped silver NPs. Aspergillus flavus and Aspergillus fumigatus were exploited for biosynthesis of silver NPs [30]. The Aspergillus flavus was claimed to be highly stable in water [75]. The morphology of the extracellularly biosynthesized silver particles, size 5 – 25 nm, was reported to be predominantly spherical with few triangular shapes; such exceptions or few changes thereof are expected to be present in bio-based synthesis of silver NPs [30].

Balaji DS et al. studied the extracellular biosynthesis of silver NPs by filtrate of Cladosporium cladosporioides fungus. The chemical compounds released by the strains of Cladosporium cladosporioides were considered to be responsible for the stability and shape of the silver NPs. Penicillium sp. J3, Penicillium fellutanum and Penicillium genus were successfully treated for the reduction of silver ions into silver NPs. Penicillium fellutanum was able to reduce silver ions into silver NPs successfully using incubation under dark conditions. Monodisperse spherical silver NPs were reported to be produced by reduction of silver nitrate solution by Coriolus versicolor [31]. The characteristics of these silver NPs were recorded through UV-visible absorption spectrophotome- try, Transmission Electron Microscope (TEM), Atomic Force Microscopy (AFM) and Fourier Transform Infrared spectroscopy (FT-IR) [31]. Sanghi R et al. reported the influence of parameters such as pH and temperature on the reaction time and characteristics of the NPs [31]. Proteins were reported to be the main cause for the stability and were suggested to be performing as a capping agent as well [31]. A list of organisms used for the biosynthesis of silver NPs and the characteristics of these silver NPs have been summarized in Table 2.

Organism	Mode	Characteristics of Silver NPs			Characterization Instrument	Microbial activity against / Applications*
		Size (nm)	Shape	Others		
Fusarium solani	extracellular	5 – 35	Spherical	Large distribution range, polydisperse	UV-vis spectrophotometer, FT-IR, TEM	–
Aspergillus clavatus	extracellular	10 – 25	Spherical few polyhedral	Highly variable, polydisperse	XRD, TEM, atomic force microscopy (AFM)	Candida albicans, Pseudomonas fluorescens and Escherichia coli
Cladosporium cladosporioides	extracellular	10 – 100	Mostly spherical	Different crystallite shapes, polydisperse	UV-vis spectrophotometer, XRD, TEM, FT-IR, Scherrer's equation	–
Penicillium fellutanus	–	5 – 25	Mostly spherical	Variable Well dispersed	UV-vis absorption spectra, TEM	–
Fusarium acuminatum Ell. and Ev. (USM-3793)	extracellular	5 – 40	Spherical	Spherical with a broad size	UV-vis spectrophotometer, TEM	Staphylococcus aureus, Salmonella typhi, Staphylococcus epidermidis and Escherichia coli
Penicillium fellutanum	–	5 – 25	Mostly spherical	Well dispersed	UV-vis absorption spectra, TEM	–

Table 2. Biosynthesis of Silver NPs using Fungi

Algae

Yellowish brown colour indicating the formation of silver NPs was observed when Spirulina platensis biomass was challenged with 0.001 M aqueous $AgNO_3$ solution. Surface plasmon absorbance, X-ray powder diffraction (XRD), High-resolution transmission electron microscopy (HRTEM) and Fourier transform infrared spectroscopic (FT-IR) measurements were utilized for recording the characteristic dispersions of nanometallic particles, confirmation of formation of silver NPs, crystalline nature, predominantly spherical shape, size range of silver NPs 7-16 nm and the possible action of proteins for reduction and capping of silver NPs respectively [32]. Iravani S et al. mentioned in their review the ability of C. Vulgaris and Oscillatoria willei to synthesize silver NPs [33]. C. Vulgaris biosynthesized silver nanoparticles in a rod-like shape with a mean length of 44 nm and width of 16- 24 nm, while Oscillatoria willei biosynthesized silver NPs with a diameter range of 100- 200 nm [33]. The efforts of a few researchers to biosynthesize silver NPs using algae have been presented in Table 3.

Organism	Mode	Characteristics of Silver NPs			Characterization Instrument	Microbial activity against / Applications*
		Size (nm)	Shape	Others		
Spirulina platensis	extracellular	7 – 16	Predominantly spherical	Relatively uniform	HRTEM, FT-IR, UV-vis spectrophotometry, XRD	Showed anti-coagulative activity
Oscillatoria willei NTDM01	extracellular	100 – 200	-	Agglomerations	HRTEM, FT-IR, UV-vis spectrophotometry, XRD	Antimicrobial
C. vulgaris	extracellular	Mean length of 44 and width of 16 – 24	Rod-like particles	-	TEM, FT-IR, UV-vis spectrophotometry, XRD, field emission scanning electron microscopy (FESEM)	Antimicrobial

Table 3. Biosynthesis of Silver NPs using Algae

Plants

Plants that were used in the experiment are described below:

1. **Bionomial Name: *Mangifera indica***

Common Name: Mango

Plant part taken: Leaves

Family Name: Anacardiaceae

Description

Mangiferin (a harmacologically active flavonoid, a natural xanthone C-glycoside) is extracted from Mango at high concentrations from the young leaves. Mangiferin shows an exceptionally strong antioxidant capacity. It has a number of pharmacological actions and possible health benefits. These include antidiabetic, antioxidant, antifungal, antimicrobal, antiinflamatory, antiviral, hypoglycemic, anti-allergic and anticancer activity.

Fig. 2 Mangifera indica

2. Bionomial Name: *Syzygium aromaticum*

Common Name: Clove

Plant part taken: Buds

Family Name: Myrtaceae

Description

Cloves are the aromatic dried flower buds of a tree. It is used as a spice in cuisines all over the world. Cloves are used in Indian Ayurvedic medicine, Chinese medicine, and western herbalism and dentistry where the essential oil is used as an anodyne (painkiller) for dental emergencies. Cloves are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis.

Fig. 3 Syzygium aromaticum

3. Bionomial Name: *Rosa indica*

Common Name: Rose

Plant part taken: Leaves

Family Name: Rosaceae

Description

Rose is a woody perennial. They form a group of erect shrubs, and climbing or trailing plants. Roses are best known as ornamental plants. Many roses have been used in herbal and folk medicines. Other species have been used for stomach problems, and are being investigated for controlling cancer growth.

Fig. 4. Rosa indica

4. Bionomial Name: *Azadirachta indica*

Common Name: Neem

Plant part taken: Leaves

Family Name: Meliaceae

Description

It is a tree in the mahogany family. The leaves are used in this manner that first they are washed thoroughly. Then 5-10 leaves along with the branch are boiled till the water turns green the water is then used for varying purposes. Elders find it useful in controlling high blood sugar level and is said to clean up the blood. The tender shoots and flowers of the neem tree are eaten as a vegetable in India. Neem gum is a rich source of protein. Products made from neem trees have been used in India for over two millennia for their medicinal properties: neem products are believed to be anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative.

Fig 5. Azadirachta indica

5. Bionomial Name-*Camellia sinensis*

Common Name – Black Tea

Plant part taken- Leaves

Family Name-Theaceae

Description

Tea is the second most commonly drank liquid on earth after water. It has numerous medicinal benefits mainly due to its antibacterial and antioxidant properties. It has been known to inhibit the growth of cancer cells and support cardiovascular health.

Fig 6. Camellia sinensis

6. Bionomial name: *Camellia sinensis*

Common Name: Green Tea

Plant part taken: Leaves

Family Name: Theaceae

Description

Green tea originates in China. Green tea has purported health benefits, with some evidence suggesting that regular green tea drinkers may have a lower risk of developing heart disease and certain types of cancer. A green tea extract

containing polyphenols and caffeine has been shown to induce thermogenesis and stimulate fat oxidation, boosting the metabolic rate 4% without increasing the heart rate. Flavonoids are a group of phytochemicals in most plant products that are responsible for such health effects as anti-oxidative and anticarcinogenic functions.

Fig 7. Green Tea

7. Bionomial name: *Coffea arabica*

Common Name: Coffee

Plant part taken: Seeds

Family Name: Rubiaceae

Description

Coffee is a genus of flowering plants whose seeds, called coffee beans, are used to make coffee. The caffeine in coffee "beans" is a natural plant defense against herbivory, i.e. a toxic substance that protects the seeds of the plant. Several insect pests affect coffee production, including the coffee borer and the coffee leafminer. Coffee is used as a food plant by the larvae of some Lepidoptera (butterfly and moth) species.

Fig 8. Coffea arabica

8. Bionomial name: *Trachyspermum ammi*

Common Name: Carom seeds

Plant part taken: Seeds

Family Name: Apiaceae

Description

The plant has a similarity to parsley. Because of their seed-like appearance, the fruit pods are sometimes called seeds. The raw fruit pod smells almost exactly like thyme because it also contains thymol. It is traditionally believed to be a digestive aid.

Fig 9. Trachyspermum ammi

9. Bionomial name: *Magnolia champaca*

Common Name: Joy Perfume Tree, Champa

Plant part taken: Leaves

Family Name: Magnoliaceae

Description

Magnolia champaca is a large evergreen tree. The flowers are used in Southeast Asia for several purposes. It is rarer and has a strong perfume, and is not that commonly or plentifully used. *Magnolia champaca* is cultivated and used as an ornamental tree in temperate climate gardens.

Fig 10. Magnolia champaca

10. Bionomial name: *Murraya koenigii*

Common Name: Curry Leaves

Plant part taken: Leaves

Family Name: Rutaceae

Description

It is a tropical to sub-tropical tree which is native to India. The leaves are highly valued as seasoning in southern and west-coast Indian cooking. The leaves are used as a herb in Ayurvedic medicine. It is valued as an anti-diebetic, antioxidant, antimicrobial, anti-infammatory, anti-hypercholesterolemic, etc. It contains carbazole alkanoid that can induce apoptosis in cancerous cells in liver.

Fig 11. Murraya koenigii

Discussion

The biosynthesis of silver NPs using biological techniques– fungi, algae, bacteria, yeast and plants has proved to be environmentally friendly and an economical approach. Although microbial species have been able to biosynthesize predominantly spherical metallic silver NPs within the range of 1-70 nm and fungi able to produce SNPs with an average size range of 13 nm, yet the lack of knowledge of the mechanism of the reduction process represents a barrier still to be overcome [34].

The suggested mechanism for the biosynthesis of intracellular and extracellular silver NPs by bacteria involves reduction of silver by sulphur-containing proteins or deoxyribonucleic acid (DNA), while in the case of fungi the mechanism is thought to occur with the involvement of carboxylic group or through nitrate dependent reductase. In the case of plants, the reduction is suggested to be carried out by a wide variety of compounds such as terpenoids, flavonoids, phenols, pinito and allantoin, present in different parts of the plants including leaves, roots, bark and latex. In the case of intracellular synthesis, the downstream

processing is difficult and expensive due to the separation and purifying steps involved, thus making extracellular synthesis preferable, owing to its easier and simpler downstream processing. Compared with bacteria or algae, fungi provide a more rational and economical approach for biosynthesis of silver NPs due to the fact that not only is the downstream processing and biomass handling much simpler and easier in the case of fungi, but the amounts of proteins known to reduce silver are also secreted in much higher amounts, thus increasing the biosynthesis productivity several fold [35]. In the case of microorganisms, not only is the strain preparation and growth intricate, but the isolation of strain is also difficult and requires too many precautions. The difficulty of maintaining the culture medium and respective conditions such as (but not limited to) pH, temperature, salinity of the culture and reaction mixture points towards the complexity of these techniques to be applied on a large scale. Plant broths or extracts, on the other hand, are quite simple and easy to handle and eliminate the complicated procedures of cell culture maintenance. Furthermore, the clear filtrate production from bacterial broths necessitates the use of complicated equipment in process technology, thus increasing investment costs to a considerable extent, which is yet another major drawback in the case of the bacterial biosynthetic approach [36]. Conversely, in the case of fungi and plants, simple equipment such as a filter press can be utilized to obtain clear filtrates, thus promising economic feasibility [37].

The synthesis of silver NPs utilizing microorganisms or their filtered cell parts creates silver NPs at rates a lot slower than the rates at which plants can biosynthesize silver NPs. The time expected for the total decrease of silver particles is known to go from 24 hours to 120 hours on account of microorganisms, while the response fulfilment time is substantially less for the situation of plants, going from a couple of hours to a limit of 48 hours, as portrayed in the examinations referenced before in the plants area [38]. This addresses one more downside as far as plausibility of microorganisms to be utilized for huge scope biosynthesis of silver NPs in examination with plants, which require less time for finishing of response. The decrease rate by plants is quick enough to cause to notice the chance of fostering a normal biosynthesis system with decrease rates proportionate to those of physical and compound methods [39]. Recent investigations were directed with the goal of tracking down an appropriate method to gain wanted sizes and morphological qualities of biosynthesized silver NPs alongside an expanded creation rate. As plainly addressed in the properties of silver NPs talked about in their particular classes,

just plants in correlation with other natural methods can exhibit better command over morphological qualities, sizes and rates of creation of silver NPs by taking advantage of basic response conditions like stock fixations, silver nitrate arrangement focus, saltiness, proportion of plant (leaf, stem, bark or plastic) concentrate to silver nitrate arrangement, pH, temperature, blending time, season of extraction, sonication and light [40]. The decrease of silver nitrate can be completed at typical temperature, despite the fact that raised temperatures are best due to the expanded pace of response and less time expected for the arrangement of silver NPs. Sonication has ended up being the best, albeit the size accomplished from sonication was accounted for to be somewhat bigger than that achieved from raised temperature, yet the consistency in shape and lessening in response time were obviously higher for sonication than any of the other two circumstances [40].

Accordingly, as per the requirement for a financially reasonable, green methodology for the combination of silver NPs, the plant-based strategy addresses a hopeful other option not exclusively to natural strategies, yet in addition to different techniques counting actual strategies, and is additionally apparent for huge scope creation. In any case, there is as yet an extraordinary need to take advantage of the plant-based biotechnique to accomplish even better command over dispersity, morphology, molecule size what's more creation rates on the off chance that it is to substitute compound techniques for creation of silver NPs on a modern scale [41].

Conclusion

Biosynthesis of silver nanoparticles can be completed by natural strategies in which the organic species range from microbes, parasites and green growth to plants fit for diminishing silver particles to metallic silver nanoparticles. Natural techniques have been ended up being all the more ecologically cordial than compound and actual strategies because of a few reasons including, however not restricted to, arrangement of hazardous/poisonous bioproducts, use of natural species as reductants and lower energy necessities. In spite of the fact that microbial species have shown successful potential for the biosynthesis of metallic silver NPs, by and by the absence of mastery to completely comprehend and control the system of the decrease cycle addresses a hindrance yet to be overcome. Furthermore, the intricacy of keeping up with the balanced-out culture medium and individual circumstances such as, however not restricted to, ideal pH, temperature achievability, or on the other hand saltiness of the way of life and response blend focuses towards the multifaceted nature of these procedures

to be applied on a modern scale. Besides, on account of plants and a couple of other organic species like green growth, some normal synthetic mixtures present in the concentrate go about as diminishing as well as covering specialists, subsequently wiping out the requirement for harmful synthetic compounds to be utilized as covering specialists. The methodology to integrate metallic silver NPs utilizing plant-inferred extricates (leaf, root, and stem) addresses the start of an eco-accommodating, simple and straightforward methodology with no economic and ecological hindrances. Further headways in the determination of plant-inferred remove bio reductant and a satisfactory information on the decrease interaction mechanism will likewise be useful in deciding a modern, cost-effective method for orchestrating silver NPs with amazing qualities, morphologies and properties, for example, yet not restricted to, antimicrobial, optical and electrical.

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Assessment of Industrial Effluent and Its Impact on Germination and Seedling Growth for Sustainable Agriculture

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Article DOI Link: https://zenodo.org/uploads/17542862

DOI: 10.5281/zenodo.17542862

Abstract

Industries are indispensable for economic development of any country; effluents are both resource and problem as it contains important nutrients which can serve as fertilizer in agriculture. But these effluents cause harmful effect to the human health and environment when released into the water bodies in its raw form. The amplitude of the damage depends on the size, nature, type of industries, raw materials used and their preliminary treatment. Harmful pollutants in the effluents not only brings about phenomenal changes in receiving system and makes them unfit for agricultural and domestic use, but can also convert them into sources of potential carcinogens and mutagens (Somashekar et al.,1984). Industrial effluent is the waste water generated in the industriesis the second major source of water pollution and are making the water unfit for both agriculture and domestic use. But these effluents with proper dilution can be used as source of fertilizer in agriculture. In the present investigation, the treated effluent from BHEL was first analyzed for 13 physico-chemical parameters. Seed germination and seedling growth study of Phaseolus vulgaris and Phaseolus mungo was conducted in the second stage using different concentrations of [10, 25, 50, 75, 100 %] effluent. The percentage germination, shoot and root length, shoot and root inhibition, vigour index, tolerance index, phyto-toxicity were measured in all these treatments. The morphological parameters showed increase in 10 and 25% concentration of effluent. The higher concentration had inhibitory effect on all morphological parameters. Vigour Index of the seedling showed decrease in higher concentration of treatment, while the phyto-toxicity exhibited

increase with increase in concentration of treatment. The tolerance index was maximum in 10 and 25% treated plants. Bangalore being land locked city faces severe water scarcity, reuse of these treated effluents in agriculture is the best solution to mitigate this problem.

Keywords: Effluent, Germination, Root and Shoot length, Pollution

Introduction

Industrial effluents are the second major source of water pollution and are making the water unfit for both agriculture and domestic use. But these effluents with proper dilution can be used as source of fertilizer in agriculture. Recent studies on industrial effluents have revealed the nutrient and irrigational potential of the effluents specially in lower concentration for sustainable agriculture. Translocation of metals to edible part was less compared to concentrations in roots (Amori et al 2022). Recycle and reuse of the effluent is one of the best solutions envisaged by scientists all over the world. Singh and Mishra (1987) in their studies on effluent have shown that they can be used in agriculture which not only removes nutrients but also provides primary, Secondary and tertiary treatment. The increasing reuse of these effluents supports sustainable agriculture preserves scarce water resource and maintain water quality. Hayat et al., (2002) noted that in oil refinery effluents containing high content of potassium, phosphate, calcium, magnesium and sulphate can serve as fertilizer. Diluted effluent not only increased chlorophyll and protein content, but also had favourable effect on overall growth and yield of crops. Besides water-saving technologies, alternative sources of irrigation water such as waste water or effluent water, are among the opportunities that can help to cope with water scarcity (Tabatabaei et al.2020). The seed germination and seedling growth test is substantiated by APHA, 1998 as an essential component of phytotoxicity testing in aquatic environment. It is in this direction the present work is taken up. In the current research the treated Industrial effluents from BHEL is used for growing crop plants to make sustainable use of waste water. The different dilution of effluent ranging from 10, 25, 50, 75 and 100% were first made using distilled water and used for growing crop plants. Impact of diluted effluent was studied on germination and seedling growth of Phaseolus vulgaris and Phaseolus mungo. The study revealed positive effect of the effluent at lower concentration (10 and 25%) on overall germination and seedling growth.

Objectives

- To Analyse the physico-chemical parameters of the Effluent
- To study the germination and seedling growth in different concentration of effluent
- To analyze Plant Growth Parameters
- To Determine optimal effluent dilutions that is beneficial for plant growth
- To Promote Responsible Effluent Management

Data and Methodology

In the present study, the treated effluent of BHEL was collected at effluent outlet of factory and analyzed for physico-chemical parameters. The effluent was then made into different concentration ranging from 10, 25, 50, 75 and 100% using distilled water and its effect on germination and overall growth of Phaseolus mungo was studied with control for reference.

The germination study was made using petriplate method. The seeds of Phaseolus mungo were treated with 0.1% Mercuric chloride as disinfectant for 10 min, then were soaked in different concentration of effluent and control for 3 hours and transferred to sterilized petriplates containing moistened filter paper. A total of 100 seeds were used and four replicates were maintained for each expt. The petriplates were kept in indoor laboratory condition under diffused light at 28+20C. Germination counts were made after 48 hrs. The shoot and root lengths were measured every alternate day for period of ten days (Mhatre and Chaphekar 1982). The root and shoot inhibition (Vaidehi et al,1985) was calculated after the completion of experiment. The Germination percentage, root and shoot length and the vigour index (Abdul-Baki and Anderson, 1973) was calculated after the completion of the experiment. Tolerance Index of the seedlings was calculated using the formula of Turner and Marshall (1972). Percentage of phytotoxicity was calculated using Chou et al (1978) formula.

Results and Discussion

The effluent was tested for 13physico-chemical parameters in Table 1. The effluent is neutral and contains 2715 mg/lt total solids. Among the heavy metals Zinc was found in higher concentration followed by Copper.

S. No.	Parameters	Values (mg/l)
1	pH	7.8
2	Turbidity	18
3	Total Dissolved Solids	3160
4	Total Suspended Solids	2260
5	Alkalinity	640

6	COD	620
7	BOD	218
8	Ammoniacal Nitrogen	10
9	Zinc	1.2
10	Copper	1.12
11	Nickel	1.1
12	Lead	0.17
13	Chromium	0.42

Table 1. Physico-chemical parameters of the Effluent water.

Germination

Seed germination and Seedling growth thevital physiological process which initiates plant growth can be taken as an important growth parameter to assess pollution level of water.

The Table 2. Depicts the impact of effluent on germination and seedling growth. Beyond 50% effluent showed significant influence on both germination and seedling growth. However, 10 and 25% effluent exerted positive influence on germination percentage, shoot and root length of the seedlings. A dose dependent relationship was observed in both the plants. Barua and Das (1997) observed similar effects when they treated Oryza seeds with Paper mill effluent.

Seed germination percentage in both tested crops gradually decreased with increasing concentration of effluent. Seedling treated with 75% and100% concentration of effluent indicated maximum reduction in germination Percentage. The higher level of total dissolved solids in 75 and 100% effluent might prevents germination by increasing salinity and conductivity of the solute absorbed by the seed. Vijayarengen and Lakshmanachary (1993) made similar observation in Vigna radiata. Both 75 and 100%concentration showed impaired seedling growth, which was observed in terms of burnt shoot and root tips, discoloration of seeds and early decaying of the seedlings. Bajji et al (2008) noted similar behaviour in their study on effluent. Generally, seedlings are more vulnerable to the environmental stresses during germination and early growth.

High germination and seedling growth was observed in 10 to 50 % effluent concentration. This depicts positive influence of effluent at lower concentration on both germination and seedlings growth. Vinod (2014) reported the increase in germination percentage over control at lower concentrations (10%) indicates the stimulation of physiologically inactive seeds by the effluent treatment.

The percentage survival of the seedlings was also more at 10 and 25%

concentration of the treatment. These results are in conformity with Madhappan (1993) in Grams. The stimulatory effect of effluent at lower concentration of effluent was due to increase in the activity of certain enzymes responsible for germination like amylase, invertase and protease (AbouZeid, 2007).

Root Length: Seedlings treated with 10% concentration showed maximum root growth followed by 25%. Similar observation was made by Sundaramoorthy and Lakshmi (2000).

Shoot Length: The growth of shoot was relatively muchless than root in all the concentrations. The diluted effluent in 10 and 25% concentration plied productive influence on shoot growth but this remained low at higher concentration. This is in accordance with Malik et al., (2014).

Root and Shoot Inhibition: Root inhibition was minimum in 10% concentration of effluent followed by 25%. Similar observations are made by Chidan kumar C.S.and Chandraju (2011) in case of distillery effluent. Both Root and Shoot inhibition increased with increase in concentration of effluent with highest inhibition was observed in 100%treatment. The interference of heavy metals decreasing the root and shoot length of plant might be due to interference of enzyme activities in physiological process of plant affecting the nutrition, water balance and alteration of hormonal balance, changed membrane permeability (Sharma and Dubey2005).

Table 2. Impact of Effluent on Germination% and Seedling Growth of Phaseolus mungo and Phaseolus. Vulgaris

Phaseolus vulgaris

S. No	**Effluent Concentration (%)**	**Germ % (Mean ±S.D.)**	**Root Length (Mean ±S.D.)**	**Shoot Length (Mean ±S.D.)**	**Root Inhibition (Mean±S.D.)**	**Shoot Inhibition (Mean±S.D.)**
1	Control Std Dev	95.00 5.00	5.85 0.89	3.05 0.32		
2	10 Std Dev	80.00 4.00	4.95 0.39	2.88 0.49	15	5.6

3	25 Std Dev	82.00 2.50	5.05 0.68	2.63 0.39	13	13.7
4	50 Std Dev	57.50 3.50	3.05 0.63	2.50 0.32	47	18
5	75 Std Dev	50.00 4.50	1.65 0.31	1.30 0.29	71	57
6	100 Std Dev	47.00 5.00	1.25 0.20	0.68 0.22	79	77.8

Phaseolus mungo

S. No	**Effluent Concentration (%)**	**Germ % (Mean ±S.D.)**	**Root Length (Mean ±S.D.)**	**Shoot Length (Mean ±S.D.)**	**Root Inhibition (Mean±S.D.)**	**Shoot Inhibition (Mean±S.D.)**
1	Control Std Dev	92.00 10.00	3.65 0.91	4.62 0.83		
2	10 Std Dev	90.00 4.79	4.10 0.28	4.62 0.82	-12	0
3	25 Std Dev	91.00 4.79	3.95 0.36	4.93 0.8	-8	-6.7
4	50 Std Dev	72.50 9.57	2.45 0.09	2.50 0.81	32	45.9
5	75 Std Dev	75.00 10.00	1.83 0.62	1.75 0.24	49	62.1
6	100 Std Dev	75.00 10.00	1.35 0.34	1.78 0.49	63	61.5

The Vigour Index (Fig 1) of both P. vulgaris and P. mungo seedlings were significantly low in undiluted effluent when compared to control. Conversely, at

lower concentration both seedlings showed marked increase in vigour index over control. Prathibha and Azra (2019) noticed similar findings in Glycine max treated with Dyeing factory effluent. There is continuous decrease in Vigour index of the seedlings with increase in concentration of effluent from 50% treatment. Tolerance index (Figure 2) of seedlings was minimum at 100% of effluent concentration and maximum at 25% concentration. Similar observation was made by David and Rajan (2015) in their studies on Dyeing factory effluent. As far as percentage of phytotoxicity (Figure 3) of effluent is concerned, minimum phytotoxicity was observed at 25% and maximum at 100% concentration. Vaithiyanathan and Sundaramoorthy (2017) noticed similar observation in sugar mill effluent.

Fig 1. Effect of Diff Conc of BHEL effluent on Vigour Index of Phaseolus vulgaris and Phaseolus mungo

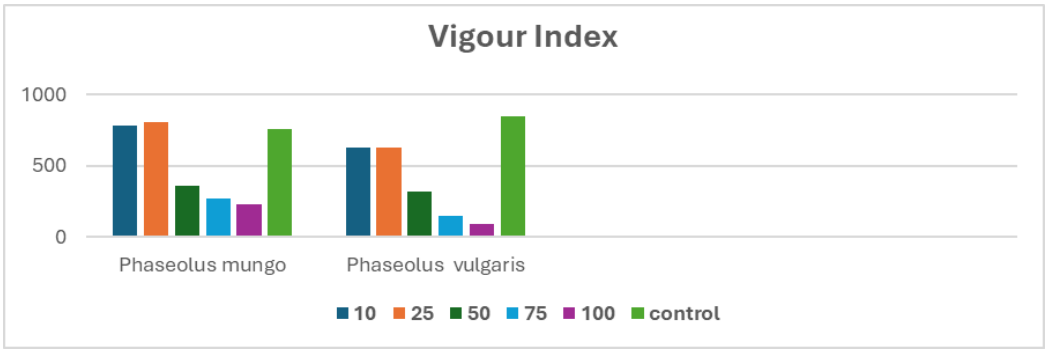


Fig 2. Effect of Diff Conc of BHEL effluent on Tolerance Index of Phaseolus vulgaris and Phaseolus mungo

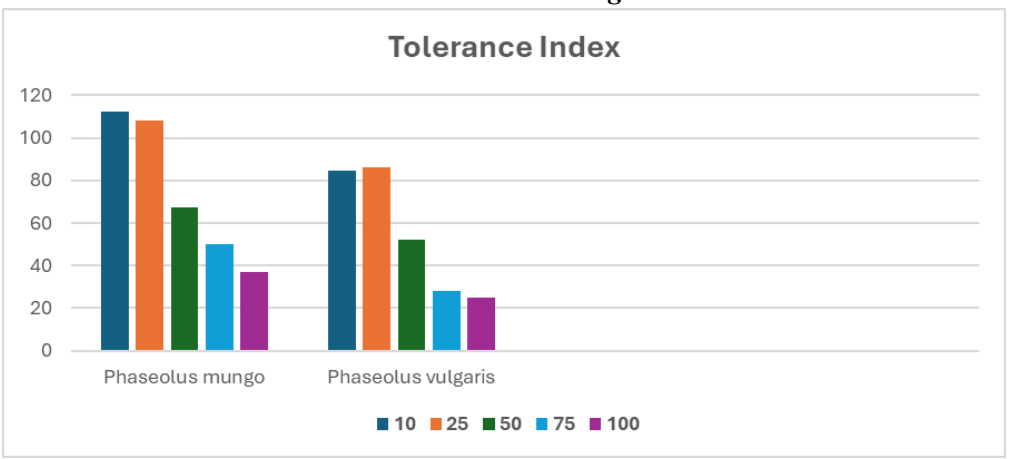
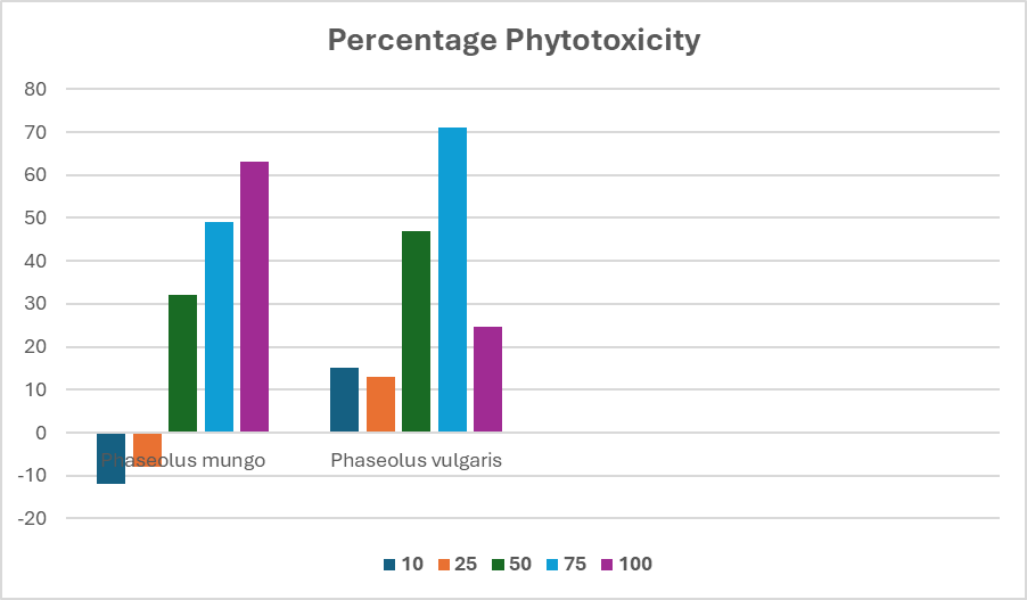


Fig 3. Effect of Diff Conc of BHEL effluent on Phyto- toxicity of Phaseolus vulgaris and Phaseolus mungo



Conclusion

The findings in the present work have revealed that the 10 and 25% treated effluent has increased both germination and seedlings growth, in some cases it is more than control. This indicates the optimum amount of nutrients at lower dilutions has positive effect ongermination and overall growth and could be recommended for irrigation, to reduce the lethality of the pollutants and as a substitute for chemical fertilizers.Hence there is every need to develop program that could convince the public and government authorities in such a way that the treated effluent should not be regarded as waste/toxicant, but as one of the finished product, which could be readily used in lower concentration for sustainable agriculture in peri urban areas of Bangalore.

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Modern Approaches and Future Perspectives in Drug Discovery and Design: Strategies, Technologies, and Challenges

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Article DOI Link: https://zenodo.org/uploads/17543156

DOI: 10.5281/zenodo.17543156

Abstract

Undoubtedly, drug design and discovery constitute an integral part of modern medicine, which has resulted in developing therapies revolutionizing healthcare. This review talks about the historical evolution of drug discovery concerning its prominence and challenges, focusing on ever-increasing technological advances. The drug discovery process is conceived in terms of target identification up to the preclinical and clinical studies, emphasizing key steps influencing the degree of success. Strategies such as structure-based drug design, ligand-based drug design, fragment-based drug design, and de novo drug design are discussed alongside the upcoming role of computational approaches like molecular docking, virtual screening, molecular dynamics simulations, and artificial intelligence. Bioinformatics and chemo informatics have made key contributions discussed in the review, followed by recent advancements like genetic editing and mRNA-based therapeutics, and the growing trend of personalized medicine. Further challenges include drug resistance, safety issues, and regulatory barriers, examined in the light of real-world examples involving both successful and failed drug candidates. Last, future perspectives will summarize the transformative possibilities enabled by emerging technologies and collaborative drug discovery models, envisaging a latitude for faster, more precise, and accessible drug

discovery in the years to come.

Keywords: Drug discovery, Drug design, Structure-based drug design (SBDD), Ligand-based drug design (LBDD), Molecular docking, Virtual screening, Molecular dynamics simulation, Artificial intelligence, Personalized medicine, Bioinformatics, Chemo informatics, CRISPR, mRNA therapeutics, Drug resistance, Regulatory challenges.

Introduction

Drug discovery means the discovery and development of new medicines. It is an essential domain of pharmaceutical science aimed at finding new therapeutic agents for eradicating diseases [1]. The new drug development entails a complex interplay of biology, chemistry, pharmacology, and technology. Advancement in drug discovery is becoming more efficient and accurate as it is progressing from the traditional methodologies of drug discovery toward more sophisticated technology-based approaches [2]. This important area truly embraces public health and offers treatments for an extensive list of medical conditions, focusing between the extremes of simple infections, complicated chronic diseases, and rare genetic disorders. Here, we will review the various stages, methods, and challenges entailed in the drug discovery process while focusing on current advances in technology, including computational drug design, bioinformatics, and machine learning [3].

Drug discovery holds great importance; that secures the very health and well-being of humanity. As this is the method by which new medicines are discovered, the society faces the fight against the disease-burden for one reason or the other-infectious or non-infectious [4]. The discovery of antibiotics, for instance, has changed the field of medicine, since it has provided cures for bacterial infections, and therefore have saved innumerable lives [5]. Similar investigational drugs are for chronic diseases such as cancer, diabetes, and other cardiovascular complications, because they have changed life expectancy tags and the quality of life. Emerging complications such as multi-drug-resistant infections or complex and rare genetic disorders make this need for novel therapeutics an even burning necessity. The economy pushes its scale of growth on drug discoveries as well [6]. The pharmaceutical sector grows by drug discovery; job creation occurs; innovations are spurred in biotech and health services and diagnostics, among many other sectors. With personalized medicine, drug discovery now caters to developing medications based on an individual's genetic makeup thus, maximizing their efficacy while reducing adverse effects [7].

It is a long journey of drug discovery and all throughout the path, it has been filled with diverse milestones ranging from old traditional remedies to the latest treatments. In the earlier days, drug discovery was done by trial and error and by using botanical, herbal, and natural substances for treating disease-like symptoms [8]. Opium, for example, dates back to the ancient civilizations in its use for relief from pain, and the bark of the cinchona tree was used to find a cure for malaria from quinine, one of the earliest remedies. They were empirical by nature and relied on what was learned from one generation to another. In fact, that era of modern drug discovery actually began in the last century of 19th century, clung to the shoulders of burgeoning chemistry and pharmacology [9]. The introduction of synthetic drugs like aspirin (acetylsalicylic acid) toward the end of the 1800s transformed the whole scenario [10].

The important part of the 20th century must have been systematic and scientific approaches to drug discovery together with the biochemistry, molecular biology, and clinical trials [11]. The development of antibiotics began with the discovery of penicillin in 1928 by Alexander Fleming, which entirely changed the course of the medicinal treatment of bacterial infections. Ant malarial drugs that were fully synthetic were developed towards the mid-20th century, and this newly born modern pharmaceutical industry changed the dimension of medicine. Late in the 20th and the early 21st centuries brought innovations in high-throughput screening and recombinant DNA technology that speeded up drug discovery, allowing one to identify newer drug candidates more quickly and accurately [12]. Most currently, it has entered the scene computerized procedures, artificial intelligence, and genetic engineering to change the entire era of drug discovery approaches into drug designing and optimization towards personalized or precision medicine [13]. Looking over the past of drug discovery, one can say that every time a new avenue was opened into medical possibilities; the very future of drug discovery is exciting and promising [14].

Drug Discovery Process

Drug discovery takes the shape of a lengthy voyage where special ideas will transform into therapeutic products through several phases of research. The first thing to do in this long journey is to understand the biological basis of a disease and to identify suitable molecular targets. Thereafter come a series of laboratory work, computational modeling, and clinical validation. Researchers want to find new compounds that can be refactored and tested into safe and effective new drugs. Each of these phases is very important; attention must be given in planning, teamwork, and regulatory compliance in every step taken. The drug

eventually is going to be taking a responsibility-an important one-for patients before long. The stages of the drug discovery process have been discussed in brief below.

Target Identification and Validation

One of the early, most critical steps in drug discovery is target identification and validation. Typically, a "target" can refer to a biomolecule that operates in a disease pathway, whereas it may include proteins, genes, or RNA. Proper target identification is really important because that step determines the entire course of drug development. Different approaches such as genomics, proteomics, and bioinformatics are employed to find obvious molecules that are altered in disease conditions [15]. After a potential target has been found, validation ensures that modulating it will provoke a therapeutic effect without harm being done to the patient. Typical validation methods include genetic manipulation (e.g., knockdown or overexpression studies), where the principal biological role of the target in relation to disease progression is evaluated using small molecules or antibodies. Validation of this target would vastly increase success in subsequent stages of drug development [16].

Hit Identification

Once a target has been validated, the next step is hit identification: identifying molecules that interact with the target and modulate activity. A hit is defined as any compound that displays a desirable biological effect on the target when screened using assays. Various methodologies are applied in hit identification; for instance, high-throughput screening (HTS), through which thousands to millions of compounds are tested against the activity of the target using automated systems. Other methods include virtual screening, which posits potential hits through modeling interactions of a target with various compounds [17]. Fragment-based drug discovery and natural product screening are familiar approaches. The focus of this stage is not to find an ideal drug, but rather to identify chemical starting points for further optimization [18].

Lead Optimization

Once identified as hits, they are subsequently selected after screening to identify promising candidates, referred to as lead compounds for the subsequent lead optimization stage. This includes a cycle of chemical modification of lead compounds to improve their potency, selectivity, pharmacokinetic properties, and safety profile [19]. The medicinal chemists will attempt enhancing the drug-like properties of a compound wherein specific properties would include better access

to the target site in the body and diminished toxicity potential of a compound [20].

A structure-activity relationship (SAR) evaluation is performed to systematically determine the different biological activities of the leads elicited through chemical modifications. Thus, lead optimization includes creating a candidate with therapeutic effectiveness, side effects as low as possible, and drug metabolism properties suitable for animal experimentation [21].

Preclinical and Clinical Studies

Once a candidate lead has been optimized, it enters the preclinical phase, where it must be tested in laboratory and animal models to ensure safety, efficacy, and pharmacokinetic behavior. These studies include identifying toxic effects, determining suitable dosages, and predicting the behavior of the drug in human beings. If the result is favorable, it passes to clinical trials, which are carried out in several phases on human volunteers [22]. Phase I trials assess safety and dosage in healthy individuals. Phase II trials evaluate efficacy and side effects in patients with the disease. Finally, Phase III trials involve larger patient populations to confirm effectiveness, monitor the adverse reaction profile of the drug, and compare it with standard treatment. If the drug is successful, approval by the regulatory authorities will permit a submission and a Phase IV post-marketing surveillance study after the drug comes onto the market. Each phase of clinical testing has been designed so that only the safest and most effective drugs get to patients [23].

Table no. 01: Drug Discovery Process

Stage	Key Activities	Key Focus	Methods/Approaches
1. Target Identification & Validation	- Identify biomolecules (proteins, genes, RNA) involved in disease pathways. - Validate targets by modulating them and observing effects.	Identify and confirm biological target for drug development	Genomics, Proteomics, Bioinformatics, Genetic Manipulation (e.g., knockdown/overexpression studies)

2. Hit Identification	- Identify compounds that interact with the validated target. - Screen compounds to find "hits" (active molecules).	Identify potential molecules for further study	High-throughput screening (HTS), Virtual screening, Fragment-based drug discovery, Natural product screening
3. Lead Optimization	- Modify chemical structure to improve potency, selectivity, pharmacokinetics, and safety. - Evaluate structure-activity relationships (SAR).	Enhance drug-like properties and safety profile	Medicinal Chemistry, SAR analysis
4. Preclinical Studies	- Test lead candidates in animal models for safety, efficacy, and pharmacokinetics. - Determine toxicology and dosages.	Ensure safety and suitability for human trials	Animal testing, Toxicology studies, Pharmacokinetic analysis
5. Clinical Trials	- **Phase I:** Test safety and dosage in healthy individuals. - **Phase II:** Evaluate efficacy and side effects in patients. - **Phase**	Test the drug in humans to validate safety and efficacy	Clinical testing on humans (healthy individuals and patients)

	III: Confirm effectiveness, monitor adverse effects, and compare with standard treatments.		
6. Post-Marketing Surveillance	- Monitor drug safety and effectiveness in the general population after release.	Ensure continued safety and efficacy post-approval	Regulatory authority monitoring, patient surveys, ongoing research

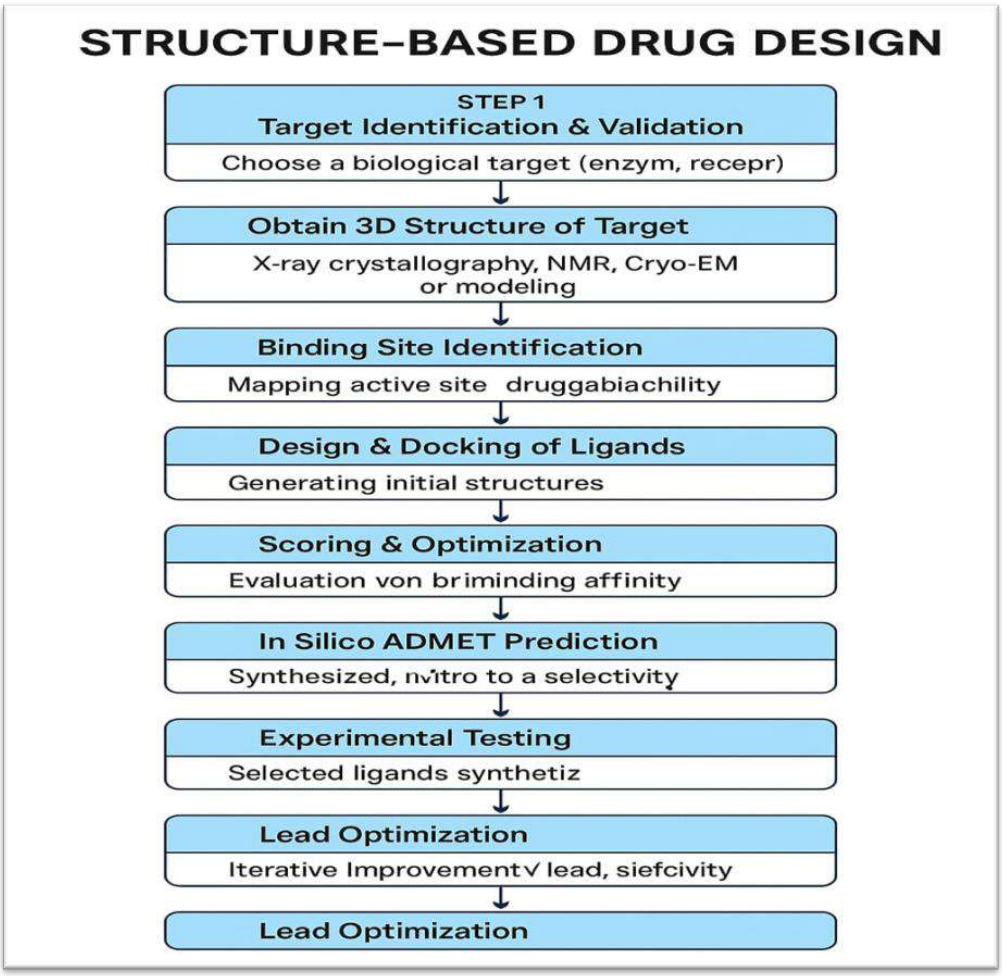
Strategies in Drug Design

Drug discovery strategies were refined and enhanced through the molecular biology, structural biology, computational science, and chemistry advancements. Modern drug design aims at understanding the typical molecular mechanisms of diseases and translating that knowledge into molecules that interact specifically with relevant biological targets. Numerous strategies are available for this, each with its methodology, pros, and cons. Among the most important strategies for drug development are structure-based drug design (SBDD), ligand-based drug design (LBDD), fragment-based drug design (FBDD), and de novo drug design. Following is an elaborate description of each of those strategies.

Structure-Based Drug Design (SBDD)

Structure-Based Drug Design (SBDD) is a methodological approach in drug discovery wherein biological targets, often proteins in nature, are harnessed through their three-dimensional (3D) structures in order to design new drug candidates [24]. The way SBDD works is that first, researchers then subject the 3D structure of the target to extensive characterization, using a combination of several biophysical techniques, which can also include chemical cross-linking and mass spectrometry, such methods can capture even the most transient states of a protein. Once the three-dimensional shape is established, the next step involves molecular docking and computational modeling on the structures to design or optimize molecules that can bind accurately to the active site or to the capabilities of the target. The "lock and key" principle here will apply, thus

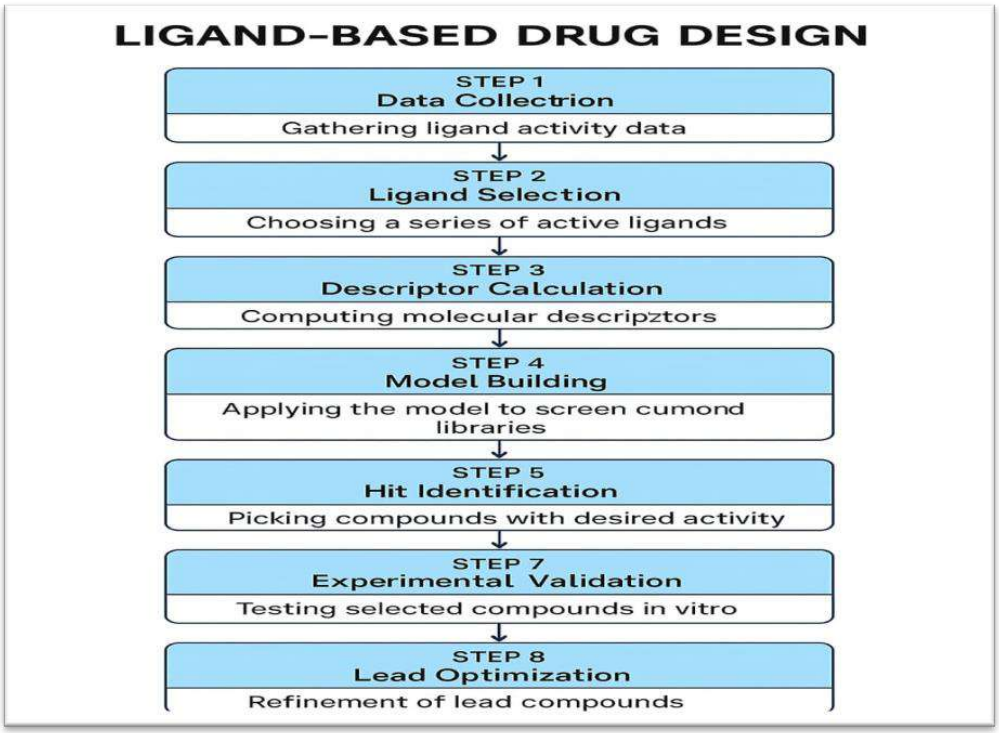
maximizing the probability of developing truly selective and potent drugs [25]. SBDD features prominently in the development of several successful drugs, including inhibitors of HIV protease. One major advantage of SBDD involves predicting binding modes and designing modifications rationally rather than through trial and error [26].



Ligand-Based Drug Design (LBDD)

When the three-dimensional structure of a target is completely unknown, ligand-based design approaches are preferred on the basis that there exists information concerning those molecules-ligands interacting with the target. In this method, researchers analyze the known ligands' chemical and structural properties to design compounds with similar or improved activity. Usually, techniques such as quantitative structure-activity relationship (QSAR) modeling, pharmacophore modeling, and similarity searching are employed in LBDD. These techniques

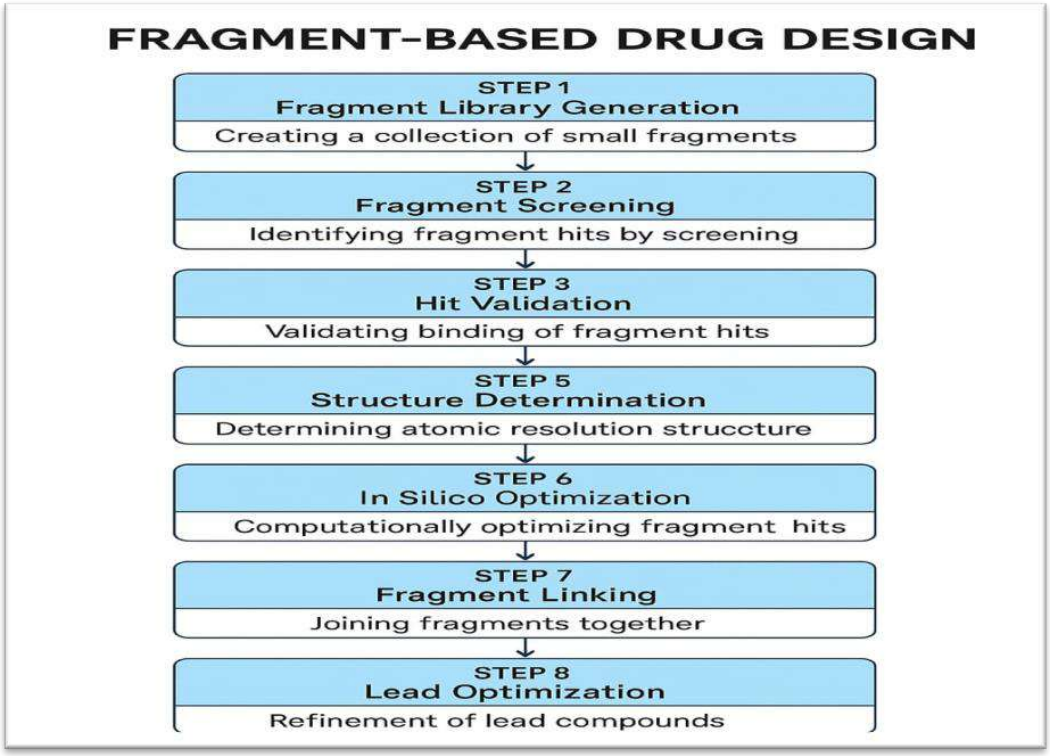
recognize the key features of the ligands such as hydrogen bond donors/acceptors, hydrophobic centers, and charged groups, required for biological activity. Ligand-based drug design can be extensively applied when the structure of the target is difficult or impossible to obtain. It serves as a very fast path for generating potential drug candidates from the knowledge accumulated by the successes and failures of existing molecules [27].



Fragment-Based Drug Design (FBDD)

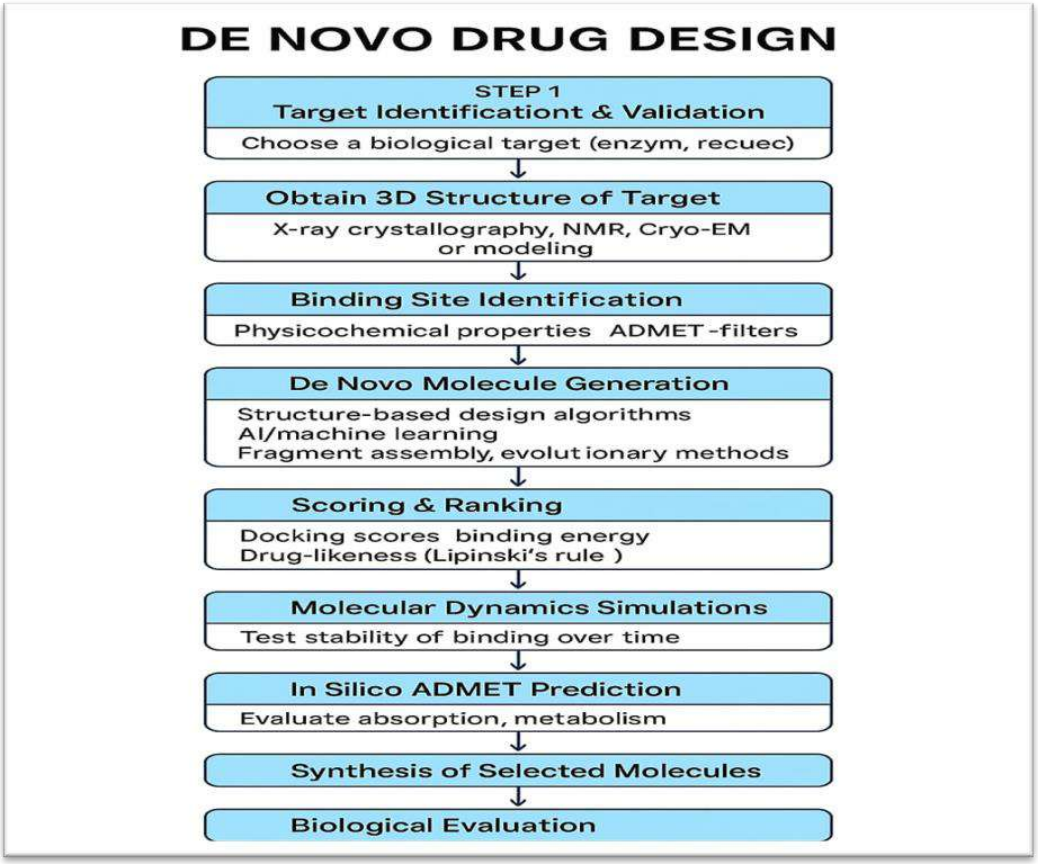
Fragment-based drug design, an approach that is rather new in the domain of drug discovery, employs very small chemical fragments-this may be referred to as smaller as and simpler than typical drug-like molecules. These fragments would bind weakly-often to different sites-of a target protein. Advanced biophysical techniques (e.g., X-ray crystallography, NMR, or surface Plasmon resonance (SPR)) would then detect these weak interactions with high sensitivity. Once the weakly interacting fragment is identified, it is optimized by biochemical manipulation, either through linking, wherein two moiety fragments are joined to make a compound, or through growing, where on-site elaboration of moiety yields a larger compound that would better fill the binding site. The advantage of FBDD is in effectively scanning chemical space to make drugs with binding

affinities that are much better and with poorly interacting properties. FBDD drug-discovery successes, vemurafenib from melanoma, not only demonstrate the power of this approach but also that of starting small in the design of increasingly large and highly potent drugs [28].



De Novo Drug Design

Typically, de novo drug designs are those that do not involve taking an existing ligand or fragment into consideration for the design of the drug molecules. The entire new chemical structures are then created by computational algorithms with the assistance of artificial intelligence to theoretically bind the target's active site. The method often combines biological structure, medicinal chemistry, and machine learning for an understanding and prediction of novel compounds. Novel compounds are predicted: de novo design allows exploring new and strange chemical spaces that may lead to unique drugs, which traditional approaches may fail to find. While it is also the most cumbersome because biological activity and predictability of pharmacokinetics are very complex, de novo design is indeed an exciting frontier in drug discovery because computational tools are now more and more powerful and more accurate [29].



Computational Approaches

Computational approaches have revolutionized the field of drug discovery by speed, cost-effectiveness, and precision. With the advent of computational techniques, researchers are no longer reliant on fully experimental methods to find and investigate how molecules can act, interact, and otherwise perform as drugs but use computer simulation and modeling to determine these characteristics. Such techniques enable rapid screening of millions of compounds, prediction of binding affinities, and even simulation of molecular behavior over time. The above-mentioned features, combined with other computational such as molecular docking, virtual screening, molecular dynamics simulations, and artificial intelligence, have substantially improved the efficiency of discovering and developing new drug candidates.

Molecular Docking

In fact, docking on a molecular level is an advanced computer modeling technique that predicts the most favorable orientation of a small molecule

(ligand) binding into active site of a target protein. The purpose of docking is to find the best alignment of the ligand with the target, much as one would look for the right key for the lock. The docking software computes and ranks on the basis of fitting the ligand into the binding site considering hydrogen bonds, hydrophobic contacts, and electrostatics while taking into account a large number of poses ("binding modes") of the ligand. Thus, a high docking score represents a strong and stable interaction between the ligand and the target, which in turn implies the likelihood of the compound being a drug candidate. Modern ways of SBDD have brought molecular docking capabilities to the fore in prioritizing molecules within the modern process of molecule-to-medicine. In other words, more and more time is saved through computation than through empirical experimentation [30].

Virtual Screening

Virtual screening is a more general computational strategy for the fast evaluation of large libraries of compounds to identify potential binders of a drug target. It can be defined in terms of docking simulations (i.e., structure-based virtual screening) or on the basis of the chemical and physical properties of known active molecules (i.e., ligand-based virtual screening). In structure-based virtual screening, hundreds of thousands to billions of compounds will be docked to the 3D model of the target protein, while ligand-based virtual screening seeks to find molecules similar to known active compounds using pharmacophore modeling or similarity scoring. Virtual screening truly accelerates the hit-to-lead stage of drug discovery by narrowing enormous chemical spaces toward just a few plausible candidates for experimental validation [31].

Molecular Dynamics Simulations

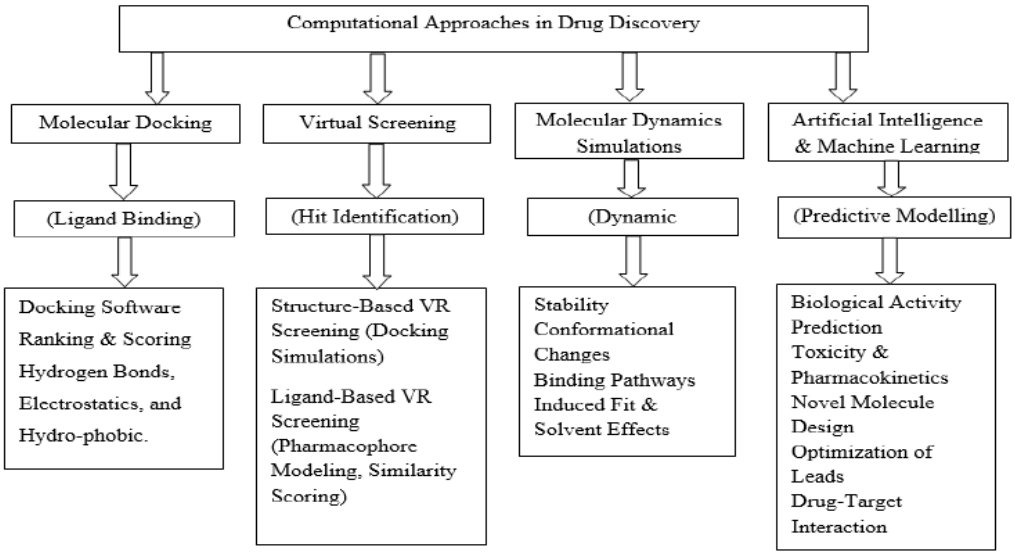
The Molecular Dynamics (MD) Simulation technique aids in comprehending molecular behavior with time at the atomic level. Whereas the molecular docking technique offers a static image of ligand-target interaction, MD simulations show how the resulting complex behaves in a more dynamic and realistic biological environment, like in water at body temperature. Using these physical and mathematical principles, MD simulations can model the displacement of all the atoms with respect to the passage of time, thereby allowing the scientist to evaluate the stability of the ligand-protein complex, conformational changes, and possible binding pathways. The MD simulation technique is also essential for spotting effects that may have otherwise been disregarded, like induced fit

binding or influence from solvent molecules onto the interaction, which beyond docking results alone [32].

Artificial Intelligence and Machine Learning in Drug Discovery

Transformative, Artificial Intelligence (AI) and Machine Learning (ML) are few such terms that have found application in modern drug discovery. AI algorithms can analyze large data sets, recognize patterns, and carry out predictions in far less time than traditional methods. In drug discovery, ML models can predict biological activity, toxicity, and pharmacokinetics of compounds; other uses include designing novel molecules and optimizing lead compounds, the simulation of drug-target interactions being another option. Deep learning-a subfield in AI-gave great strides like the generative model that proposes entirely new chemical structures with desired properties, now widely used by companies and researchers to facilitate the design, screening, and optimization of drug candidates, bringing down to a considerable extent the time and cost that were traditionally incurred in the process of drug development. The ongoing evolution in AI could offer an even better chance to personalize, precision, and efficiency for drug discovery [33].

Branched Structure (Mind Map) for Computational Approaches:



Role of Bioinformatics and Chemo informatics

Bioinformatics and chemo informatics in drug discovery have made a great change in the way researchers identify drug targets, design new molecules, and optimize therapeutic candidates. Drug discovery is an iterative, computational

field in which researchers manage and analyze large biological and chemical databases to produce results that are faster, more accurate, and more innovative. Bioinformatics mainly handles biological data such as genes, proteins, diseases, and pathways, whereas chemo informatics handles chemical information, including molecular structures, properties, and activities. In union, bioinformatics and chemo informatics form a potent basis for rational drug design, target validation, lead discovery, and optimization.

Bioinformatics in Drug Discovery

Bioinformatics acts an important role in the initial stages of drug discovery especially regarding target identification and validation. Bioinformatics refers to the computational applications for the analysis of genomic, proteomic, and transcriptomic data to decipher the molecular mechanisms underlying diseases. It assists in revealing potential drug targets that are important for the disease development by studying gene expression profiles, protein-protein interaction networks and signaling pathways. Bioinformatics tools also help support comparative genomics with which conserved regions can be identified that might be amenable to use during drug development. Verification of critical aspects of targets with respect to their structure, function, and dynamics relies on use of sequence alignment algorithms, structural prediction software, and database mining - common methods of bioinformatics. For example, bioinformatics also does biomarker discovery, which again plays a role on patient stratification and implementing personalized approaches in medicine [34].

Chemo Informatics in Drug Discovery

Chemo informatics involves the storage, retrieval, analysis, and visualization of chemical information, which is essential for designing and optimizing drug candidates. The discipline employs molecular modeling, quantitative structure-activity relationship (QSAR), similarity searching, and chemical database mining techniques for predicting biological activity, toxicity, and drug-likeness of compounds. Chemo informatics enables an in-silico screening process of vast chemical libraries prior to selecting a subset for synthesis and testing and enabling the design of new molecules with desirable properties. In addition, it can predict pharmacokinetic behavior of xenobiotics and drug metabolism to discard compounds with poor profiles early in development. Advanced chemo informatics tools apply machine learning patterns of chemical structure and biological activity to enable better decision making at the lead optimization

stages. In general, chemo informatics enables discovery of the new drug rationally as well as data-driven approaches towards chemical exploration [35].

Recent Advances and Emerging Trends

Born out of revolutionary technologies, the landscape of drug discovery and development has transformed fast. Drug discovery is now not only about speed and efficiency but also about designing therapies that are highly targeted and personalized. CRISPR for gene editing, mRNA-based therapeutics, artificial intelligence (AI), and personalized medicine are all teaching the pharmaceutical industry new tricks for the cure of complex and often incurable diseases. These breakthroughs mark a departure from conventional approaches to more precise, data-driven, and individualized treatment strategies.

CRISPR and Gene Editing

CRISPRs (clustered regularly interspaced short palindromic repeats) and descriptions of gene editing techniques have transformed biotechnology and drug discovery. This has become possible by bringing accuracy in gene editing for specific genes in living organisms using CRISPR-Cas9, the method through which scientists do their work concerning the editing of genetic defects. Gene function can be studied, and the development of gene-based therapies can be undertaken. In drug discovery, the main application of CRISPR is in making disease models more realistic so that the mutations causing disease can be introduced into cells or animals. It also allows the running of high-throughput genetic screens to discover new targets and resistance mechanisms for drugs. Finally, the CRISPR technology has also great potential for directly curing genetic disorders since it can repair faulty genes in patients. As research progresses, gene editing will open up new therapy-dedicated personalized and curative approaches, especially for genetic disorders, cancer, and infectious diseases [36].

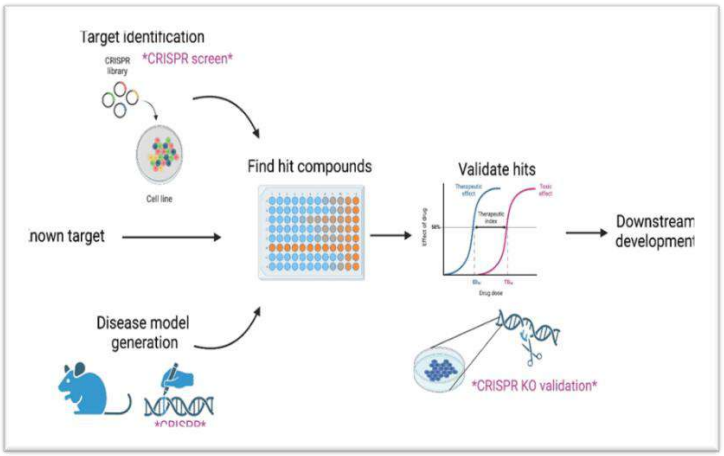


Fig. 01: CRISPR and Gene Editing

mRNA-based Therapeutics

After the development of mRNA vaccinations against COVID-19, from Pfizer-BioNTech and Modern, mRNA-based therapeutics and their applications have attracted a lot of attention worldwide. This technology employs messenger RNAs (mRNA) to instruct cells to produce an exact protein, which will either elicit an immune response or help in curing the disease. This way, unlike traditional therapies, mRNA therapies provide rapid and flexible platforms, enabling them to quickly develop vaccines and treatments for a wide range of various diseases including infectious diseases, cancers, and rare inherited disorders. mRNAs are being actively developed for use in personalized cancer vaccines, where an individual's unique tumor mutations are taken into consideration. Their safety profile, scalability of manufacture, and potential targeting of pathways previously termed as "undruggable" make mRNA therapeutics possibly one of the most exciting frontiers in modern medicine [37].

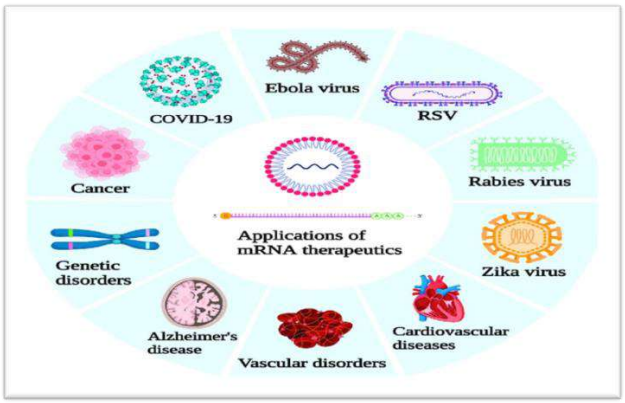


Fig. 02: mRNA-based Therapeutics

AI-Driven Drug Discovery

AI has emerged as a revolutionizing change in drug discovery processes-bringing efficient decision making, molecular behavior prediction, and entire research pipeline acceleration. AI algorithms analyze vast datasets such as chemical libraries, genomics data, and clinical trial information to explore hidden patterns and predict the most successful compounds. Machine learning models assist medicinal chemists in de novo drug design, optimization of molecular structures, pharmacokinetic properties prediction, off-target effects identification, and so forth. Artificial intelligence platforms are now offering the proposal of new drug candidates within days at a fraction of the cost over traditional methods. Integration of AI is expected to further reduce overall failure rates in clinical trials by filtering the drug candidates better in an early stage of the process. With the ongoing advancements in AI, the future is bright for automating and personalizing drug discovery, making the process faster, more efficient, and more precise than ever before [38].

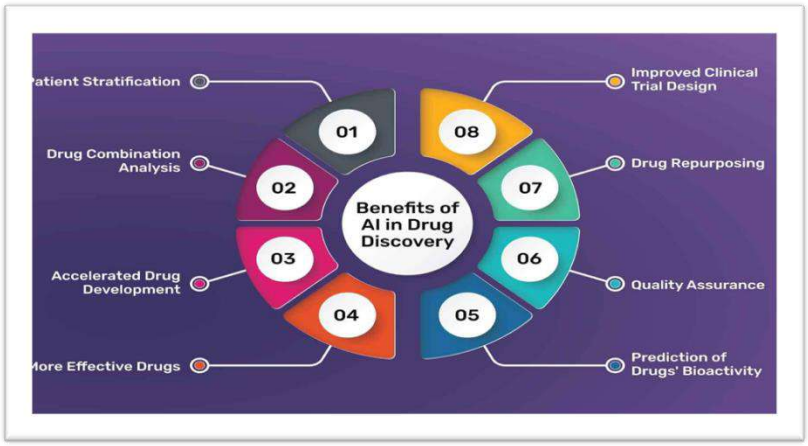


Fig. 03: AI-Driven Drug Discovery

Personalized Medicine

Individualized medicine, or precision medicine, is the concept that a medical treatment must be modified by a patient's individual characteristics, such as genetic profile, lifestyle, and environment. With regard to drug discovery, personalized medicine deals with therapy developed for a subgroup of patients sharing certain biomarkers or carrying specific genetic mutations. This offers an improvement of therapy efficacy, reduced adverse effects, and greater patient satisfaction. Technologies such as next-generation sequencing (NGS), bioinformatics, and molecular diagnostics are important to determine which patients really will benefit from a given therapy.

Personalized medicine has changed the management of diseases like cancer-a process of assigning therapies according to the patient's tumor genetic makeup. With an ongoing investigation, personalized medicine is certainly expected to replace the traditional model of healthcare into a better and more customized therapeutic strategy as compared to the "one-size fits-all" [39].

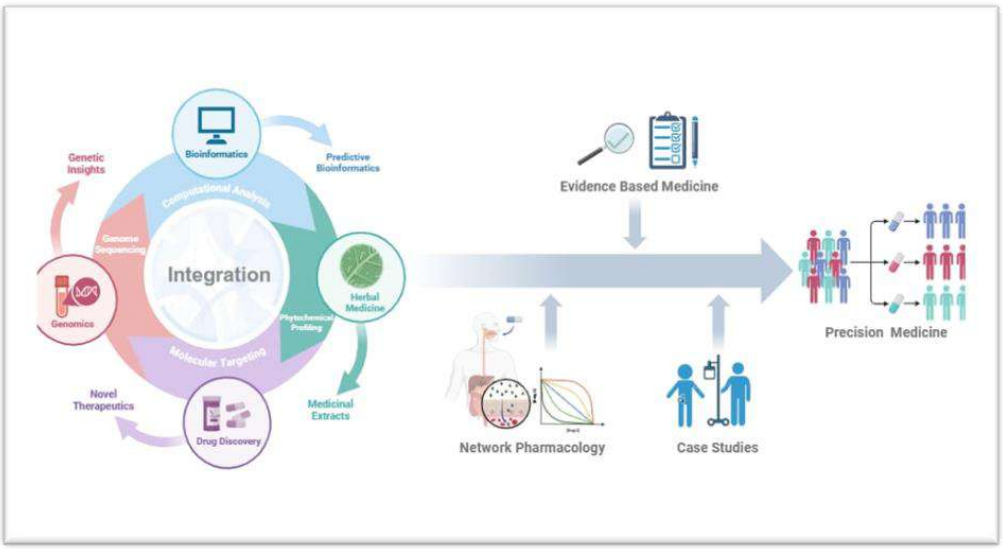


Fig. 04: Personalized Medicine

Challenges in Drug Design and Discovery

Even with the phenomenal improvements in technology, drug design and discovering remain complicated and expensive processes with the capacity for long time frames. Researchers have to deal with various salient challenges that can hamper successful new therapy design. These mainly include drug resistance, safety and toxicity, and ever-growing regulatory hurdles. These hurdles not only rate a project for possible failure but add to the cost and time to bring a drug to market. This then emphasizes the need to overcome these barriers to speed up innovation and get safe and effective medicines to the patients in a timely manner.

Drug Resistance

The resistance of drugs, which has been more prevalent in infectious diseases and cancers, is one of the biggest obstacles in drug discovery. Micro-organisms and even malignant cells obtain mechanisms that are fully capable of counteracting the effect of the drug so that the course treatment will be ineffective at one point in time. Some examples of such mechanisms include: the resistance development of bacteria to an antibiotic through modification of the drug action site, enzymes

production capable of degrading drugs or increasing their efflux from bacterial cells. Similarities would be found with the capacity of tumors to develop mutations rendering them ineffectively targeted by specific therapies. Drug resistance generally results in a treatment failure, recurrence of the disease, and establishment of alternative or combined therapies to use. Hence, development of new drugs with innovative mechanisms of action and establishing strategies such as drug combinations or cycling of therapy to prevent or delay the development from this battle are strategies that must be conducted [40].

Safety and Toxicity Issues

Drug safety assurances and toxicity minimization are arguably one of the most important challenges in drug development. A significant number of promising compounds fail during preclinical or clinical trials as a result of unexpected toxic effects on vital organs, such as liver, heart, or kidneys. A toxicity prediction solely based on chemical structure is not always right; hence, it has become a major concern in drug pipelines. Majority of times, even effective drugs can cause severe adverse effects which outweigh their benefits. The advances in predictive toxicology, in vitro testing, and computational modeling aim to identify toxic liabilities at the earliest stages of discovering a payment, but that is still a thing quite difficult to predict because great numbers related to the compound safety profile are not easy. Balancing efficacy and safety in the development of a drug is very critical when it comes to approval for use [41].

Regulatory Challenges

Even the brawny contenders find welcoming obstacles in regulatory navigation hurdles of drug design and discovery. The U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) require an extensive amount of evidence from testing to secure a drug's approval regarding safety, efficacy, and manufacturing quality. Investigational New Drug (IND) applications, clinical trials (Phase I-III), New Drug Applications (NDA)-the regulatory process comprise several stages, all requiring serious documentation and compliance. The regulatory needs keep changing depending on the new safety risks, new technology, and public health needs, so they keep evolving as per paragraph companies. The expense and long duration that come with regulating this and that serve as disincentives for innovations from a smaller biotech house. The effort to streamline regulatory pathways really moves through speedier designations and adaptive licenses, but the challenges are again enormous [42].

Case Studies

Case studies have a huge input in the practical realities of drug design and discovery. They hold great examples of success or failure, thereby providing further insight into what strategy worked or pitfalls to be avoided. Studies in successful drug discoveries help showcase the effective techniques or innovative approaches or critical decision-making factors that led to eventual approval for marketing. On the contrary, studies of failed drugs present insights into common mistakes, unexpected problems, or lessons that can make drug design in future more meaningful and fruitful. Together, these case studies would amplify the understanding of the drug discovery complexities and how to handle them by the scientific community.

Successful Drug Discovery Examples

One of the mostly talked-about success stories in drug discovery concerns the development of Imatinib (Gleevec) for chronic myeloid leukemia (CML). It is a small molecule tyrosine kinase inhibitor that selectively inhibits a BCR-ABL fusion protein formed because of chromosome translocation characteristic of CML. CML had a dismal prognosis with very few therapeutic options before the advent of Imatinib. The rational design of Imatinib acting on the basis of the molecular understanding of the disease mechanism marked a major breakthrough in targeted therapy. It did not only change the face of cancer treatment; it also showed the strength of structure-based drug design and personalized medicine in general. Another example was development for hepatitis C virus (HCV) infection: Sofosbuvir (Sovaldi) boasts a cure rate of over 90%, thus greatly enhancing patient welfare compared with older therapies [43].

Failed Drugs and Lessons Learned

High-profile drug failures in the pharmaceutical industry present the important opportunity for learning. Drug development by Pfizer for cholesterol modification under the name Torcetrapib is a relevant example. Though Torcetrapib successfully raised high-density lipoprotein (HDL) levels (so-called "good" cholesterol), the drug was later withdrawn after extensive clinical trials due to the unexpected rise in blood pressure and associated cardiovascular risks, which, in turn, translated into increased mortality rates. This hallmark case expressed the exigency of thorough safety evaluation and the risks associated with the uncritical reliance on surrogate markers (like cholesterol levels) while truly having little understanding of the full physiological impact of the drug [44]. Rimonabant, another odious example, was an anti-obesity drug withdrawn after

inciting serious side effects on psychiatric health like depression and suicidal ideation [45]. These failures show that efficacy is not sufficient; safety, tolerability, and holistic assessment of the patient's wellbeing must be guiding criteria for any drug to obtain approval and long-term success.

Table no. 02: Case Studies Highlighting Successful and Failed Drug Discoveries with Key Lessons Learned

Section	Description
Case Studies	Case studies play a crucial role in understanding the practical realities of drug design and discovery. They highlight successful techniques, innovative approaches, decision-making factors, as well as the pitfalls and failures, thus providing deep insights to improve future drug development strategies.
Successful Drug Discovery Examples	- **Imatinib (Gleevec):** Developed for chronic myeloid leukemia (CML), Imatinib is a small molecule tyrosine kinase inhibitor that selectively targets the BCR-ABL fusion protein. It revolutionized cancer treatment, showcasing the power of structure-based drug design and personalized medicine. - **Sofosbuvir (Sovaldi):** Developed for hepatitis C virus (HCV) infection, Sofosbuvir achieved cure rates of over 90%, drastically improving patient outcomes compared to earlier therapies.
Failed Drugs and Lessons Learned	- **Torcetrapib (Pfizer):** Although it raised HDL cholesterol levels, it unexpectedly increased blood pressure and mortality, leading to its withdrawal. This case highlighted the importance of comprehensive safety evaluations and cautious interpretation of surrogate markers. - **Rimonabant:** An anti-obesity drug withdrawn due to serious psychiatric side effects, including depression and suicidal tendencies. It emphasized that efficacy alone is insufficient; safety, tolerability, and holistic patient wellbeing are critical for drug approval and success.

Future Perspectives

Changes in drug design and discovery are all very fast, propelled by novel technology and an increasingly profound understanding of human biology. The things that are likely to influence future drug discovery are many, including the emergence of precision medicine, integrating artificial intelligence into drug discovery, the growing availability of gene therapies, the introduction of new drug delivery systems, and a heinous increased sustainability and global accessibility. Each of these trends is expected to speed up, simplify, and personalize the development of drugs.

Expansion of Precision and Personalized Medicine

The next future drug development efforts will heavily focus on personalized medicine. Using genomic, proteomic, and metabolomic data, the scientist can come up with a specially designed medication for an individual based on his/her unique biological profile. This approach not only increases the effectiveness of therapy but also reduces the incidence of undesirable side effects. With the new advancement in next-generation sequencing and biomarker discovery, health care providers will be able to predict disease risk, select the most appropriate treatment, and monitor responses to treatment with near-surgical precision. Personalized medicine will move pharmaceutical industry thinkers out of their traditional frameworks of thinking in terms of "one-size-fits-all" toward a more individualized and effective form of treatment [45].

Greater Integration of Artificial Intelligence and Machine Learning

It is predicted that the use of artificial intelligence as well as machine learning would phenomenally increase in every aspect of the drug discovery phases-from target discovery to lead optimization design trial design up to the point of the patient selection. It is a matter of fact that in a very fast manner, it shall process altogether huge datasets without requiring manual labor, which involves the discovery of unseen materials, as well as the derivation of predictive models to guide investigators to headings for much more promising materials. Advanced newer developments in AI are probably very soon going to be directed toward designing entirely new molecular entities, predicting off-target effects much earlier, and downsizing regulatory submissions. This integration could significantly shorten the time between drug conception and its availability in the market, thereby decreasing the costs of development and increasing the probabilities of success [47].

Rise of Gene and Cell Therapies

Gene editing technologies, CRISPR-Cas9 among them, and newer ones like base editing and prime editing are opening new frontiers in drug discovery. Gene therapy aimed at correcting or replacing faulty genes could potentially yield forever cures for genetic maladies such as cystic fibrosis, muscular dystrophy, and certain cancers. Likewise, cell-based therapy, which by its CAR-T cell therapies-for-cancer definition has a more limited use today, is expected to be applied to other therapeutic areas soon. Research likely would center on safety, efficiency, and affordability improvements to increase their feasibility among larger patient populations [48].

Development of Advanced Drug Delivery Systems

Drug discovery in the future will not only be about the new molecules, but also how they will get into the body. Advanced delivery systems such as nanoparticles, liposomes, microneedles, and implantable devices are being developed to enhance their bioavailability, targeting, and controlled release. These systems drastically minimize side effects, improve compliance, and even allow treatment by means of non-invasive or localized ways. Innovative delivery technologies will be particularly important for biologics, gene therapies, or therapies requiring actions in particularly specific locations [49].

Emphasis on Sustainable and Ethical Drug Development

While the global health scenario increasingly transforms and keeps changing, the focus will eventually come into more emphasis on producing medicines that will not be effective, but sustainable and ethically produced. Pharmaceutical enterprises can also be looked upon by directing all possible efforts towards reducing environmental damage by using green chemistry, waste minimization, and renewable resources. Furthermore, issues such as multi-faceted access to drugs, especially in lower- or middle-income countries, will change the way drugs are priced, distributed, and regulated. Drug discovery in future will need to be balanced between the excitement of the very new scientific innovation and the reality of being responsible to all people [50].

Conclusion

It is sure though, that the field of drug discovery has in fact metamorphosed from ancient empirical remedies into modern, technology-driven modalities. This pillar remains critical in public health, addressing an array of diseases and, in the process, creating an avenue for many times revenues augmenting from the pharmaceutical industry. The process comprises a set of meticulously designed

steps, starting with understanding the disease biology and identification of targets to hit discovery, lead optimization, and clinical evaluation, each requiring scientific rigor and technological support. Blessed with advanced new-age approaches to structure-based and ligand-based drug design, fragment-based strategies, and de novo ones, coupled with the radical changes brought about by computational tools such as molecular simulations, artificial intelligence, and machine learning, drug discovery is increasingly becoming faster, more accurate, and highly personalized. The future seems inviting, with the expectation of innovative therapies- ones that could not only be effective but would also be very safe, customized individualized therapies available for the globe.

Integration of bioinformatics and chemo informatics has transformed the process of drug discovery, now faster, smarter, and more personalized therapeutic formulation. Bioinformatics works toward accelerated disease mechanism understanding and target validation while chemo informatics supports molecule design, optimization, and prediction of pharmacological properties. And now, what we see is CRISPR gene editing, immunization through mRNA, AI-assisted drug discovery, helping reshape traditional models and opening new gates for precision medicine. That fact remains; even though the milestones achieved are huge, there are still definite roadblocks like drug resistance, safety, and regulatory concerns. Lessons should be derived from successes and failures to improve future strategies. To answer all these changes, drug discovery will be revolutionized in time for the personalized medicine explosion, deeper AI integration, emergence of gene and cell therapies, novel drug delivery systems, and enhanced sustainability and global accessibility. Accelerated regulatory pathways and collaborative models will well further reduce the gap between scientific discovery and patient benefit, bringing them to a new age of smarter, faster, and more ethical drug development.

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Metal-Organic Frameworks (MOFs): Structure, Bonding Principles and Sustainable Applications

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Article DOI Link: https://zenodo.org/uploads/17543378

DOI: 10.5281/zenodo.17543378

Introduction

Inorganic chemistry has always been essential to design the materials that have both structural order and functional versatility. Over the past two decades, one of the most significant developments in this field and it has been the emergence of metal–organic frameworks (MOFs). MOFs are crystalline porous materials formed through coordination of metal ions or polynuclear clusters with multidentate organic linkers/lgands to form extended network. These MOFs are differentiated from conventional porous solids such as zeolites and activated carbons due to their unique structural and chemical tunability which is afforded by combining the principles of coordination chemistry with organic synthesis methodologies. Today, MOFs are one of the fastest-growing fields in material sciences, which bridging traditional inorganic chemistry with advanced applications in catalysis, gas storage and biomedicine. Their ability to combine structural order, high porosity, and chemical functionality makes them one of the most promising families of next-generation functional materials [1-3].

Structure and Bonding in MOFs

The structural diversity of MOFs arises from the interaction between metal nodes and organic linkers. The metal nodes in MOFs can be individual cations (e.g., Zn^{2+}, Cu^{2+}, Zr^{4+}, Fe^{3+}) or polynuclear secondary building units (SBUs) such as paddlewheel dimers [$M_2(COO)_4$] or oxo-clusters (e.g., $Zr_6O_4(OH)_4$), which act as nodes in the extended framework. These nodes interact with polyfunctional organic ligands—typically carboxylates, azolates, or phosphonates—resulting in networks stabilized by a combination of coordination bonds, hydrogen bonds, and π–π interactions. This mixture of strong and weak interactions imparts MOFs with both rigidity and flexibility. For example, MIL-53 can 'breathe,' expanding or contracting depending on which guest molecules are adsorbed. This unusual

flexibility challenges the traditional idea of crystals as rigid solids and is now being used to design responsive materials [4]. The coordination environment around the metal is governed by principles familiar from VSEPR theory, Crystal Field Theory (CFT), and Ligand Field Theory (LFT), allowing chemists to rationalize geometries such as tetrahedral, octahedral, or square-planar arrangements.

Dynamic Behavior and Flexibility

Unlike classical inorganic solids, MOFs often display stimuli-responsive structural dynamics:

- **Breathing MOFs:** Expand/contract pores with guest adsorption.
- **Gate-opening MOFs:** Selective adsorption triggered by pressure or concentration.
- **Stimuli-responsive frameworks:** Changes induced by temperature, light, or pH.

This dynamic behavior arises from the balance of strong coordination bonds with flexible organic linkers and weak supramolecular interactions. Such properties enable MOFs to function as smart materials in sensing, separation, and controlled release systems.

Significance and Applications

The multifunctionality of MOFs derives from their ultrahigh surface areas (often >5000 $m^2\ g^{-1}$), uniform pores, and exceptional chemical tunability. Their significance extends to several key application areas:

- **Gas Storage and Separation:** MOFs outperform conventional adsorbents in hydrogen and methane storage, as well as selective CO_2 capture [5]. Their tunable pore structure and surface chemistry make them ideal candidates for gas storage and separation, including hydrogen storage for clean energy, methane capture for natural gas technologies, and carbon dioxide sequestration to mitigate climate change.
- **Catalysis:** MOFs act as heterogeneous catalysts, leveraging metal nodes as Lewis's acid sites or incorporating functional groups for selective organic transformations, biomass valorization, and CO_2 reduction [6]. MOFs are increasingly studied as heterogeneous catalysts, where the combination of accessible active sites and structural regularity allows selective transformations ranging from biomass valorization to CO_2 reduction.
- **Environmental Remediation:** Functionalized MOFs capture heavy metals, dyes, and pollutants from wastewater [7]. Environmental remediation

represents another frontier, with MOFs showing promise in the removal of toxic heavy metals, dyes, and industrial pollutants from aqueous and gaseous media. The incorporation of functional groups or catalytic centers within the framework can significantly enhance their selectivity and efficiency in such processes.

- **Biomedicine:** Biocompatible MOFs serve as drug delivery carriers, imaging agents, and antimicrobial platforms [8]. In the biomedical domain, MOFs have been explored as drug delivery platforms, benefiting from their biocompatibility, tunable degradation rates, and ability to encapsulate therapeutic molecules within their pores.
- **Energy Conversion:** MOF-derived carbons and oxides function as electrode materials in batteries and supercapacitors, retaining high porosity and conductivity [9]. The versatility of MOFs also extends to energy storage and conversion. MOF-derived materials, obtained through thermal or chemical transformation, often yield porous carbons or metal oxides that retain aspects of the parent structure. These derivatives have demonstrated remarkable performance in batteries, supercapacitors, and electrocatalytic processes, underscoring the broader impact of MOF chemistry beyond the frameworks themselves.

Conclusion

The field of metal-organic frameworks exemplifies how fundamental principles of structure and bonding in inorganic chemistry can be translated into transformative real-world applications. By integrating modular metal nodes with versatile organic linkers, MOFs achieve a unique balance of rigidity, flexibility, and tunability. Their dynamic behavior such as breathing and gate-opning, expand the scope of crystalline solids beyond static structures, enabling the design of responsive and adaptive materials.

MOFs offers a broad spectrum of sustainable applications due to their remarkable properties such as- high surface area, tunable porosity and chemical functionality. In summary, MOFs represent a powerful convergence of inorganic chemistry fundamentals with applied material science, and they are poised to remain at the forefront of innovation in sustainable chemistry for decades to come.

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Herbo-metallic Preparations: An Ethnopharmacological Bridge Between Traditional Wisdom and Modern Medical Science

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Article DOI Link: https://zenodo.org/uploads/17543496
DOI: 10.5281/zenodo.17543496

Abstract

Ethnopharmacological Relevance: Herbometallic preparations (HMPs) are Ayurvedic formulations used to treat chronic diseases such as neurological problems, cancer, and metabolic diseases. Despite their historic benefits, safety concerns remain due to the presence of heavy metals like mercury and arsenic.

Herbo-metallic preparations, which combine purified metals and minerals with herbal ingredients to treat chronic and complex diseases like neurological disorders, cancers, and metabolic conditions, have long been an essential part of traditional medical systems like Ayurveda, Siddha, and Unani. These formulations go through rigorous purifying procedures designed to improve medicinal efficacy and detoxify metals. Their incorporation into contemporary medical practice has been hindered by worries about heavy metal toxicity, a lack of standardization, and inadequate clinical validation, despite their historical significance and therapeutic application. Many of these preparations exist as nano-sized particles, which may improve bioavailability and decrease toxicity, according to recent developments in nanotechnology and analytical methods. This evolving scientific evidence opens avenues for rigorous evaluation and standardization, promoting the safe incorporation of herbo-metallic formulations into contemporary healthcare. This chapter explores the ethnopharmacological background, challenges, and future prospects of herbo-metallic medicines,

highlighting their potential as complementary therapeutic agents within modern medicine.

Conclusion: Herbometallic medicines are regarded safe when taken in low doses and should be administered with caution as prescribed by physicians. However, additional safety analysis and thorough scientific research are required to properly demonstrate their long-term safety and efficacy.

Keywords: Herbo-metallic Preparations, Ayurveda, heavy metals, safety analysis, Toxicology.

Introduction

Herbo-metallic preparations have been used in ancient medical systems like Siddha, Unani, and Ayurveda for centuries, and they are valued for their unique blend of refined metals and minerals and plant-based substances. These formulations are commonly used to treat chronic and complex diseases such as neurological disorders, malignancies, metabolic syndromes, and inflammatory ailments. Metals and minerals are rigorously purified and processed, including Shodhana and Marana, which are thought to cleanse and increase their therapeutic effects when mixed with plant extracts (Maurya et al., 2015). Your paragraph is clear and well-informed, but it could be polished a bit for flow and clarity. Despite their long history of use and reported benefits, herbometallic medications have faced significant challenges in gaining widespread acceptance within modern medical science. Concerns about heavy metal toxicity remain prevalent, largely due to the inclusion of elements which includes arsenic, mercury, and lead compounds that are traditionally processed but continue to be viewed with skepticism by the biomedical community (Bhalla & Pannu, 2022; Hardin et al., 2023). Ayurvedic medicines, often associated with these metals, have been linked to several cases of heavy metal toxicity, raising serious safety concerns (Mikulski et al., 2017). One recognized human carcinogen is arsenic, which can cause malignancies of the skin, liver, lungs, bladder, kidney, and other organs, as well as various other severe toxic effects from both acute and chronic exposure (Liu et al., 2008). Additionally, practitioners and regulators remain cautious due to inconsistent manufacturing practices, variability in formulations, and a lack of robust clinical trials. Modern pharmacology requires unequivocal evidence of safety, standardized quality control, and well-designed clinical validation, all of which herbometallic formulations now lack. However, recent advances in analytical technologies and nanoscience have begun to shed new light on these traditional medicines, revealing that many metal-based

formulations exist as nano-sized particles that may be less toxic and have higher bioavailability than their raw counterparts (Gomes et al., 2014). This accumulating data suggests a possible way to integrate traditional herbometallic remedies with modern healthcare, emphasizing the importance of rigorous scientific examination while also respecting centuries-old knowledge systems. As a result, there is a rising desire to re-examine and maybe harmonize ancient formulations within modern medical frameworks in order to realize their full therapeutic potential safely and efficiently.

This study takes an ethnopharmacological approach to analyzing herbometallic formulations, looking at historical use, production techniques (such as Shodhana and Marana), pharmacodynamics, and pharmacokinetics. Modern analytical techniques, such as spectroscopy, chromatography, and nanotechnology, are used to evaluate these old medications and determine their safety and efficacy.

1. Herbo-metallic preparations (HMPs) and their historical significance in traditional medical systems: The foundational Ayurvedic texts like Charaka Samhita and Ashtanga Hridaya document ancient medical knowledge rooted in the Vedas, flourished under Indian rulers, declined during colonial times, and revived in the 20th century through modern research and integration with conventional medicine (Sikder,2024.). Minerals and rocks have been used in Ayurveda for over 5,000 years. Common examples include mica, realgar, orpiment, pyrite, and chalcopyrite. Others are magnetite, hematite, galena, salts, chalk, clays, and gems. These are purified before use. Mica ash (Abhraka Bhasma) is used in herbometallic medicines. It helps treat worm infections. With Picrorhiza kurroa (Kutuki Kawath), it shows mild blood sugar-lowering effects (Wijenayake et al., 2014). Nityananda Rasa (NR)is a herbometallic preparation used to cure numerous medical conditions including, hypothyroidism, hemorrhoids, gout, obesity and elephantiasis (AFI,part II, 2000). It is believed to balance the doshas i.e. pitta, vata and kapha. NR includes a combination of medicinal plant extracts along with metals like copper, bronze, tin, mercury, arsenic and iron, and non-metals such as sulphur. All ingredients are purified and processed using traditional Ayurvedic methods to form a refined, roasted product. Another formulation Arshakuthar Rasa (AR) is a mercury-based Ayurvedic drug used for treating hemorrhoids. The name means "axe for piles" in Sanskrit. It helps relieve constipation, anorexia, indigestion, and also acts as a laxative (Kumari et al., 2022).

2. Traditional Preparation and Detoxification of Herbo-Metallic Formulations: Ayurveda experts believed that some toxic drugs and metals routinely used in Rasa Shastra undergo "Shodhana" for purification or detoxification to enhance their medicinal values. In Ayurveda, toxic herbs can be made safe and effective through purification processes called Śodhana. These methods remove harmful substances, enhancing the safety and efficacy of the medicine. Modern science supports this traditional approach, showing that purification helps reduce toxicity and side effects (Maurya et al., 2015). Along with metals like tin, iron, arsenic, copper, bronze, mercury, and sulfur, it also contains a variety of medicinal plant extracts. All of these elements undergo a special ayurvedic process to produce its refined roasted form (AFI Part II, 2000). Purified sulphur (Sodhita Gandhakam) reduces mercury toxicity while exhibiting immunostimulant and anti-inflammatory effects (Kumar et al., 2006). Mica ash, used traditionally for liver, kidney, and skin ailments, requires thorough purification through repeated heating (850–1000 °C) and quenching to remove impurities and ensure safety, utilizing mica's stability up to 1100 °C (Parmar et al., 2010).
3. Safety challenges of herbo-metallic preparations: Herbo-metallic compounds, which are fundamental to ancient medical systems such as Ayurveda, have been used for centuries to treat a variety of disorders because of their alleged therapeutic properties. These formulations frequently include metals and minerals that have been processed using sophisticated purifying (shodhana) and incineration (marana) processes targeted at detoxifying and increased bioavailability. However, the inclusion of heavy metals such as mercury, lead, and arsenic in these formulations has created serious safety issues in current pharmacovigilance and toxicology. Heavy metals are known to accumulate in biological tissues, potentially causing nephrotoxicity, neurotoxicity, and cancer.

   Given these hazards, it is critical to systematically analyze the safety profiles of herbomineral preparations using standardized toxicological evaluations, such as acute, subchronic, and chronic toxicity tests. Toxicity evaluation and safety analysis are especially important because they determine the potential of these substances to induce genetic damage, which could lead to mutagenesis or carcinogenesis. Recognizing this, regulatory agencies such as the Government of India have required genotoxicity testing of metal- and mineral-based Ayurvedic medications to assure patient safety and support their reasonable therapeutic usage.

Despite the lengthy history of herbo-mineral use, scientific data on their genotoxic potential is few, with only a few formulations having undergone complete genotoxicity investigations. This gap underlines the critical need for systematic research that uses proven in vitro and in vivo genotoxicity assays, such as the Ames test, micronucleus assay, and chromosomal aberration tests, to determine the safety of these formulations at clinically relevant levels.

4. Toxicological Evaluation of Selected Herbo-Metallic Preparations: Herbo-mineral formulations have been used therapeutically for centuries, but concerns about heavy metal content have raised safety questions. To address this, the Government of India has emphasized the need for toxicological studies. Hridayarnava Rasa is used to support heart health, improve circulation, and treat respiratory and digestive disorders. The study conducted by Jagtap et al., (2014) evaluated the genotoxic potential of Hridayarnava Rasa. Results showed no structural deformities or genotoxic effects in treated groups, indicating that the formulation is safe under the tested conditions. Nityananda Rasa (NR) is a mercury-based Ayurvedic herbometallic compound that has been used to cure hypothyroidism, gout, obesity and elephantiasis. Kshirsagar et al. (2023) studied the subchronic oral toxicity of NR in albino Wistar rats. The study found no mortality or major behavioral changes in the animals, and their body weight and food intake remained unchanged. However, at higher levels, the study found biochemical, histopathological, and genotoxic consequences. These hazardous effects were dose-dependent and only occurred at doses significantly higher than those utilized therapeutically. Because NR is often administered at modest doses in clinical practice, it is considered safe when used sparingly and under professional supervision. A similar sub-chronic toxicity study on Arshakuthar Rasa (AR) was conducted by Kumari et al. (2022). The study observed mild changes in serum biochemical parameters that remained within normal limits, with histopathological findings consistent with these biochemical changes. Significant alterations were noted only at medium and high doses, which exceeded therapeutic levels. Hematological and genotoxicity parameters showed no changes. Additionally, gene expression analysis revealed activation of oxidative stress scavenger enzymes, suggesting cellular homeostasis was maintained. The absence of mercury in the blood likely contributed to the lack of toxicity observed. Overall, the study concluded that AR exhibits moderate toxicity only at doses above 600 mg/kg in rats, indicating it is likely safe for long-term human use at therapeutic doses. LD50

of AR is above 2000 mg/kg body weight, placing it in Category 4—considered to be non-toxic (Hodge & Sterner, 2005). Chandramrit Rasa (CR) is a mercury and iron-based HM Ayurvedic compound intended to treat chronic cough and other respiratory disorders (Duggal et al., 2023). Along with its metallic components, CR incorporates Adhatoda vasica leaf, which has been shown to have therapeutic effects on the respiratory system, as well as dicalcium phosphate, maize starch, and gelatin, which act as excipients to improve the formulation's stability and effectiveness. Further research is required to determine the specific mechanism of action of CR in animal and human models before recommending this product as a therapeutic treatment.

Future Directions for Safe HMP

Future research on herbo-metallic preparations (HMPs) must adopt an integrated, multidisciplinary framework that combines traditional knowledge with advanced scientific methodologies to enhance their safety and therapeutic efficacy. A critical area of focus should be the implementation of sophisticated analytical techniques—such as X-ray diffraction (XRD), electron microscopy, inductively coupled plasma mass spectrometry (ICP-MS) and spectroscopy—to accurately characterize the physicochemical properties, elemental composition, and bioavailability of metals within these formulations. Such detailed characterization is essential to detect residual toxic metals, understand their chemical forms, and ensure batch consistency, which directly impacts safety and efficacy. Parallel to analytical advancements, there is an urgent need to standardize classical purification processes (shodhana) and incineration methods (marana), which detoxify metallic components and transform them into biologically assimilable forms. Developing validated quality control protocols and pharmacopeial standards will facilitate reproducibility and regulatory acceptance of these medicines. Toxicological evaluation must also be comprehensive and systematic, encompassing acute, sub-chronic, and chronic toxicity assessments, along with focused studies on genotoxicity, reproductive toxicity, immunotoxicity, and pharmacokinetics to map metal biodistribution and potential bioaccumulation. Furthermore, the exploration of nanotechnology-based formulations offers promising avenues to improve the delivery, targeting, and safety profiles of HMPs, given that nanoparticle size and surface characteristics profoundly influence biological interactions and toxicity. Mechanistic studies using in vitro cellular models and in vivo animal systems, supported by omics technologies such as transcriptomics, proteomics, and metabolomics, can elucidate the molecular pathways modulated by herbo-metallic components, enabling a clearer

understanding of their therapeutic actions and adverse effects. Importantly, integrating traditional knowledge with modern scientific validation through ethnopharmacological studies and collaboration with experienced practitioners will ensure that research remains culturally relevant and clinically applicable. To translate preclinical findings into clinical practice, there is a pressing need for rigorously designed clinical trials that assess safety, efficacy, and optimal dosing regimens in human populations. Additionally, strengthening regulatory frameworks specific to herbo-metallic medicines—including clear guidelines for safety testing, quality assurance, and post-market surveillance—will support the responsible integration of these formulations into healthcare. Overall, fostering collaboration among pharmacologists, toxicologists, chemists, material scientists, clinicians, and traditional medicine experts is essential to drive innovation, address safety challenges, and unlock the full therapeutic potential of herbo-metallic preparations, thereby bridging the gap between ancient wisdom and modern medicine for the benefit of patients worldwide.

Conclusion

Herbo-metallic preparations have been integral to traditional medical systems like Ayurveda, Siddha, and Unani for centuries, valued for their unique combination of purified metals, minerals, and plant extracts. These formulations are widely used to treat chronic and complex diseases such as neurological disorders, cancers, metabolic syndromes, and inflammatory conditions. Traditional purification processes like Shodhana and Marana are believed to detoxify metals and enhance their therapeutic effects. Despite their long history and reported benefits, herbometallic medicines face skepticism in modern medicine due to concerns about heavy metal toxicity, inconsistent manufacturing standards, and a lack of robust clinical evidence. Recent advances in analytical technologies and nanoscience have revealed that many of these formulations contain nano-sized particles, which may offer improved bioavailability and reduced toxicity. To improve formulations and deepen understanding of their mechanisms of action, interdisciplinary collaboration between traditional medicine, pharmacology, toxicology, and material science is essential. Furthermore, establishing strong regulatory frameworks and conducting well-designed clinical trials will confirm safety and efficacy, promote quality control, and support responsible use. By bridging traditional knowledge with modern science, herbo-metallic preparations can be safely integrated into contemporary healthcare, offering patients effective therapeutic options with minimized risks.

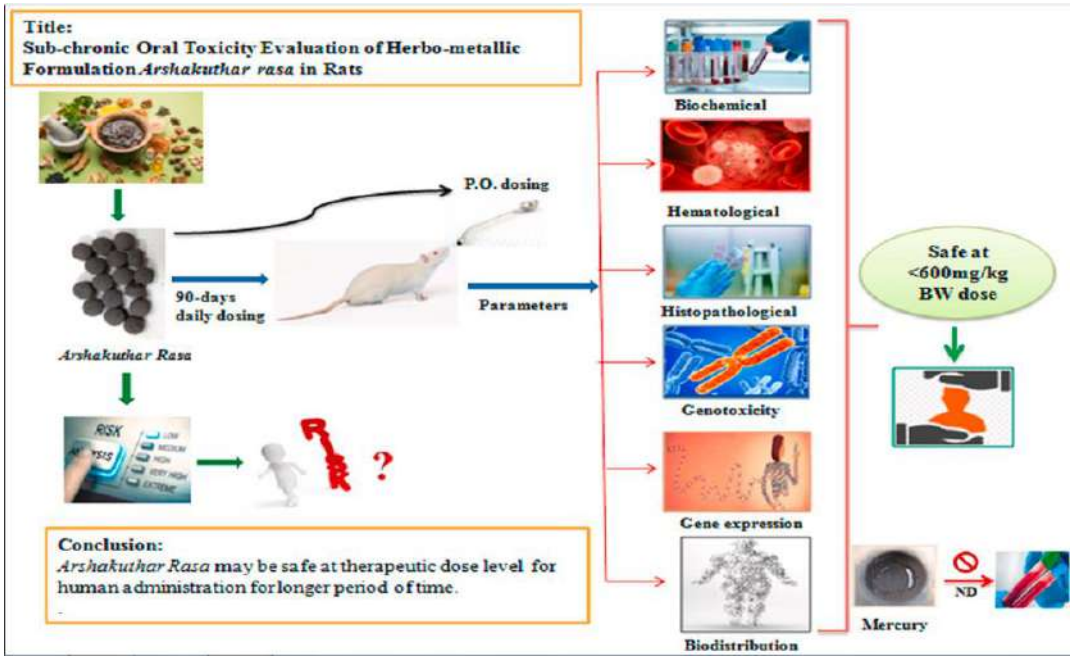

Image showing schematic presentation of safety analysis of Arshakuthar rasa in albino wistar rat (Kumari et al., 2022)

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Structural, Morphological CdS Nanomaterial Using the Chemical Precipitation Method

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Article DOI Link: https://zenodo.org/uploads/17543609
DOI: 10.5281/zenodo.17543609

Abstract

CdS nanomaterial were synthesized by chemical precipitation method and characterized by XRD and absorption spectra and SEM imageries. XRD spectra were recorded in terms of 2θ versus counts. The absorption spectra, consisting of several absorption bands, have been recorded for 300–1000 nm wavelength. The presence of the high intensity peaks shows that the prepared sample were highly crystalline in nature. The UV-visible spectra peak of band gap is suitable for optoelectronics, biological solar cell fabrication application.

Introduction

In the recent years, some of the properties due to CdS nanomaterial have novel electronic, structural, and thermal properties of high scientific value in basic and applied research [1]. CdS nano particles are also used as pigment in paint and in engineered plastic due to their stable thermal character [2]. CdS have high band gap energy of 2.42eV at room temperature that enables its nanoparticles to be superior in optoelectronics, photonics, photovoltaic can be utilized in optoelectronics for photocell labelling, light emitting diode (LED) [3], and Lasers field effect transistor [4]. In photonics, since its photo conducting and electric properties can be harnessed for sensors, photo detectors, optical filters, and optical switches, its band gap is in the visible range [5,6,7]. Here, stable and strong CdS nanomaterials have been synthesized and prepared through the simple chemical precipitation method. In the present investigation, the spectral characterization absorption nanomaterials possess interesting research in past years due to their novel chemical and physical properties. Under the physical properties in low dimensional and to investigate their immense potentiality for use in spectroscopy. In 1993 CdS high quality quantum

dots were prepared for the first time. They glowed various colors based on their size, morphology and band gap [6,8,9].

Structural and Morphological Properties

It can attain three types of crystal structures namely wurtite blend high pressure rock salt phase. Among the wurtzite is the most stable of the three phases and can be normal synthesized. Wurtzite phase has been observed in both the bulk nanocrystalline CdS while cubic and rock –salt phases are observed only in nanocrystalline CdS [10]. CdS wurtzite from comprises of hexagonal close packing (hcp) in hexagonal wurtzite and cubic zine blend, each atom is co-ordinated to four other atoms in tetrahedral fashion such that atom has four neighboring atoms of the opposite type.

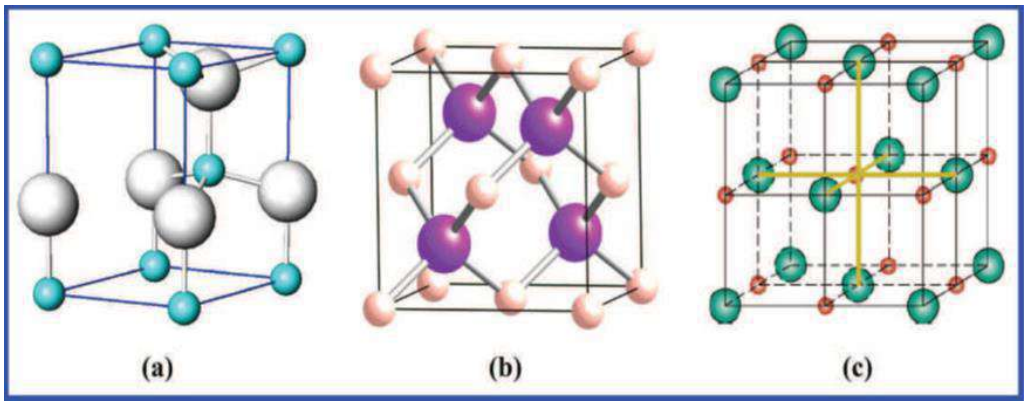

Fig.1: Diagram for the Unit cell for crystal structure of CdS (a) Wurtzite(hcp) (b) Zinc blend (c) Rock salt (ccp)phases

The CdS nanoparticle used physical and, chemical and structural properties. The melting point, electronic absorption spectra, band gap energy crystal structure and other properties of cadmium sulfide nanoparticle (CdS Nanomaterial) are affected by size. Thus, CdS on the whole is an attractive system for practicing synthetic chemistry for nanocrystals and for understanding the chemistry, growth history of nanomaterials and also for technical application [11-12]. Colloidal dispersions of CdS semiconductor nanoparticle can display color change of fluorescence depending on size of the particle. The CdS nanoparticles show quantum size effect, due to which the size of the cadmium sulfide particles is directly related to the absorption wavelength. The structure of the nanocrystalline CdS can play an important role in determining the electric properties. It can crystalline in different structures upon size reduction, depending upon reaction conditions. Physical properties of Cadmium Sulfide were collected in table 1. [13,14]

Table 1: Physical Properties of Cadmium Sulfide

Property	Value
Solubility	Insoluble in hot and cold water
Color	Yellow or brown
Physical state and appearance	Solid (solid power)
Molecular weight	144.46 g/mole
Melting point	Sublimes/9800c or 17960F
Specific gravity	4.82g/cm3

Synthesis of Cadmium Sulfide (CdS) Nanomaterials

cadmium sulfide nanoparticles synthesized using a simple chemical precipitation method of cadmium nitrate and sodium sulfide and particles size protected by diethylence Glycol 50 ml 0.1M(0.325gm) cadmium nitrate tetrahydrate (Cd (NO3)2.4H2O) was taken in Borosil Biker. Around 5 ml of Diethylene Glycol (DEG) was added to cadmium Nitrate tetrahydrate solution under constant stirring. After 30 minutes ,100 ml 0.1M (0.823gm) Sodium sulphide solution under constant stirring, reaction was kept 3hrs(800c) at constant stirring and yellow precipitate of CdS formed, washed with ethanol and distilled water dried at room temperature given Fig.1 illustrates [15,16].

Fig.2: A flow chart Diagram of synthesis of CdS Nanomaterial

Characterization and Discussion

CdS nanomaterials have been characterized by SEM, XRD and Absorption Optical properties of nanomaterial have been discuses.

Scanning Electron Microscopy (SEM)

The SEM image CdS nanoparticles prepared by simple chemical pricipetation method at room temperature. The image shows in fig.3 that approximate spherical shape to CdS nanoparticle and size of the particles around 1μ-100nm.It demonstrates clearly the formation of spherial CdS nanoparticles, and change of morphology of the nanoparticles.

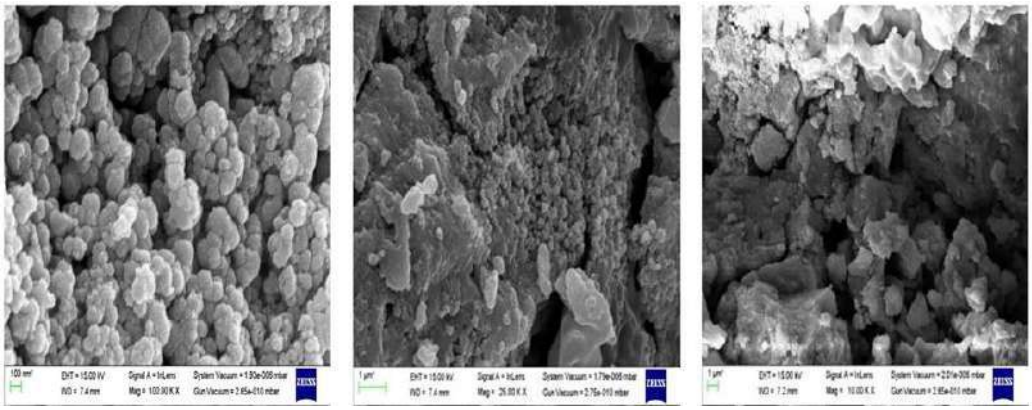

Fig. 3: SEM micrograph CdS nanomateral .

XRD (X-ray Diffraction)

Sharp peaks in the XRD patterns indicate crystalline nature of the samples. XRD pattern of CdS nanomaterials with Nd3+ have been shown in Fig.4.The variations of peak position and Sharp diffraction (2θ0) have been collected XRD patterns indicated that successfully incorporated into the crystal lattice of CdS matrix.

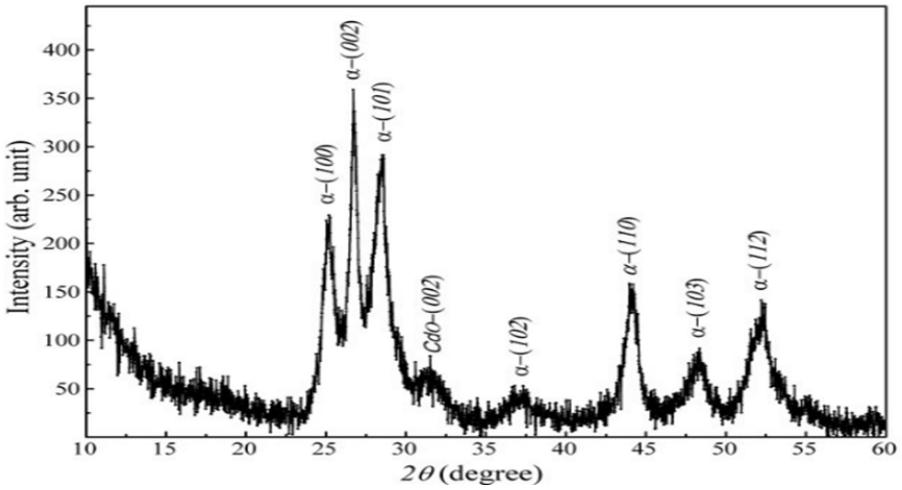

Fig.4: XRD micrograph CdS nanomaterial

The XRD results also confirmed the proper spherical phase formation and improved crystalline. It can be seen that diffraction patterns CdS show only diffraction peaks corresponding to hexagonal wurtzite CdS Joint Committee on Powder Diffraction Standards (JCPDS card # 36-1451).

UV-Visible Absorption Spectra

The Uv-Visible absorption spectra CdS nanoparticles were at room temperature. The absorption spectra recorded visible region in wavelength range 300-1100 nm fig.5. and correspond to transitions from the level to excited levels. The Uv-Visible spectra region from energy transistions involve the outer orbital or valence electrons [17]. This spectra in liquid media are usually broad, relatively featureless bands a result indicated a blue shift absorption. Uv-visible spectrophotometer used for primary application in quantitative analysis and calculated different parameters [18].

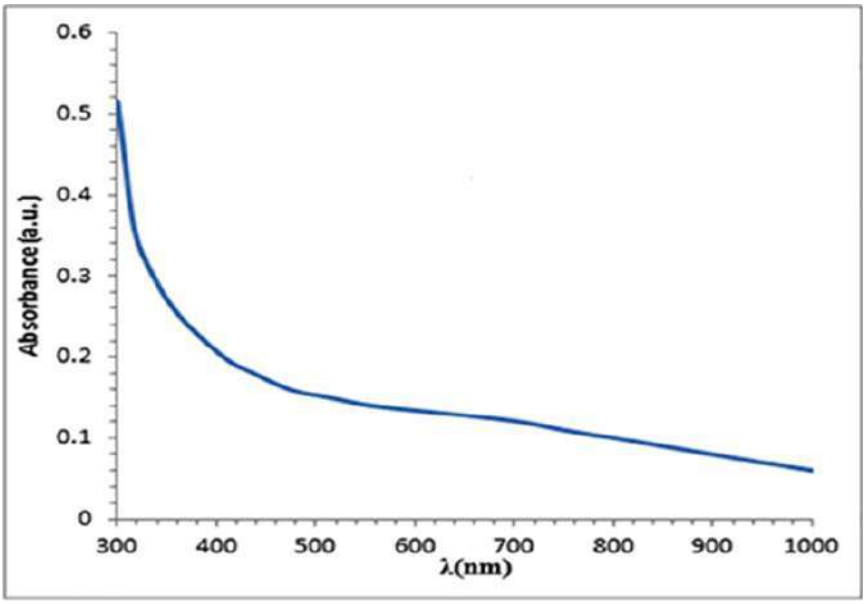

Fig.5: –Absorption spectrum of CdS nanomaterial [12].

The energy level structure has to be studied for spectra interpretation .The visible absorption spectra consist of narrow weak band .this chapter has been dedicated to the along with explanation of the observed optical absorption spectra of doped CdS nanomaterial ions in terms of energy state and transition intensity [19]The UV-visible absorption spectra CdS nanoparticles were at room temperature .The recorded visible region in wavelength range 300-1000 nm and corresponds to transitions from the level to excited levels.

Conclusions

Cadmium nanomaterials were successfully prepared simple chemical precipitation synthesis. The XRD pattern exhibit a spherical phase structure of CdS nanomaterial. The SEM images clearly signifying the change of morphology of the spherical and Absorption spectrum has high absorption in UV-visible region due to obtained range of band gap is suitable for useful materials-based application on optoelectronics biological medical sensor LED etc

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Regulatory Affairs, Quality Control, and Good Manufacturing Practices (GMP)

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DOI: 10.5281/zenodo.17543735

Abstract

Quality assurance (QA) in pharmaceutical manufacturing plays a vital role in guaranteeing the safety, effectiveness, and consistency of medicines. With the expansion of global pharmaceutical markets and increasingly complex regulations, a comprehensive approach is needed that integrates compliance requirements, supply chain resilience, and modern technologies to maintain high-quality standards. This paper discusses the importance of regulatory bodies such as the FDA, EMA, and GMP guidelines in ensuring international compliance, while also addressing challenges arising from changing regulations and extensive documentation. It further explores supply chain vulnerabilities, including issues with raw material sourcing, counterfeit products, and cold chain logistics, and emphasizes strategies like serialization, traceability, and real-time monitoring to reduce these risks. Additionally, the paper highlights how advancements such as AI, automation, blockchain, and continuous manufacturing are transforming quality assurance by enhancing transparency, predictive control, and efficiency. In conclusion, it recommends aligning regulatory frameworks with technological innovations, adopting AI-based compliance systems, and advancing automation to safeguard product quality and patient safety in the rapidly evolving pharmaceutical industry.

Keywords: Regulatory Affairs, Central Drugs Standard Control Organization.

Introduction

Regulatory Affairs (RA) is a key field in pharmaceuticals, biotechnology, and medical devices that combines science and law to support drug development and market approval. It prevents issues from poor data management or presentation and ensures compliance with regulatory requirements. RA professionals are

increasingly influenced by global changes such as geopolitical shifts, the green economy, and the COVID-19 pandemic, which shape how new therapies are developed and brought to market.[1] The Regulatory Affairs department plays a vital role in the organizational structure of pharmaceutical companies. Regulatory Affairs is actively involved in every stage of new drug development as well as in post marketing activities for approved medicinal products. Regulatory affairs professionals ensure pharmaceutical products follow industry regulations, handling applications, licensing, and marketing while maintaining safety and efficacy standards.[2]

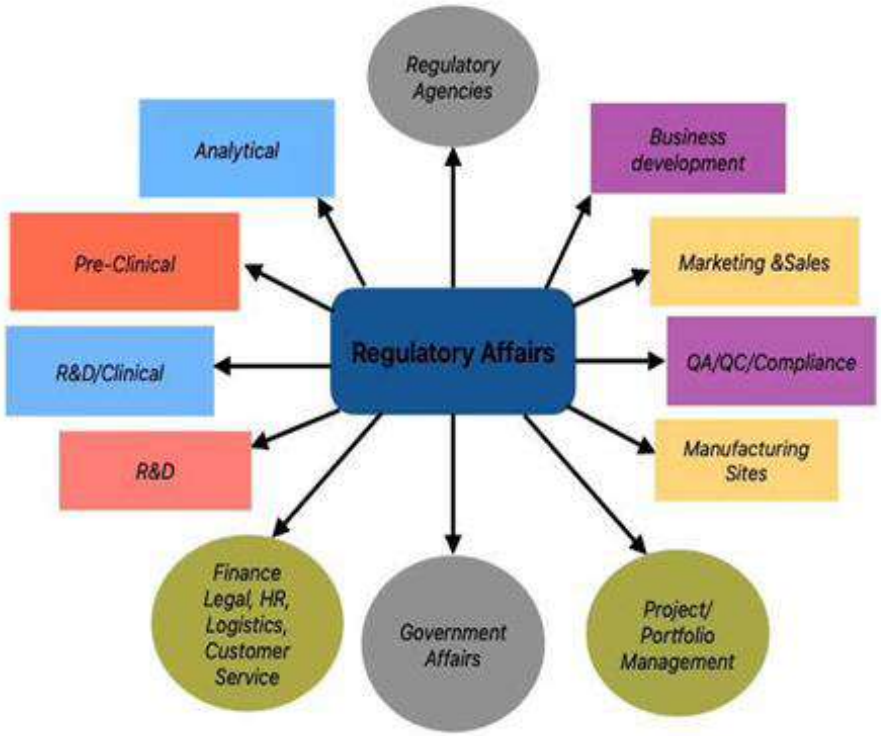


Fig no:1 Roles and Responsibilities

Drug Regulatory Authorities in India:

The Central Drugs Standard Control Organization (CDSCO), headquartered in New Delhi, is India's main regulatory authority for drugs, medical devices, and cosmetics. It is responsible for drug approvals, clinical trials, licensing, and import registrations. CDSCO also monitors the market, conducts drug testing, and bans unsafe products when required. Additionally, it issues guidelines and certifications to maintain safety and quality standards in collaboration with the

Drugs Controller General of India (DCGI).[3]

Objectives of Regulatory Affairs

- How and why the pharmaceutical industry and drug regulations have developed in USA.
- The Rules Governing Medicinal Products in the European Union.
- Major Regulations of USA.
- Framework of EU and its regulatory.
- Pharmaceutical Legislations of EU.
- Indian Pharmaceutical Industry & Drug Regulations development in different Era.
- Types of Marketing Authorization Procedure in EU Market.
- Major Rules and Act of India.
- Roles of Regulatory Affairs Professional in Health Authorities as well as Pharmaceutical Industry.
- Ensuring that their companies comply with all of the regulations and laws pertaining to their business.
- Working with federal, state and local regulatory agencies and personnel on specific issues affecting their business.
- Advising companies on the regulatory aspects and climate that would affect their proposed activities.

Good Manufacturing Practice (GMP) is a key aspect of quality assurance that ensures products are consistently manufactured and controlled to meet approved quality standards and intended use, as required by marketing authorization. It is a system that confirms compliance with set quality guidelines and aims to reduce risks in pharmaceutical production that cannot be completely addressed by testing or inspecting the final product.[4]

Six System Approach to Pharmaceutical CGMP Regulations

The FDA has enacted several pharmaceutical cGMP regulations. These are key concepts that are critical to quality systems. Some of the concepts by which the FDA and other regulatory bodies ensure cGMP regulations are Quality, Quality by Design (QbD) and product development, Quality Risk Management, Corrective and Preventive Action (CAPA), Change Control, and the Six Systems Approach.[5]

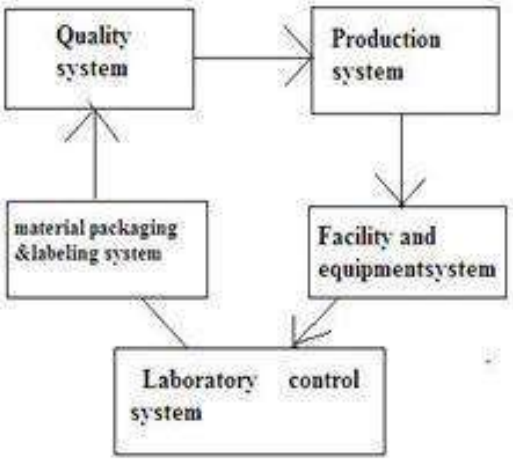
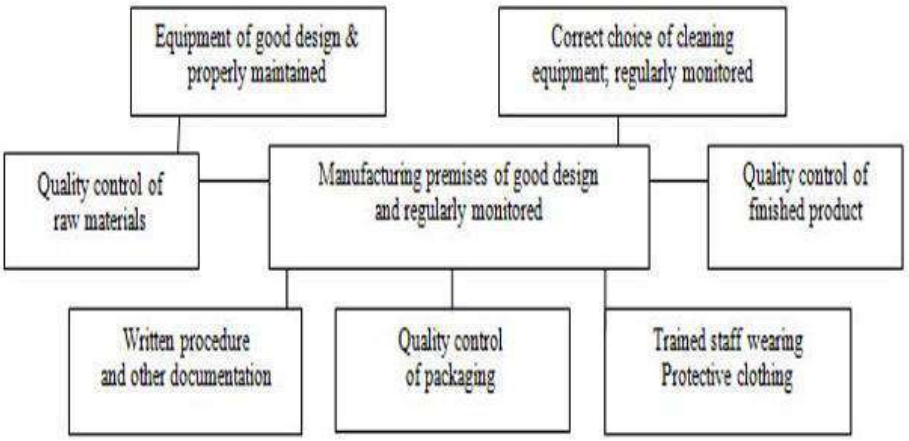


Fig no:2 Six system approach

GMP requires that the manufacturing process is fully defined before being initiated and all the necessary facilities are provided. In practice, personnel must be adequately trained, suitable premises and equipment used, correct materials used, approved procedures adopted, suitable storage and transport facilities available, and appropriate records made.[6]



Regulatory Approval & Submission Procedure in India

The Drugs and Cosmetics Act of 1940, along with the associated Rules of 1945, was enacted by the Indian Parliament to oversee the importation, production, distribution, and sale of drugs and cosmetics. Through these laws, the Central Drugs Standard Control Organization (CDSCO) and its chief officer, the Drugs Controller General of India (DCGI), were set up to enforce and administer these

regulations.[7]

In 1988, India incorporated Schedule Y into the Drugs and Cosmetics Rules, establishing guidelines for clinical trials. In 2005, it was revised to align with global standards, introducing definitions for Phase I–IV trials and clarifying investigator and sponsor duties. In 2006, trials were split into two groups: Category A (those already approved in countries like the U.S., U.K., EU, etc.) eligible for fast-track approval in about eight weeks, and Category B, which undergo greater scrutiny and take 16–18 weeks for approval. The application to conduct clinical trials in India must be submitted to the DCGI along with data on chemistry, manufacturing, control, and animal studies, plus the trial protocol, investigator's brochure, and informed consent forms. A copy also goes to the ethics committee, and trials may begin only after both the DCGI and ethics committee grant approval. Phase I trials on healthy volunteers help determine the maximum tolerated dose and adverse reactions, while Phase II trials in small patient groups identify therapeutic uses and effective dosing ranges. Confirmatory (Phase III) trials must be carried out on at least 500 patients across 10–15 centres (if the drug is not marketed elsewhere) to validate its efficacy and safety. After trials, a new drug application is submitted (Form 44) with full preclinical and clinical data. Additional required details include the drug's marketing status abroad, prescription use, sample plans, testing protocols, product monograph, labelling, and packaging.[8]

After NDA approval, the drug enters Phase IV trials to study long-term effects, new uses, or populations. Drug approval processes differ across countries: in the USA, the FDA handles all regulatory functions, while in India these are divided between central and state authorities. Differences also exist in approval timelines, fees, review types (normal/accelerated), and dossier formats. For example, CTD format is mandatory in the USA, EU, and Japan, but optional in India.[9]

Conclusion

The Regulatory Affairs function continually expands and is among the most resilient during mergers, acquisitions, and economic downturns. As regulatory demands grow, companies often outsource these tasks. Speeding a product's market entry is vital for success, making Regulatory Affairs efficiency economically critical. Using CTD and eCTD formats greatly streamlines electronic submissions and cuts down the time and effort needed to assemble registration dossiers.

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Green Innovations: A Comprehensive Analysis of Plant-Based Bioadhesives and Their Sustainable advantages over Synthetic Alternatives

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Article DOI Link: https://zenodo.org/uploads/17543887
DOI: 10.5281/zenodo.17543887

Abstract

Synthetic stickers are less environmentally friendly than vegetable bioadesis. Natural vegetable and polysaccharide proteins make these stickers eco-friendly. Vegetable protein-based biological stickers have advanced commercially, according to a study. Renewable bioadhesives perform comparable to synthetic adhesives (Yue et al., 2023). Plant-based bioadesives are in food and packaging. Alternative packaging adhesives derived from tannic acid, chitosan, and shellac are useful and sustainable. A unique water-based approach makes an eco-friendly bio-adhesive from soluble soy and soy protein polysaccharide. These materials can be used in several markets, according to Yuan et al. (2017). Plant-based bioadhesives are greener than synthetic ones. Organic decomposition and fossil fuel reduction make the economy more sustainable. Plant-based meat mimics are versatile because pH-controlled tannic acid and separated soy protein make them stickier and persist longer (Xue et al., 2024). Soy protein isolate and tea polyphenol stickers had better traction resistance with vegetable proteins. The environmental health industry may benefit from these materials. A vegetable-based bioadhesive helps businesses go green. Development of plant-based materials will benefit industry and the environment.

Keywords: Bio-adhesive, synthetic adhesives alternatives, biomedical adhesives, polyphenol stickers.

Introduction

Bioadhesives based on plants, defined as adhesives derived from renewable resources such as plant extracts, starchs and proteins, represent an innovative and durable alternative to traditional oil -based adhesives. While the pivot towards ecological materials is intensifying in various sectors, the contemporary adhesive market has started to adopt plant bioadhesives as a viable solution to increasing

concerns surrounding plastic pollution and environmental degradation. The growing consciousness of sustainability and the harmful effects of synthetic adhesives on health and the environment catalyzes an evolution of paradigm towards biodegradable alternatives. An important underlying factor to increase interest in plant-based bioadhesives is the alarming rates of plastic pollution worldwide. According to the United Nations Environment Program (UNP), around 300 million tonnes of plastic are produced each year, a substantial part contributing to environmental degradation and negatively affecting ecosystems (UNP, 2021). As around 80% of this plastic is not reused or recycled, accumulation in discharges and oceans constitutes a serious threat to biodiversity and public health (Heinrich, 2019). Plant bioadhesives can provide an innovative counter-action to these challenges, offering a sustainable membership solution because they are biodegradable and can be produced from easily available agricultural by-products. Recent studies reveal that the global bioadhesive market is expected to reach 8 billion USD by 2025, extending to an annual growth rate (TCAC) of 12% compared to the 2020 levels, reflecting the growing demand for environmentally friendly materials (Marketsandmarkets, 2021). This arrow interest echoes increasing regulatory pressures and consumer preferences promoting sustainable products. Industries ranging from wooden packaging and work to automobile and biomedical applications now explore the incorporation of plant bioadhesives which not only meet the membership performance standards but also align with the evolution of sustainability objectives.

Literature

The functional advantages of plant -based bioadhesives include strong binding capacities, thermal stability and a reduction in toxic emissions, promoting a healthier working environment during manufacturing processes (XU et al., 2020). In addition, due to their renewable nature, plant bioadhesives reduce the exhaustion of fossil resources while simultaneously supporting agricultural economies, thus promoting a more circular economy in adhesive production. Despite these advantages, the adoption of plant -based bioadhesives involves certain challenges which must be met to improve their market penetration. The performance, the conservation time and the water resistance of the plant -based bioadhesives are often cited as limiting factors compared to their synthetic counterparts. For example, certain formulations could face stability problems leading to the degradation of performance in various environmental conditions, which can dissuade manufacturers from going to organic alternatives (Heinrich, 2019).

In addition, supply of raw materials can raise concerns about competition with food production, especially in regions that depend on agricultural results.

In the light of these challenges, in progress research strives to improve the formulation, sustainability and application of bioadhesives based on plants while considering scalability and profitability for industrial applications. This exploration underpins the potential of bioadhesives to alleviate the considerable environmental impacts associated with traditional adhesive technologies, thus advancing towards a more sustainable future in material science.

In summary, plant-based bioadhesives represent a significant change towards sustainable membership solutions. Their growing relevance on contemporary markets not only reflects a response to environmental challenges, but also a proactive approach in the redefinition of material sciences and production practices within various industries., The development and use of bioadhesives based on plants confer numerous advantages over traditional synthetic adhesives, mainly derived from petrochemical sources.

These benefits can be classified into three key areas: renovability, biodegradability and lower toxicity levels, all of which contribute to a more positive environmental impact.

Plant-based bioadhesives come from renewable materials, aligning with sustainable practices that seek to minimize dependence on fossil fuels. Primary raw materials for bioadhesives may include natural polymers derived from plants, such as starches, proteins and lignin. According to Dhawale et al. (2022), the use of renewable resources not only promotes a circular economy, but also reduces the carbon footprint inherent in the extraction and processing of oil -based products. As the global emphasis changes towards sustainability, dependence on biological adhesives can reduce ecological impacts associated with the depletion of resources and environmental degradation. Another significant advantage of plant bioadhesives is their biodegradability. Traditional synthetic adhesives, such as epoxy or polyurethane based, can persist in the environment for prolonged periods after consumption, contributing to soil and water pollution. In contrast, bioadhesives are designed to decompose naturally, facilitated by microbial activity in various environments (Khan et al., 2021). This characteristic not only improves environmental sustainability, but also aligns with the increase in regulatory expectations aimed at reducing plastic waste and promoting designs of disposable products that minimize ecological footprints (Dhawale et al., 2022).

In addition, compared to synthetic adhesives, plants-based counterparts generally exhibit lower levels of toxic compounds. The production of many synthetic

adhesives often implies hazardous chemicals, such as formaldehyde and volatile organic compounds (VOC), which can represent risks for both human health and for the environment during their life cycle, from manufacturing to elimination. On the contrary, bioadhesives generally consist of safer non -toxic ingredients, thus minimizing health risks associated with prolonged exposure. Studies indicate that the least toxicity of bioadhesives can improve security in the workplace for manufacturers and end users equally (Khan et al., 2021). This aspect is particularly relevant in industries where adhesives should be applied with minimal protection measures, such as food and products for children. The environmental impact of adopting bioadhesives instead of synthetic options can be visually represented. The biodegradation rates of various adhesive types, which shows the rapid breakdown of bioadhesives compared to the persistent nature of their synthetic counterparts.

The advantages of plant bioadhesives are multifaceted and critical to advance sustainable practices in various industries. By taking advantage of renewable resources, adopting biodegradability and minimizing toxicity, these innovative adhesives not only contribute to environmental administration, but also satisfy a growing demand for sustainable materials that are aligned with contemporary ecological objectives (Dhawale et al., 2022)., The functional properties of vegetable -based bioadesives are basically anchored in their molecular composition, in particular the inclusion of proteins and polysaccharides. These biomolecules have adhesive characteristics through various mechanisms, including the hydrogen bond, Van der Waals forces and hydrophobic interactions. Proteins, mainly derived from sources such as soy, casein and gluten, contribute to adhesive formulations forming complex networks that can improve both the strength of the bond and elasticity. The structure of these proteins, in particular their sequences of amino acids and folding models, plays a fundamental role in dictating their adhesive properties.

Polisaccharides, such as cellulose, migling and starch, are equally significant in bio -adhesive formulations. Their vast capacity of hydrogen bond and the ability to form gel when you develop appropriately allow them to act as effective adhesive matrices. For example, the use of starch -based stickers is prevalent in the packaging sector due to their biodegradable nature and tiring viscosity. If hydrated, starch granules swell, leading to viscosity increases, which can be manipulated to meet specific application needs. This property depicts the structure of Amilosio and Amilopectin, the two main components of the starch, highlighting their ability to form intermolecular interactions when gelatinized.

Recent innovations in molecular design have focused on improving vegetable -based bioadesive performance. A remarkable progress is the incorporation of chlorogenic acid, a polyphenolic compound derived from various plants, which has shown to increase strength and adhesive function. According to Zhang et al. (2025), chlorogenic acid demonstrates the double functionality by acting both as the reticulated agent and improving the resistance to the humidity of the adhesive. This compound promotes the formation of covalent bonds between the adhesive components, thus creating a more robust and lasting adhesive interface. The molecular structure of chlorogenic acid, shows its unique attributes that facilitate these improvements if integrated into adhesive systems. In addition, the molecular design of bioadesives can also be perfected through the manipulation of the degree of polymerization and branching of the polysaccharides. These changes can influence the fluidity, viscosity and mechanical properties of the adhesive, ultimately determining its performance in various conditions. For example, increasing the molecular weight of the polysaccharides generally involves greater viscosity, which can be useful in applications that require greater cohesion and duration against external stresses. The emerging approaches to molecular engineering involve biosynthetic methods, in which specific enzymes are exploited to modify polysaccharides and proteins at the molecular level. This biocatalitic approach not only provides a more ecological synthesis path, but also allows the production of bio -adhesive bio -adhesive for the distinct requirements of various applications in sectors such as construction, packaging and biomedical devices.

The complexity of the molecular design in vegetable -based bioadesives underlines the potential for the development of high -performance stickers that align with the modern objectives of sustainability. However, continuous research is essential to overcome the challenges relating to the variability of the properties of raw materials and the downsizing of the production methods that adhere to environmental practices. These efforts further strengthen the applicability and acceptance of the vegetable -based bioadesive market, opening the way to a wider innovation in adhesive technologies.

Vegetable -based bioadesive saw a growing adoption in various sectors due to their ecological profile and the characteristics of improved performance. This section examines their applications in packaging, construction and biomedical fields, supported by relevant cases of study that highlight their innovative uses and effectiveness. In the packaging sector, vegetable -based bioadesives are emerging as oil -based adhesives as well as practicable alternatives. Their ability

to adhere well to a variety of substrates, biodegradability and non -toxic properties make them particularly attractive. For example, the research conducted by Xie et al. (2022) demonstrates the successful integration of stickers derived from starch in the production of biodegradable packaging materials. This study shows how these bioadesive can improve the sustainability of packaging solutions without compromising performance, facing the demand for consumers of ecological products. The use of natural polymers from sources such as corn, manoca and potato starch contributes to significant reductions in plastic waste, a problem of fundamental importance in today's environmental landscape.

In the construction sector, vegetable -based bioadesives play a fundamental role, especially in the production of wooden products in wood. An important case of Yue et al. (2023) illustrates the use of soy protein stickers for the production of plywood. In this research, the mechanical performance, the resistance to humidity and the overall duration of the soy adhesive compensated compensated were evaluated against the traditional adhesive adhesives. The results showed that the soy sticker not only satisfied the standards of the sector for the bond force, but also offered better resistance to degradation induced by water. This progress presents the construction and furniture industries with a sustainable option that aligns with the growing regulatory pressures and consumer preferences, ultimately contributing to a reduced formaldehyde emissions and better internal air quality.

The biomedical field also benefits from the unique properties of vegetable -based bioadesive. These stickers are particularly precious in applications in which biocompatibility is essential. Nuswantoro et al. (2022) have explored the use of bioadesives based on chitosan in orthopedic applications, focusing in particular on the engineering of bone tissues. The study highlighted the ability of the material to promote adhesion and cell proliferation while providing an impaling for bone regeneration. The results showed that the chitosan sticker has effectively facilitated the integration of synthetic systems with the guest bone tissue, demonstrating the potential to improve surgical outcomes and reduce recovery times in patients. This application marks significant progress in the development of bioactive stickers that are not only safe for human use, but also improve therapeutic efficacy.

However, the transition to plant -based bioadesivos is nonetheless challenges. Current limitations around the performance, consistency and cost-effectiveness of these materials require continuous research and development. Questions such as moisture sensitivity, union force under varied conditions and long -term

durability should be addressed to improve their competitiveness in relation to synthetic counterparts. In addition, biomass supply should be evaluated to ensure that it does not lead to adverse ecological consequences such as destruction of dwelling or food safety (Dunky and Mittal, 2023).

Summary and Conclusions

Given these issues, biotech innovation must be promoted. Synthetic biology and biopolymer engineering research can improve bioadhesives' performance and marketability. Novel biological components like lignin, cellulose, and chitosan can improve formulation resilience. Hybrid adhesives that combine synthetic and biological benefits may help solve performance issues.

To promote bio-bio-nod, research institutions, industry players, and government must work together. Standards and certifications for bioadhesives can boost market acceptance. Consumer and producer education can change perceptions of plant-based glue systems' efficacy and reliability.Vegetable bioadhesives promote sustainability. Plant-based bioadditives may help companies lessen their environmental impact as they prioritize environmental responsibility. Its ability to provide efficient connectivity solutions for ecological protection emphasizes the need for rapid R&D. The evolution of plant -based bioaddests represents a critical component in the movement towards sustainable adhesive technologies. Continuous investment in this area is essential to advance material science, support environmental conservation and promote a more sustainable future. Applying insights obtained with ongoing research can catalyze a change in adhesive patterns, leading to the establishment of plant -based bioadesivos as a conventional alternative in various industries.

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Global Importance of Biodiversity Conservation and Ecological Restoration

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Article DOI Link: https://zenodo.org/uploads/17543948
DOI: 10.5281/zenodo.17543948

Abstract

This paper examines India's initiatives for biodiversity conservation and ecological restoration in the context of global sustainability goals, comparing them with efforts in developed countries. It analyses international frameworks, including the three Rio Conventions, and highlights India's progress in land restoration and biodiversity conservation through regulatory frameworks, dedicated policies and programmes, and community-driven conservation practices. Through a comparative analysis, the paper contrasts India's initiatives with those in the EU and North America, focusing on differences in technological adoption, financial resources, and community engagement. The findings provide insights into potential opportunities for collaboration, particularly in integrating traditional knowledge with modern conservation techniques.

Keywords: Biodiversity conservation, ecological restoration, developed countries, global sustainability, community-based conservation.

Introduction

Biodiversity

Biodiversity, or biological diversity, refers to the variety of life on Earth. It includes the different plants, animals, and microorganisms, the genes they contain, and the ecosystems they form. This diversity is essential for the stability and resilience of ecosystems, providing vital services that sustain life on our planet. Biodiversity can be categorized into three main levels: genetic diversity, species diversity, and ecosystem diversity. Many of the world's ecosystems have undergone significant degradation with negative impacts on biological diversity and peoples' livelihoods. There is now a growing realisation that we will not be able to conserve the earth's biological diversity through the protection of critical areas alone. Conservation of biodiversity involves protecting, managing, and

restoring ecosystems, species, and genetic diversity. It is crucial because biodiversity provides a wide range of ecological, economic, and cultural benefits. Biodiversity supports ecosystem functions and services like air and water purification, nutrient cycling, and climate regulation. It also promotes resilience against environmental changes.

Ecological Restoration

Ecological restoration or ecosystem restoration, is the process of assisting the recovery of an ecosystem that has been degraded, damaged, destroyed [1] or transformed.[2] It is distinct from conservation in that it attempts to retroactively repair already damaged ecosystems rather than take preventative measures.[3][4] Ecological restoration can help to reverse biodiversity loss, combat climate change, support the provision of ecosystem services and support local economies.[5] The United Nation has named 2021-2030 the Decade on Ecosystem Restoration.[6]This paper explains what is meant by the term "ecological restoration" and outlines how it can provide enhanced biodiversity outcomes as well as improve human well-being in degraded landscapes. In this way ecological restoration becomes a fundamental element of ecosystem management, although until recently, its potential has not always been fully recognised. Given that many people now depend on what have become degraded ecosystems to sustain their livelihoods, ecological restoration needs to address four elements. These elements are critical to successful ecosystem management.
Ecological restoration should:

- Improve biodiversity conservation
- Improve human livelihoods
- Empower local people
- Improve ecosystem productivity.

It means ecological restoration can be a primary component of conservation and sustainable development programmes throughout the world. The conservation benefits of restoration are obvious. This paper has been produced by a joint working group of the Society for Ecological Restoration (SER) International and the IUCN Commission on Ecosystem Management. The primary motivation for this paper has been to establish a joint rationale for both organizations as to why ecological restoration is a critical tool for biodiversity conservation and sustainable development. Much of this document was derived from the SER Primer on Ecological Restoration (SER 2002 and 2004). Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded,

damaged or destroyed. Many healthy ecosystems are a product of human endeavours over very long time periods and therefore restoration commonly requires the participation of resource dependent communities. In this respect ecological restoration supports conservation and sustainable development efforts worldwide. There are two major challenges involved when undertaking ecological restoration. One is how to undertake restoration across large areas comprising a variety of land-uses. The second is how to equitably balance the trade-offs between improving biodiversity conservation and improvements in human well-being. Principles of Good Ecological Restoration Practice Ecological restoration is a well-established practice in biodiversity conservation and ecosystem

In this context, in particular with the United Nations Decade on Ecosystem Restoration and the unprecedented global changes in the coming decades, the world is increasingly committed to preventing, halting and reversing the degradation of fragile ecosystems, conserving biodiversity and regenerating ecosystem services for both nature and humanity (Hobbs and Harris 2001; Wu et al. 2023). The 2025–2030 Global Strategic Framework for Wetland Conservation adopted by the 14th Meeting of the Conference of the Contracting Parties to the Ramsar Conservation on Wetlands (COP 14) also emphasizes restoring vulnerable ecosystems as a priority, such as mangroves and small-scaled wetlands (Li 2022).

Discussion

Ecological restoration should:

- Improve biodiversity conservation
- Improve human livelihoods
- Empower local people
- Improve ecosystem productivity.

It means ecological restoration can be a primary component of conservation and sustainable development programmes throughout the world. The conservation benefits of restoration are obvious. What is less apparent, but which is at least as important, is that in many instances, ecological restoration has also been able to renew economic opportunities, rejuvenate traditional cultural practices and refocus the aspirations of local communities.

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establish a joint rationale for both organizations as to why ecological restoration is a critical tool for biodiversity conservation and sustainable development. Much of this document was derived from the SER Primer on Ecological Restoration (SER 2002 and 2004). Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed. Ecological restoration has as its goal an ecosystem that is resilient and self-sustaining with respect to structure, species composition and function, as well as being integrated into the larger landscape and supporting sustainable livelihoods. In this respect ecological restoration supports conservation and sustainable development efforts worldwide. There are two major challenges involved when undertaking ecological restoration. One is how to undertake restoration across large areas comprising a variety of land-uses. The second is how to equitably balance the trade-offs between improving biodiversity conservation and improvements in human well-being. Principles of Good Ecological Restoration Practice Ecological restoration is a well-established practice in biodiversity conservation and ecosystem management. We have itemized fourteen principles of good ecological restoration practice based on experience gained over several decades. These principles, and the Attributes of Restoration Progress below, are consistent with both the scope and intent of the Convention on Biological Diversity's Principles for the Ecosystem Approach.

Principles of Good Ecological Restoration Practice Include:

Ecosystems

- Incorporating biological and environmental spatial variation into the design.
- Allowing for linkages within the larger landscape.
- Emphasizing process repair over structural replacement.
- Allowing sufficient time for self-generating processes to resume.
- Treating the causes rather than the symptoms of degradation.
- Include monitoring protocols to allow for adaptive management. Human systems
- Ensuring all stakeholders are fully aware of the full range of possible alternatives, opportunities, costs and benefits offered by restoration.
- Engaging all relevant sectors of society and disciplines, including the displaced and powerless, in planning, implementation and monitoring.
- Involving relevant stakeholders in the definition of boundaries for restoration.
- Considering all forms of historical and current information, including scientific and indigenous and local knowledge, innovations and practices.

- Providing for the accrual of ecosystem goods and services.

A degraded ecosystem can be considered to have been restored when it regains sufficient biotic and abiotic resources to sustain its structure, ecological processes and functions with minimal external assistance or subsidy. It will interact with contiguous ecosystems in terms of biotic and abiotic flows and social.
India, home to over 1.3 billion people, faces significant challenges with 29.77% of its geographic area (97.85 Mha) under degradation as of 2018–19, an increase of 3.32 Mha. since 2003–05 (SAC, 2021). Factors such as water erosion, vegetation degradation, and wind erosion are primary drivers of this degradation, which is concentrated in states like Rajasthan, Maharashtra, and Telangana. Percent degraded land in India as per land use type Data Source: SAC (2021) Unirrigated agricultural land stands out with the highest degradation rate at 38%, largely due to soil erosion, nutrient loss, and water stress. In contrast, irrigated agricultural land shows a lower degradation rate of 8%, benefiting from irrigation but still facing challenges like salinization. Forests (22%) suffer from deforestation and overgrazing, and scrublands (14%) are affected by land clearing and desertification. Water erosion is the most critical driver, affecting 11% of the area, often exacerbated by deforestation and poor agricultural practices. Vegetation degradation follows closely at 9.15%, driven by deforestation and overgrazing, leading to increased soil erosion and loss of biodiversity.

Key Highlights of India's Achievements in Biodiversity & Land Restoration

Megadiversity Ranking: India ranks 12th among the world's 17 megadiverse countries, housing about 8% of the global species diversity.

Species Diversity: Home to approximately 45,000 plant and 91,000 animal species, despite covering only 2.4% of the world's land area.

Forest Cover: Total forest cover stands at 21.71% of India's geographical area, with a target to reach 30% as per the Kunming Montreal GBDF.

Biodiversity Hotspots: Contains 4 out of 36 global biodiversity hotspots, which host over 30% of the country's plant and animal species.

Biosphere Reserves: India has 18 Biosphere Reserves covering about 5% of the total land area, with 12 included in UNESCO's World Network.

Forest Growth: Forest and tree cover has consistently increased, with 21,000 sq. km added in the last decade, reflecting a growth rate of 2.91%.

Tiger Population: Achieved a 42.3% increase in the tiger population from 2014 to 2022.

Wildlife Corridors: Developed 104 wildlife corridors to mitigate habitat fragmentation, marking an 18% increase over the last decade.

Mangrove Restoration: Expanded mangrove covers to 4,992 sq. km, indicating a significant increase of 7% (or 4662 sq. km) since 2010.

Marine Protected Areas: 1.07% of India's Exclusive Economic Zone (EEZ) is designated as Marine Protected Areas (MPAs), representing a 114% increase over the past decade.

Protected Areas: Established 998 protected areas, covering 5.3% of the total land area.

Restoration Initiatives: 19 Mha (73%) of the 26 Mha target under the Bonn Challenge have already been restored.

Ramsar Sites: Increased Ramsar sites from 26 in 2014 to 85, covering 1.3 Mha of wetlands.

Community Involvement: A total of 47 BHSs have been declared, showcasing local community involvement and indigenous knowledge in biodiversity protection. Functioning 2,000 Eco-Development Committees (EDCs) for community-led conservation, showing a 300% increase in numbers over the last decade. 18,000 Joint Forest Management Committees (JFMC) managing 22 Mha of degraded forestlands.

The Society for Ecological Restoration International (SER) is a non-profit organization infused with the energy of involved member individuals and organizations who are actively engaged in ecologically sensitive repair and management of ecosystems. Our mission is to promote ecological restoration as a means of sustaining the diversity of life on Earth and reestablishing an ecologically healthy relationship between nature and culture. It means ecological restoration can be a primary component of conservation and sustainable development programmes throughout the world. The primary motivation for this paper has been to establish a joint rationale for both organizations as to why ecological restoration is a critical tool for biodiversity conservation and sustainable development. Much of this document was derived from the SER International Primer on Ecological Restoration (SER 2002 & 2004). The paper

has been also been written to further the Principles of the Ecosystem Approach as endorsed by the Convention on Biological Diversity.

Conclusion

Biodiversity is fundamental to the health and functioning of our planet. It encompasses the genetic variation within species, the variety of species themselves, and the diversity of ecosystems. Protecting biodiversity is essential not only for the survival of individual species but also for the stability and sustainability of entire ecosystems. Biodiversity, the variety of life on Earth, is essential for the health of our planet and human well-being. It encompasses the diversity of genes, species, and ecosystems. The values of biodiversity are manifold, encompassing ecological, economic, social, cultural, and ethical dimensions. One significant aspect of biodiversity's value is its consumptive use, which refers to the direct utilization of biological resources by humans.The social values of biodiversity are multifaceted and deeply intertwined with human culture, recreation, education, creativity, and health. As we face increasing environmental challenges, it is crucial to appreciate and protect the rich biodiversity that supports not only the natural world but also the fabric of human society.

The Society for Ecological Restoration International (SER) is a non-profit organization infused with the energy of involved member individuals and organizations who are actively engaged in ecologically sensitive repair and management of ecosystems. Our mission is to promote ecological restoration as a means of sustaining the diversity of life on Earth and reestablishing an ecologically healthy relationship between nature and culture. It means ecological restoration can be a primary component of conservation and sustainable development programmes throughout the world. The primary motivation for this paper has been to establish a joint rationale for both organizations as to why ecological restoration is a critical tool for biodiversity conservation and sustainable development. Much of this document was derived from the SER International Primer on Ecological Restoration (SER 2002 & 2004). The paper has been also been written to further the Principles of the Ecosystem Approach as endorsed by the Convention on Biological Diversity.

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Nature- 64

ISBN: 978-93-49938-17-5

Price- 750/-