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PROGRESSIVE TRENDS IN PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES VOLUME IV

Editors:
Dr. Mangal Kadam
Dr. Mandar Gaikwad
Dr. Yogita G. Bodkhe
Dr. Pollobi Duara

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Progressive Trends in Pharmaceutical, Chemical and Biological Sciences

Volume IV

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Editors

Dr. Mangal S. Kadam

P. G Department of Zoology,

Yeshwant Mahavidyalaya,

Nanded, Maharashtra

Dr. Mandar S. Gaikwad

Department of Chemistry,

Yeshwantrao Chavan Mahavidyalaya,

Tuljapur, Maharashtra

Dr. Yogita G. Bodkhe

Department of Chemistry,

Guru Nanak College of Science, Ballarpur,

Gondwana University, Gadchiroli

Dr. Pollobi Duara

Department of Zoology,

Majuli College,

Majuli, Assam

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E-mail: bhumipublishing@gmail.com

PREFACE

We are delighted to present this book titled Progressive Trends in Pharmaceutical, Chemical and Biological Sciences, an edited volume that brings together emerging perspectives, innovative research, and recent advancements across these interconnected disciplines. The rapid pace of scientific progress in pharmaceutical, chemical, and biological sciences has led to significant breakthroughs impacting human health, environment, and industrial applications. This compilation aims to provide a comprehensive platform for academicians, researchers, industry professionals, and students to understand current developments and future directions in these fields.

The chapters included in this book address a diverse range of topics, including novel drug delivery systems, synthesis and characterization of new chemical entities, green chemistry approaches, molecular biology techniques, biotechnological applications, and interdisciplinary research trends. Each contribution has been rigorously reviewed to maintain academic integrity and relevance. The authors have shared their expertise, experimental insights, and critical analyses to foster an integrated understanding of concepts and their practical implications.

This book is designed to inspire young researchers to undertake multidisciplinary studies that address global challenges such as sustainable development, environmental safety, drug discovery for unmet medical needs, and improvement of quality of life. It serves as a valuable reference for postgraduate students, research scholars, and faculty members seeking updated information and research methodologies in these fast-evolving domains.

We express our sincere gratitude to all the contributing authors for their timely submissions and scholarly efforts, and to the editorial board members and reviewers for their meticulous evaluation, constructive suggestions, and support throughout the publication process. We are thankful to the publisher for their encouragement and professional assistance in bringing this book to fruition.

We hope that Progressive Trends in Pharmaceutical, Chemical and Biological Sciences will motivate its readers to explore new ideas, initiate collaborative research, and contribute effectively towards scientific advancement. We welcome constructive feedback and suggestions for future editions to further enrich the academic value of this work.

- Editors

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MULTICOMPONENT ACCESS TO PHENANTHRIDINE AND ACRIDINE FRAMEWORKS VIA FISCHER CARBENE COMPLEXES

Priyabrata Roy
Department of Chemistry,
Victoria Institution (College), 78B, A.P.C. Road, Kolkata 700009, India
Corresponding author E-mail: priyo_chem@yahoo.co.in

Abstract:

This chapter presents a comprehensive study on the multicomponent synthesis of nitrogen-fused heterocycles, specifically phenanthridine and acridine derivatives, using Fischer carbene complexes and heteroaromatic o-alkynyl carbonyl substrates. The key feature of this approach lies in the in situ generation of furo[3,4-c]isoquinoline and furo[3,4-b]quinoline intermediates, which are efficiently trapped through inter- or intramolecular Diels–Alder reactions. The methodology accommodates a wide range of functionalized aldehydes and ketones and displays excellent compatibility with various dienophiles, including dimethyl maleate and N-substituted maleimides. Additionally, the use of γ,δ-unsaturated Fischer carbenes enabled the synthesis of tetracyclic frameworks, representing aza analogues of triterpenes. This versatile and atom-economical strategy offers valuable synthetic access to complex heterocycles of medicinal and natural product relevance.

Keywords: Fischer Carbene Complex, Phenanthridine, Acridine, Multicomponent Reaction, Diels–Alder Cycloaddition.

1. Introduction:

Nitrogen-fused polycyclic heterocycles constitute a highly significant class of bioactive compounds, known for their structural complexity and wide-ranging pharmacological potential. Their biological efficacy is often attributed to the planarity and rigidity of their fused ring systems, which enhance interactions with biological targets. Within this domain, substituted phenanthridines and acridines have gained considerable prominence as key structural motifs in the design and development of therapeutic agents. Their widespread occurrence in natural products and broad spectrum of bioactivities—ranging from anticancer and antiviral to other pharmacologically relevant properties[1]—has rendered them attractive synthetic targets for organic and medicinal chemists alike.

Additionally, numerous acridine-containing compounds, including both naturally occurring alkaloids and synthetic analogues, have been reported to exhibit potent antibacterial and antimalarial activity.[2] These multifaceted biological applications underline the importance of efficient synthetic approaches for accessing these azaheterocyclic frameworks.

2. Synthetic Methodology and Mechanistic Insight

Limitations of Traditional Synthetic Approaches

Conventionally, the synthesis of phenanthridine and acridine systems has relied on multistep condensation strategies, typically involving cyclization and oxidation protocols that, although well-established, come with notable limitations.[3] [4] These classical methodologies often demand harsh reaction conditions, such as high temperatures, strong acids or bases, and reagents incompatible with sensitive functional groups. As a result, their utility becomes limited when dealing with structurally complex or highly functionalized substrates. Moreover, the overall efficiency and step-economy of these protocols often fall short, necessitating the search for more robust and modular synthetic alternatives.

Emergence of Multicomponent Reactions (MCRs)

In recent years, multicomponent reactions (MCRs) have emerged as a powerful alternative to traditional multi-step synthesis.[5] These reactions enable the simultaneous combination of three or more starting materials in a single reaction vessel, significantly improving atom economy, reducing waste, and streamlining synthetic operations. Importantly, MCRs offer access to molecular diversity and structural complexity in a time- and cost-effective manner, making them particularly valuable in the rapid generation of compound libraries for pharmaceutical screening and lead identification.

In a previous communication from our laboratory, we reported a novel multicomponent strategy for the synthesis of phenanthridine derivatives, based on the reactivity of Fischer carbene complexes with heteroaromatic alkynyl carbonyl compounds.[6] Building on this foundation, the current chapter presents an extended and unified multicomponent approach that also enables the synthesis of acridine analogues, through a similar Fischer carbene-based coupling strategy.

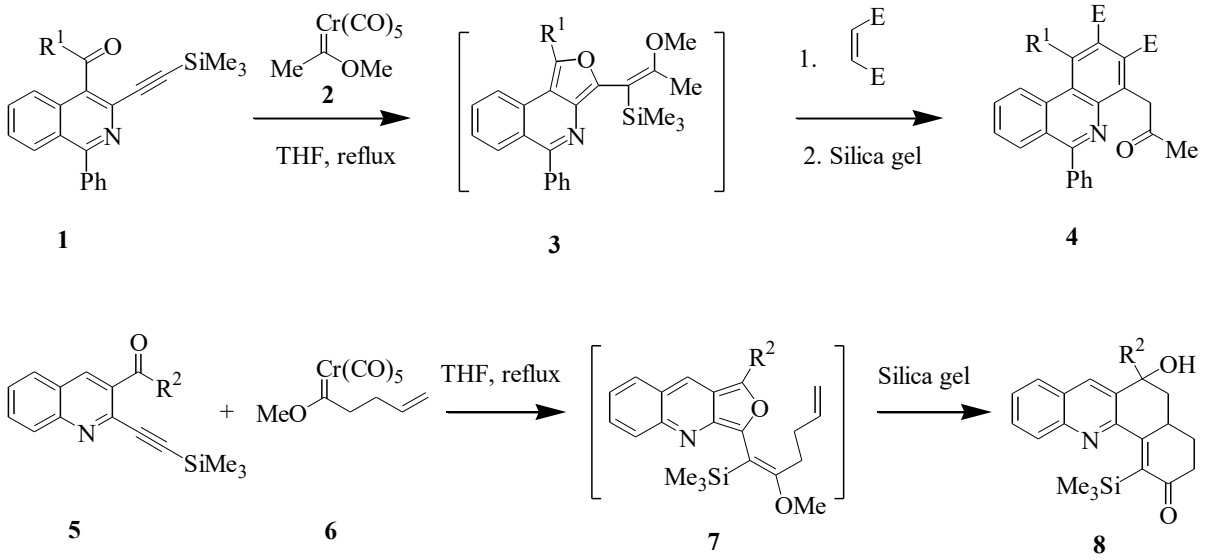
Synthetic Strategy: Fischer Carbene Coupling with Alkynyl Carbonyls

The methodology described herein represents the first account of a synthetic sequence involving the in situ formation and cycloaddition of reactive intermediates—namely, furo[3,4-c]isoquinoline and furo[3,4-b]quinoline derivatives. These intermediates are generated via the coupling of Fischer carbene complexes[7] with o-alkynyl carbonyl-substituted heteroaromatic systems, and possess inherent reactivity suited for both intra- and intermolecular cycloaddition reactions.

As illustrated in Scheme 1, this synthetic protocol relies on the generation and subsequent trapping of:

- Furo[3,4-c]isoquinoline intermediates via the reaction between o-alkynylisoquinoline carbonyl derivatives 1 and carbene complex 2, and
- Furo[3,4-b]quinoline intermediates through coupling of o-alkynylquinoline carbonyl derivatives 5 with carbene complex 6.

In both cases, the transient heterocyclic intermediates are intercepted by suitable dienophiles to deliver phenanthridine and acridine frameworks, respectively, with high structural complexity and synthetic efficiency.



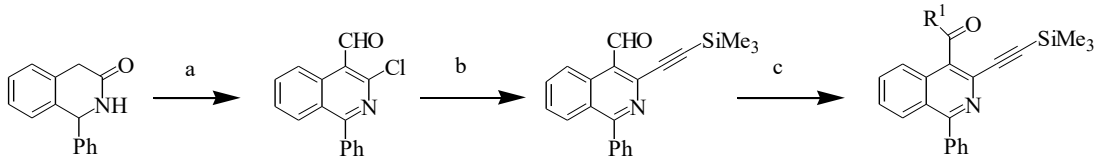
Scheme 1: Tandem generation and trapping of furo[3,4-*c*]isoquinoline and furo[3,4-*b*]quinoline intermediate

Synthesis of Key Alkynyl-Isoquinoline Carbonyl Precursors

The synthesis of essential 3-alkynyl-4-isoquinoline carbonyl building blocks, which served as pivotal intermediates for our multicomponent coupling studies, is depicted in Scheme 2. The synthetic route commenced from dihydroisoquinoline 98, a commercially available or readily accessible precursor. Through a Vilsmeier–Haack formylation reaction, the isoquinoline nucleus was first functionalized at the 4-position with an aldehyde group. Subsequent oxidative treatment using potassium permanganate in acidic medium led to the formation of 3-chloro-1-phenyl-4-isoquinoline carboxaldehyde (10) in good yield.[9]

To install the alkynyl functionality necessary for downstream Fischer carbene coupling, compound 10 was subjected to a Sonogashira cross-coupling with trimethylsilylacetylene, furnishing the silyl-protected alkyne derivative 11—namely, 1-phenyl-3-trimethylsilylethynyl-4-isoquinoline carboxaldehyde.

Next, this terminal alkyne intermediate was converted into a series of carbonyl compounds suitable for Fischer carbene reactivity. Upon desilylation and subsequent reaction with various Grignard reagents, the resulting propargylic alcohols were oxidized using pyridinium dichromate (PDC) to yield the key alkynyl ketones 12–14. These ketonic intermediates were found to be highly effective in undergoing the desired multicomponent coupling reactions with Fischer carbene complexes, as discussed in the following sections.



9 **10** **11**

12, R^1 = Ph
13, R^1 = $CH_2(CH_2)_2CH{=}CH_2$
14, R^1 = $CH_2(CH_2)_3CH{=}CH_2$

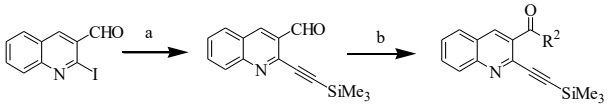
Scheme 2: Synthesis of isoquinolines substituted with an aldehyde or ketone group. *Reagents and conditions*: (a) $POCl_3$/DMF, THF, 0 °C, then $KMnO_4$, H_2SO_4, r.t. 42 %; (b) Trimethylsilylacetylene, $(Ph_3P)_2PdCl_2$, PPh_3, CuI, THF, Et_3N, r.t., 67%; (c) (i) R^1MgBr; (ii) CrO_3, 2Py, 12 (72%), 13 (52%), 14 (47%)

Synthesis of Alkynyl Quinoline Carbonyl Derivatives

In a parallel synthetic approach, the corresponding alkynylated quinoline derivative 16[10] was prepared via a palladium-catalyzed Sonogashira cross-coupling reaction, employing 3-formyl-2-iodoquinoline (15) as the key halogenated precursor.[11] The coupling with a terminal alkyne under standard Sonogashira conditions afforded intermediate 16, bearing the requisite alkynyl functionality at the 2-position of the quinoline ring (see Scheme 3).

Subsequent transformation of intermediate 16 was achieved through Grignard addition reactions, wherein treatment with either aryl or alkyl Grignard reagents in a diethyl ether/THF solvent system provided the corresponding propargylic alcohols. These intermediates were then subjected to oxidation using pyridinium dichromate (PDC) to furnish a series of structurally diverse alkynyl carbonyl derivatives 17–19.

These ketonic substrates served as crucial partners in the Fischer carbene-mediated multicomponent coupling reactions, enabling the generation of furo[3,4-b]quinoline intermediates for the subsequent construction of acridine-based heterocycles, as discussed in the following sections.



15 **16**

17 R^2=Ph
18 R^2=*p*-Tol
19 R^2=$(CH_2)_3CH{=}CH_2$

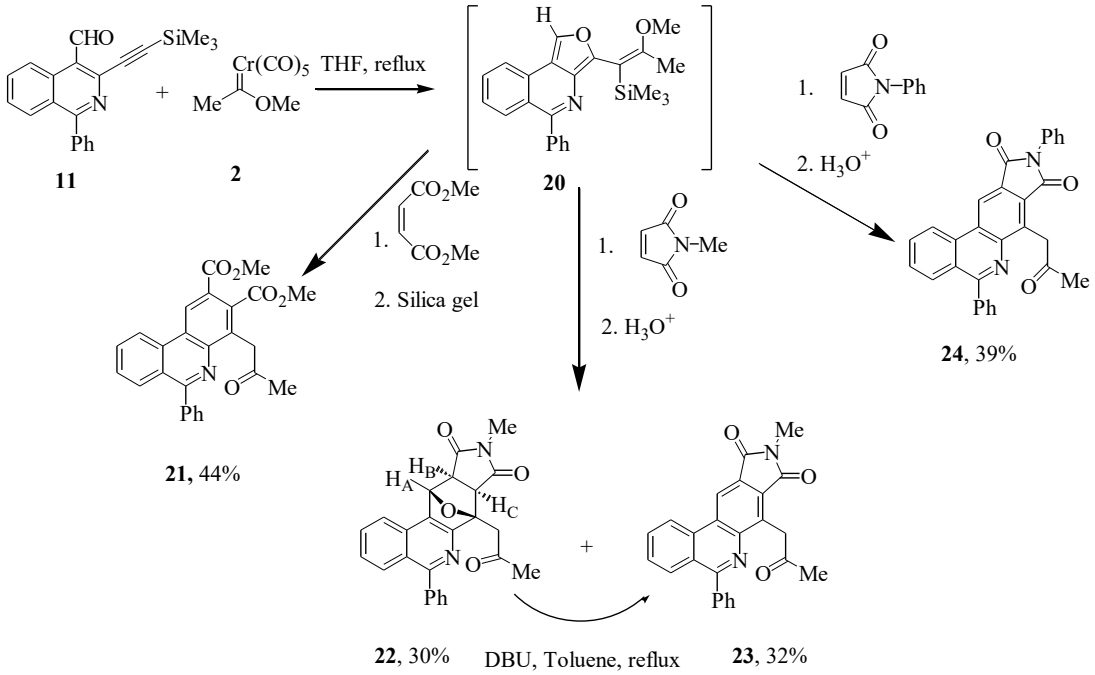
Scheme 3: Synthesis of functionalized quinolines. *Reagents and conditions*: (a) Trimethylsilylacetylene, $(Ph_3P)_2PdCl_2$, CuI, THF, Et_3N, rt, 86%; (b) (i) R^2MgBr; (ii) CrO_3, 2Py, 17 (70%), 18 (69%), 19 (45%).

Alder Cycloaddition

To explore the synthetic potential of the designed multicomponent strategy, an initial three-component coupling reaction was carried out using Fischer carbene complex 2, 3-alkynyl-4-isoquinoline carboxaldehyde 11, and dimethyl maleate in refluxing THF. The reaction mixture

was subjected to purification over silica gel, which afforded the targeted phenanthridine derivative 21 in good yield, as depicted in Scheme 4.

Mechanistically, the transformation is proposed to proceed through the in situ formation of the reactive furo[3,4-c]isoquinoline intermediate 20, generated via the coupling of the Fischer carbene complex with the alkynyl carbonyl compound. This intermediate then undergoes a Diels–Alder cycloaddition with dimethyl maleate, functioning as the dienophile, to construct the phenanthridine framework in a single synthetic operation. This result validated the feasibility of the designed multicomponent protocol and encouraged further exploration with alternative substrates and dienophiles.



Scheme 4: Synthesis of phenanthridine derivatives

Formation and Conversion of Oxa-Bridged Intermediate 22

In a variation of the multicomponent reaction, N-methylmaleimide was employed in place of dimethyl maleate as the dienophile. Under the same reaction conditions, this led to the formation of a mixture of products, comprising both the [4+2] oxa-bridged cycloadduct 22 and the phenanthridine derivative 23.

Detailed ^{1}H NMR spectroscopic analysis provided strong evidence in support of the exo-stereochemistry of the oxa-bridged product 22. This was indicated by the chemical shift values of protons HB and HC, both appearing at less than 4 ppm, and the absence of spin-spin coupling between protons HA and HB (i.e., 0 Hz coupling constant). Additionally, a distinct N-methyl singlet at approximately 3.0 ppm further supported the proposed structural assignment.[7a,12]

Interestingly, when compound 22 was treated with DBU in refluxing toluene, the oxa-bridged ring system underwent ring opening, resulting in clean conversion to phenanthridine

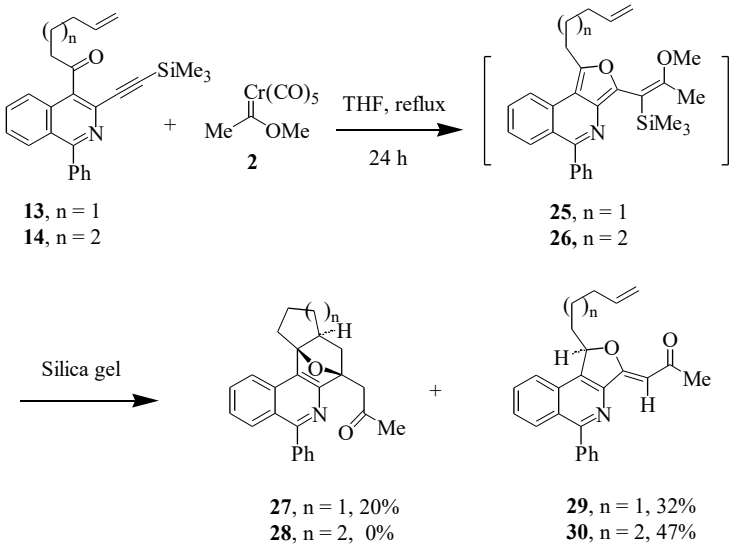
derivative 23.[13] This transformation illustrates the synthetic utility of the oxa-bridged intermediate as a masked precursor for phenanthridine construction under mild basic conditions.

Substrate and Dienophile Variation: Formation of Phenanthridine 24 and Rearranged Products

In a contrasting outcome, when N-phenylmaleimide was employed as the dienophile in the three-component coupling of aldehyde 11 with carbene complex 2, the reaction proceeded with remarkable selectivity. Under the same optimized conditions, the process cleanly afforded phenanthridine derivative 24 as the exclusive product, without the formation of any oxa-bridged intermediates or side products. This result highlights the influence of the electronic and steric nature of the dienophile on the reaction pathway and product profile.

Encouraged by these findings, the study was extended to examine the tandem generation and intramolecular trapping of reactive furo[3,4-c]isoquinoline and furo[3,4-b]quinoline intermediates, using ketonic substrates bearing unactivated alkenyl tethers of varied lengths. When carbene complex 2 was reacted with ketone 13, which features a non-activated alkenyl side chain, the reaction delivered a mixture of two distinct products: the oxa-bridged cycloadduct 27 and the [1,7]-sigmatropic rearrangement product 29, as shown in Scheme 5.

This observation suggested competing intramolecular pathways for the reactive intermediate under the given conditions, influenced by the nature of the tethered alkene and its ability to participate in the Diels–Alder step.



Scheme 5: Generation and Diels-Alder trapping of furo[3,4-*c*]isoquinoline intermediates using an intramolecular approach

Effect of Alkenyl Tether Length on Reaction Outcome

Interestingly, when the structurally related ketone 14, bearing an alkenyl side chain extended by one additional methylene unit, was subjected to the same reaction conditions, the outcome diverged significantly. In this case, the expected intramolecular cycloaddition failed to produce the anticipated oxa-bridged adduct 28. Instead, the reaction proceeded selectively to furnish the [1,7]-hydrogen shift product 30 as the sole isolated compound.

The formation of rearranged products 29 and 30 through [1,7]-hydride migration points to the operation of alternative mechanistic scenarios. Two plausible explanations can be proposed:

(i) The non-activated alkene in the tether may reduce the efficiency of the Diels–Alder cycloaddition, thus enabling competing rearrangement pathways to dominate; or

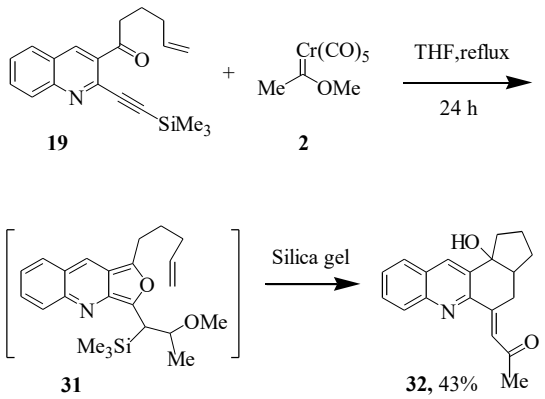
(ii) The initially formed furo[3,4-c]isoquinoline intermediates 25 and 26 may undergo direct hydrolytic cleavage, leading to the rearranged products 29 and 30, respectively.[14]

These findings underscore the sensitivity of the reaction pathway to subtle structural changes, particularly the length and nature of the alkenyl tether, which governs the fate of the reactive intermediates.

Reactivity of Quinoline-Based Substrate with Unactivated Alkenyl Chain

However, when the same Fischer carbene complex 2 was reacted with alkynyl quinoline ketone 19, which also bears a non-activated alkenyl side chain, the reaction outcome differed notably from the isoquinoline analogues. Under identical reaction conditions, the transformation led exclusively to the formation of alcohol product 32, with no trace of any [1,7]-hydride shift product observed (Scheme 6).

This selective outcome suggests that the quinoline-based framework exhibits distinct reactivity compared to its isoquinoline counterpart, possibly due to differences in electronic distribution or steric orientation within the fused heterocycle. The suppression of rearrangement pathways in this case further highlights the influence of the heteroaromatic core on the fate of the intermediate species and suggests that careful structural tuning can direct the reaction outcome toward specific products.



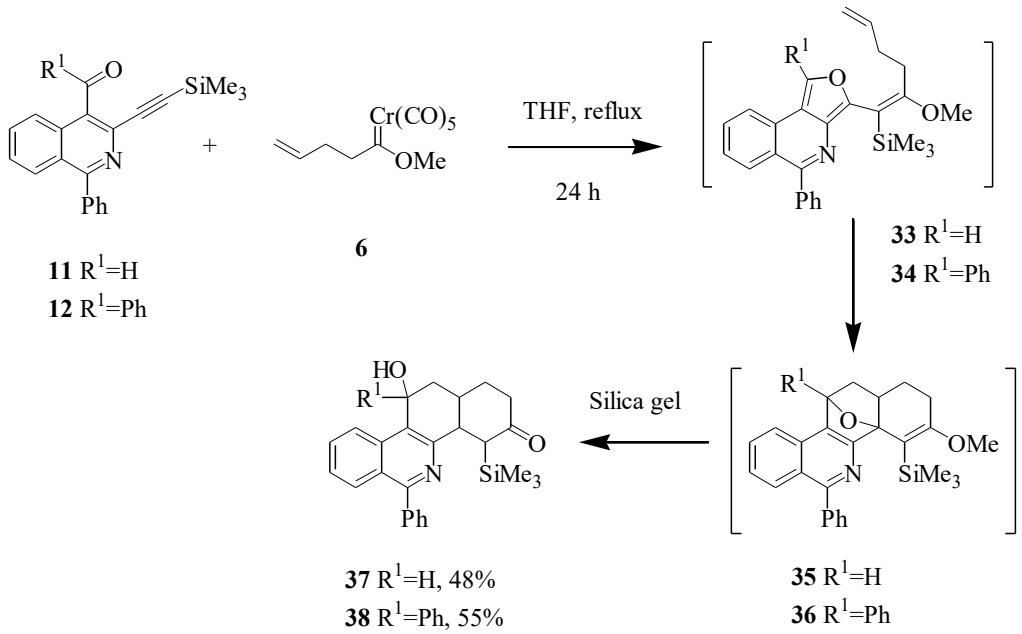
Scheme 6: Generation and Diels-Alder trapping of furo[3,4-*b*]quinoline intermediates in intramolecular cases

Construction of Tetracyclic Frameworks Using γ,δ-Unsaturated Carbene Complex

To further expand the scope of the methodology, the reaction pathway was examined using a γ,δ-unsaturated Fischer carbene complex. Specifically, the monoprenylated carbene complex 6 was employed in coupling reactions with alkynyl carbonyl derivatives 11 and 12 under the previously optimized conditions.

These reactions proceeded smoothly through the in situ generation of furo[3,4-c]isoquinoline intermediates 33 and 34, which underwent an efficient intramolecular Diels–Alder cycloaddition. This tandem sequence ultimately led to the formation of tetracyclic products 37 and 38, respectively, as shown in Scheme 7.

The successful construction of these fused polycyclic ring systems highlights the versatility of the multicomponent approach when applied to tethered dienophile systems and demonstrates its potential for generating complex molecular scaffolds in a concise and modular fashion.



Scheme 7: Synthesis of phenanthridines by using monoprenylated Fischer carbene complex

Tetracyclic Products as Nitrogen-Containing Triterpene Analogues

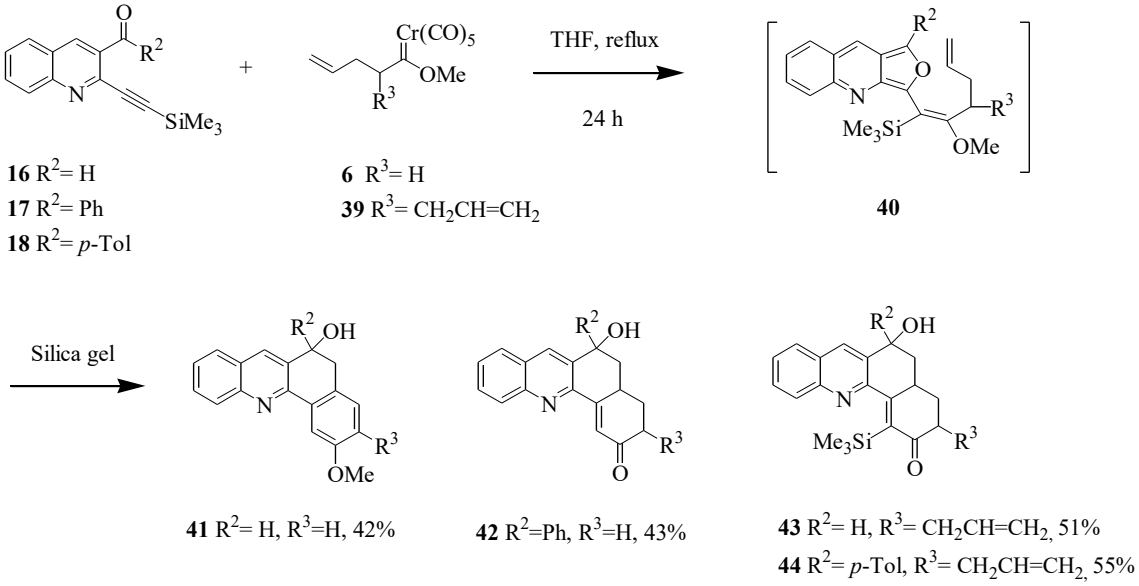
The initially formed cycloadducts 35 and 36, derived from furo[3,4-c]isoquinoline intermediates 33 and 34, appear to be transient species, likely prone to ring-opening transformations under the reaction conditions.[15] These intermediates eventually rearranged to give the stable tetracyclic products 37 and 38, each featuring a densely fused ring architecture.

Structurally, compounds 37 and 38 can be considered as aza analogues of tetracyclic triterpenes, a class of natural products known for their diverse biological activities. The ability to access such frameworks through this Fischer carbene-mediated multicomponent strategy opens up new possibilities for the design and synthesis of triterpene-inspired heterocyclic analogues, potentially useful in medicinal chemistry.

Acridine Formation via Trapping of Furo[3,4-b]quinoline Intermediates

Building on this success, the intramolecular trapping of short-lived furo[3,4-b]quinoline intermediates was next explored using both Fischer carbene complex 6 and its bis-prenylated derivative 39.[16] These complexes were reacted with o-alkynylquinoline carbonyl derivatives 16, 17, and 18 to investigate the extension of this methodology to acridine synthesis.

Under the optimized conditions, both carbene complexes 6 and 39 promoted the generation of the reactive intermediate 40, which underwent intramolecular cyclization to afford a series of acridine derivatives 41–44, as illustrated in Scheme 8. This set of transformations demonstrates the robustness and adaptability of the strategy across different heteroaromatic scaffolds and tethered dienophile systems.



Scheme 8: Synthesis of acridine derivatives by using mono/bis(prenylated) Fischer carbene complexes

Conclusion:

This study establishes a versatile and efficient one-pot multicomponent synthetic approach for the construction of phenanthridine and acridine ring systems, employing the reactivity of Fischer carbene complexes with heteroaromatic o-alkynyl carbonyl derivatives. Central to this strategy is the in situ generation and interception of highly reactive furo[3,4-c]isoquinoline and furo[3,4-b]quinoline intermediates, which serve as crucial platforms for subsequent inter- or intramolecular Diels–Alder cycloadditions.

The methodology proved effective across a range of substrates, including isoquinoline- and quinoline-based ketones and aldehydes, and was adaptable to different dienophiles such as dimethyl maleate, N-methylmaleimide, and N-phenylmaleimide, thereby enabling the selective formation of structurally diverse fused polycyclic heterocycles. Importantly, the stereochemical outcomes, supported by NMR data, confirmed the formation of oxa-bridged adducts and their subsequent rearrangement to phenanthridine derivatives under mild base treatment.

Further extension of this strategy to γ,δ-unsaturated carbene complexes, including monoprenylated and bis-prenylated variants, led to the successful synthesis of tetracyclic aza analogues of triterpenes, as well as acridine derivatives via the efficient trapping of short-lived quinoline-based intermediates.

Overall, this multicomponent protocol offers a modular, atom-economical, and functionally tolerant route to access biologically relevant nitrogen-containing heterocycles, showcasing its potential in the development of natural product-inspired scaffolds and medicinally valuable molecules.

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ISOLATION AND ANTIFUNGAL SUSCEPTIBITY TESTING OF *CANDIDA ALBICANS* ISOLATES FROM VARIOUS CLINICAL SPECIMEN

Mahesh Karale*[1], Datta Phule[2], Mohan Karuppayil[3] and Rahul More[1]

[1]Department of Microbiology,
Dayanand Science College, Latur-413512, Maharashtra, India.
[2]Department of Microbiology,
Homi Bhabha Cancer Hospital, Varanasi-221010, Uttar Pradesh, India.
[3]DST-FIST & UGC-SAP sponsored School of Life Sciences,
Swami Ramanand Teerth Marathwada University, Nanded-431606, Maharashtra, India.
*Corresponding author E-mail: karale.mahesh@gmail.com

Abstract:

To isolate and determine *in vitro* antifungal susceptibility pattern of *Candida albicans* to anti-fungal agents. A total of twenty *C. albicans* species were isolated from various clinical specimens. The preliminary diagnosis of specimens were performed by colony appearance, microscopic examination, sugar assimilation and utilization test and germ tube test on Sabouraud dextrose agar for identification of *Candida albicans*. Further, they were processed for *Candida* speciation on CHROMagar *Candida*. Anti-fungal susceptibility of clinical isolates were evaluated by determination of minimum inhibitory concentrations (MIC) by using broth micro-dilution method. The result discovered that clinical isolates were susceptible to fluconazole (85%), voriconazole and amphotericin B (100%) and the lowest sensitivity shown to ketoconazole (15%). The study revealed higher rate of ketoconazole resistance seen in *C. albicans* (65%). The detection of resistance in *C. albicans* to ketoconazole which is a species typically susceptible to antifungals, is of great importance for clinical practice.

Keywords: *Candida albicans*, Susceptibility, CHROMagar *Candida*, Antifungal Agent.

Introduction:

In most clinical settings, *Candida albicans* is the prevailing cause of invasive candidiasis, accounting for 50-70% of cases. In recent years, the frequency of infections with *Candida* has increased, including transplant recipients, cancer patients and other patients receiving immunosuppressive therapy[1]. *Candida* microorganisms belong to the normal microbial flora of the oral cavity, gastrointestinal tract, and the vagina. While human mucosal surfaces are usually colonized, genus members can cause a broad range of infections, characterized by different clinical manifestations and severity[2, 3]. The differentiation of *Candida albicans* and non-albicans species has been accepted for the needs of clinical practise. A shift to non-albicans species is now observed, but *C. albicans* of the most prevalent species[4-6].

The normally used antifungal drugs show significant variation in the susceptibility pattern among the types of *Candida* species. The most commonly used anti-fungals are azoles,

which act by inhibiting the enzyme lanosterol 14-alpha-demethylase dependent on cytochrome P-450. This enzyme is involved in fungal-specific membrane sterol ergosterol biosynthesis. The most commonly used agent for the treatment of Candida infections is fluconazole, a triazole group member. It has been revealed that *C. albicans* developed high-level resistance to azoles associated with the type of infection and previous fluconazole application[7].The emergence of drug resistance for *Candida* species in global scenarios has been reported in several previous studies[8, 9]. The CHROM agar medium is a simple, fast and reliable method for isolating and differentiating types of *Candida* species[10].

Thus, isolation, identification, characterization and susceptibility testing of *Candida* species in clinical specimens has become increasingly important for the management of fungal infections. The objective of this study was to isolate and identify the in vitro susceptibility of *C. albicans* isolates to antifungal agents.

Materials and Methods:

Chemicals

The laboratory uses chemicals such as CHROM agar medium, RPMI 1640 medium, peptone, dipotassium hydrogen phosphate, chromogenic mixture, chloramphenicol, yeast extract, Sabouraud dextrose agar (SDA), bovine serum albumin (BSA) agar and horse serum were obtained from Hi Medialaboratory Ltd Mumbai, India. The antifungal agents; fluconazole, ketoconazole, voriconazole and amphotericin B were obtained from local market.

Collection of Sample

A total of 20 different clinical specimens (urine, sputum and blood) were collected from Dr ShankarraoChavan Government Medical College and Suyog laboratory Nanded, Maharashtra state of India.The distribution of the isolates according to the patient specimens was as follows: Urine (8), sputum (11) and blood (1). The preliminary specimen diagnostics were performed and the isolates diagnosed to be different from *Candida albicans* were excluded from the study.

Identification and Isolation

All samples were inoculated on chloramphenicol-supplemented Sabouraud dextrose agar (SDA) slants and aerobically incubated for 24-48 h at 37°C. The specimens were analyzed by microscopy for the clinical significance of *Candida* isolates from sputum and urine, as well as for evidence of budding yeast cells with pseudohyphae and significant pus cells[11, 12]. Any visible growth seen on the SDA has been processed for the identification of the species. Microscopic examination, gram staining, and germ tube testing were carried out from an isolated colony. The pasty and creamy colony that showed gram-positive budding yeast cells with pseudohyphae on microscopic examination were further processed for *Candida* speciation on CHROM agar. The *Candida* species differentiated on the basis of the growth type and colour of the isolates on CHROM agar *Candida*[13, 14]. The colour of the colonies on CHROM agar was observed after incubation at 37°C for 24-48 h. On the basis of typical green coloured colonies on CHROMagar

Candida, the isolates were preliminarily identified. These *Candida albicans* isolates (20) were chosen for further antifungal susceptibility test studies.

Antifungal Susceptibility Testing

In vitro antifungal susceptibility testing of *Candida* isolates was performed according to the guidelines of the Clinical and Laboratory Standards Institute by broth micro-dilution method. (CLSI)[15]. By suspending 5 colonies of growth in 5 ml of sterile saline, the inoculum was prepared and the turbidity was compared to 0.5 McFarland Standard. The yeast dilution was carried out in a medium of RPMI 1640 containing a final inoculum concentration of $0.5\text{-}2.5\times10^3$ cells/ml. In RPMI-1640 medium, the various concentrations of the chosen drugs were prepared by double dilution in the 96-well plates. An inoculum of 1×10^3 cells/ml was contained in each well and the final volume of RPMI-1640 medium maintained in each well was 200 µL. The wells served as a control without adding drugs[16]. The micro plates were incubated for 48 hours at 35°C and read at 620 nm spectrophotometrically, using a micro plate reader (Multiskan-EX; Thermo Elect. Corp., USA). *C. albicans* ATCC 90028 was used as controls. The minimum inhibitory concentration (MIC) was considered the lowest concentration of the drugs that caused a 50% reduction in absorbance compared to the control.

Interpretive criteria for susceptibility to antifungal agents were as follows: for fluconazole, MICs ≤ 8 µg/ml were considered susceptible for *C. albicans*after 24 h of incubation, and MICs ≥ 64 µg/ml were resistant, 16-32 µg/ml susceptible-dose dependent (SDD). whereas the clinical breakpoints for *C. albicans* were MICs ≤ 0.125 µg/ml susceptible, 0.25–0.5 µg/ml intermediate, and ≥ 1.0 µg/ml considered resistant for ketoconazole. For amphotericin B susceptibility breakpoints were susceptible if the MICs ≤ 1.00 µg/m and MICs ≥ 2.00 µg/ml were resistant. The resistance breakpoints for antifungals were, fluconazole ≥ 64; amphotericin B ≥ 2.00; voriconazole ≥ 8.00; ketoconazole ≥ 1.00 µg/ml[15, 16].

Results:

A total of 20 isolates of *Candida albicans* were identified and isolated from 20 various clinical specimens. When distributed according to the sources, they were as; 11 (55%) isolates from sputum, 8 (40%) from urine and 01 (5%) from the blood. *C. albicans* was the most prevalent species of *Candida* isolated from sputum, urine and blood. Isolation and identification of *Candida albicans* was done by germ tube formation and carbohydrate assimilation test. The formation of germ tube was observed in horse serum at 37^0C after 2 hrs of incubation and growth and fermentation profile on various sugars in carbohydrate assimilation test confirmed the presence of *C. albicans.* Further isolation was done on CHROMagar *Candida* which shown green coloured colonies of *C. albicans*. The green-coloured colonies were isolated and tested for antifungal susceptibility study.

The antifungal susceptibility of clinical isolates of *C. albicans* shown in Table I. The results revealed that *C. albicans* were susceptible (S) to fluconazole (85%), amphotericin and

voriconazole (100%), and the lowest sensitivity shown to ketoconazole (15%) as shown in Fig. 1. *Candida albicans* showed highest sensitivity (S- 100%) to amphotericin and voriconazole. Whereas, 20% (4 isolates) and 65% of isolates were susceptible dose dependent (SDD) and resistance to ketoconazole respectively. The *Candida albicans* isolates shown 15% (3 isolates) SDD to fluconazole (MICs 16 µg/ml). out of 20 isolates, no one shown resistance to fluconazole, voriconazole and amphotericin B. The standard strain, *C. albicans* ATCC 90028, was sensitive to all antifungal drugs tested in this study. MIC values for the antifungals- fluconazole, ketoconazole, voriconazole and amphotericin B were 2, 0.125, 0.125 and 0.5 µg/ml respectively.

Table I: Antifungal susceptibility testing of 20 clinical isolates of *Candida albicans*

Isolate No	Clinical specimens	MICs in µg/ml			
		Fluconazole (0.125-64)	Amphotericin B (0.125-2)	Voriconazole (0.125-8)	Ketoconazole (0.125-1)
1	Sputum	4	0.50	0.25	0.25
2	Sputum	2	1	4	0.50
3	Sputum	1	1	0.125	0.50
4	Sputum	1	0.50	1	1
5	Sputum	16	0.125	1	0.50
6	Sputum	16	0.50	2	1
7	Urine	0.50	0.25	2	0.25
8	Sputum	1	0.125	2	0.50
9	Urine	0.25	0.25	2	0.25
10	Sputum	2	0.25	2	0.50
11	Sputum	8	0.25	1	0.125
12	Urine	16	0.125	1	1
13	Sputum	1	0.50	2	0.25
14	Urine	8	0.50	2	0.125
15	Urine	2	0.50	4	0.50
16	Urine	1	0.50	2	0.25
17	Urine	2	0.25	0.125	0.125
18	Sputum	4	0.125	4	0.50
19	Blood	1	1	4	2
20	Urine	8	0.125	2	0.50

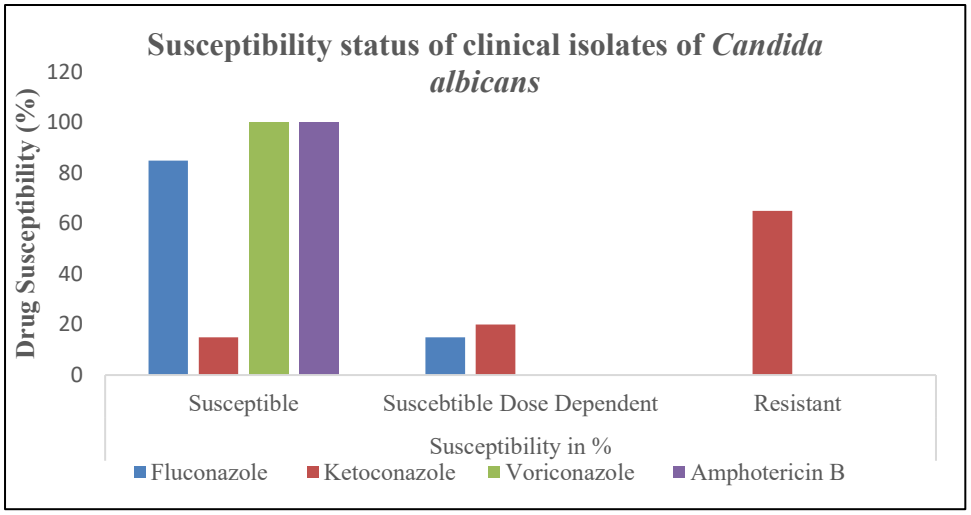

Fig. 1: Susceptibility status of all clinical isolates of *Candida albicans*

Discussion:

The current study presents information about antifungal susceptibility profiles of 20 *C. albicans* isolates, obtained from different clinical specimen. Speciation of *Candida* species by CHROMagar on the basis of colour differentiation offered a rapid, convenient and reliable method for identification of clinically important *Candida* species when compared with clumsy traditional techniques. In developing countries, CHROMagar can be taken as a simple phenotypic test alternative to molecular based assay. For the identification of *Candida* species, CHROMagar has high sensitivity and specificity[17, 18]. It facilitates mixed culture detection and identification of *Candida* species and provides results within 24-48 hours.

The data published on the resistance of fluconazole in *Candida albicans* varied widely. According to specimen types and wards, various susceptibilities were found[19].The present study found that 85% of the isolates were shown to be susceptible to fluconazole, which differs from the different studies which concluded that the *Candida* species had the greatest potential for resistance to fluconazole[20-22]. The deviation of the MIC breakpoints according to the CLSI also affects the resistance rate. Interpretive criteria are therefore important when comparing the results.

The study also found that a higher rate of resistance (65%) to ketoconazole was observed in *Candida albicans*. This high level of resistance of ketoconazole may be due to overuse of antifungal agents and also to their empiric therapy in our scenario. Our result appears to be similar to the findings of Khadka *et al.,* 86% *C. albicans* isolates were resistant to ketoconazole[23].These findings indicate the rapid increase in resistance to ketoconazole among *Candida* species and the need for speciation and susceptibility to antifungal drugs prior to antifungal therapy. The first systemic antifungal agent for the treatment of invasive fungal infections was amphotericin B and has been the drug of choice[24].As similarly demonstrated in the present study, all *Candida* isolates were sensitive to amphotericin B found in the Tseng *et al.* study[25].

Conclusion:

In conclusion, the sensitivity pattern of *Candida albicans* as shown in this study, amphotericin B, voriconazole and fluconazole with the lowest MIC appear to be appropriate empiric therapy drugs, while a few isolates have shown high resistance to ketoconazole. Detection of resistance to antifungal agents in *Candida albicans*, an alarming sign of emerging common nosocomial fungal infections. Continuous surveillance of antifungal susceptibility in clinical isolates of *Candida* species at national and international levels is needed to control the spread of resistance and to provide effective strategies for the prophylaxis and treatment of humans with fungal infections. Therefore, in order to effectively manage patients, we need to know the rates of antifungal susceptibility in each region to the available agents.

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AN OVERVIEW ON PHARMACEUTICAL SOFTWARE

Akhilesh Saini
CSE Department, RNB Global University, Bikaner (Raj.)- 334601
Corresponding author E-mail: akhilesh.saini@rnbglobal.edu.in

Abstract:

The pharmaceutical industry is experiencing rapid growth, highlighting the need to address its various scientific, production, and regulatory challenges. Innovation is essential not only for advancing drug development but also for improving compliance with Good Manufacturing Practices (cGMP) and other regulatory standards. In this context, computer-based programs and digital solutions have emerged as vital tools for efficiently monitoring and managing pharmaceutical operations and documentation. To meet the stringent requirements of regulatory authorities, pharmaceutical companies are increasingly adopting specialized software systems. This article provides an overview of pharmaceutical software, emphasizing its importance, applications, and the growing role of artificial intelligence in modern drug design and regulatory compliance.

Keywords: Pharmaceutical Software, Artificial Intelligence, Drug Design, Regulatory Compliance, cGMP.

Introduction:

Software has become an indispensable tool in the pharmaceutical industry, playing a critical role in data management, process optimization, and ensuring regulatory compliance. High quality in pharmaceutical systems is achieved through the application of structured development methodologies and rigorous Verification and Validation (V&V) protocols throughout the software development lifecycle (Mannam & Mubeen, 2018).

Modern pharmaceutical software significantly reduces the burden of routine documentation and clerical tasks for medical professionals. It enhances data accuracy, minimizes errors, reduces reliance on animals and chemicals, and increases productivity. Additionally, such software facilitates standardized processes, continuous monitoring of transactions, and provides quick and remote access to vital information (Mali, 2022).

In the digital era—where software performance is mission-critical—merely delivering functional software is no longer sufficient. Companies must now ensure consistently high-quality software solutions delivered at unprecedented speeds (Upadhyay, 2012).

In recent years, significant advancements have been made in adopting innovative methodologies to deepen our understanding of pharmaceutical processes and product development. Despite these advancements, the industry continues to face increasing pressure to improve drug safety and quality while simultaneously reducing production costs. Addressing these challenges requires the adoption of well-organized, technology-driven pharmaceutical development and manufacturing practices (R & K, 2015).

An Overview – Pharma Software

Automated Systems

An automated system integrates data input, computer processing, and information output, either for reporting or for automated control purposes. These systems refer to regulated patterns of interrelated activities and processes that operate in coordination to produce organized and functional outcomes (Hoffmann, 1998). In the pharmaceutical context, such systems streamline operations, enhance precision, and support real-time monitoring and decision-making.

Digitalization in the Pharmaceutical Industry

Digitization represents a transformative step in optimizing industrial production processes. Within the pharmaceutical industry (PI), digitalization encompasses the adoption of robotics, automation solutions, and software-based systems, leading to enhanced productivity, operational efficiency, cost savings, and improved adaptability to market changes.

Despite these advantages, the PI has traditionally been slow to embrace digital transformation due to its reliance on established procedures and the inherently complex nature of drug development and manufacturing. However, with the rising demand for both conventional and novel therapeutics, there is an urgent need to accelerate digital integration across the sector.

Contract Development and Manufacturing Organizations (CDMOs), in particular, face unique challenges in this transformation journey. The digitalization of pharmaceutical operations—especially within CDMOs—must be meticulously aligned with the core principles of Good Manufacturing Practices (GMP) to ensure quality, compliance, and reliability (Hole, Hole, & McFalone-Shaw, 2021).

Compliance Criteria for Computerized System Validation

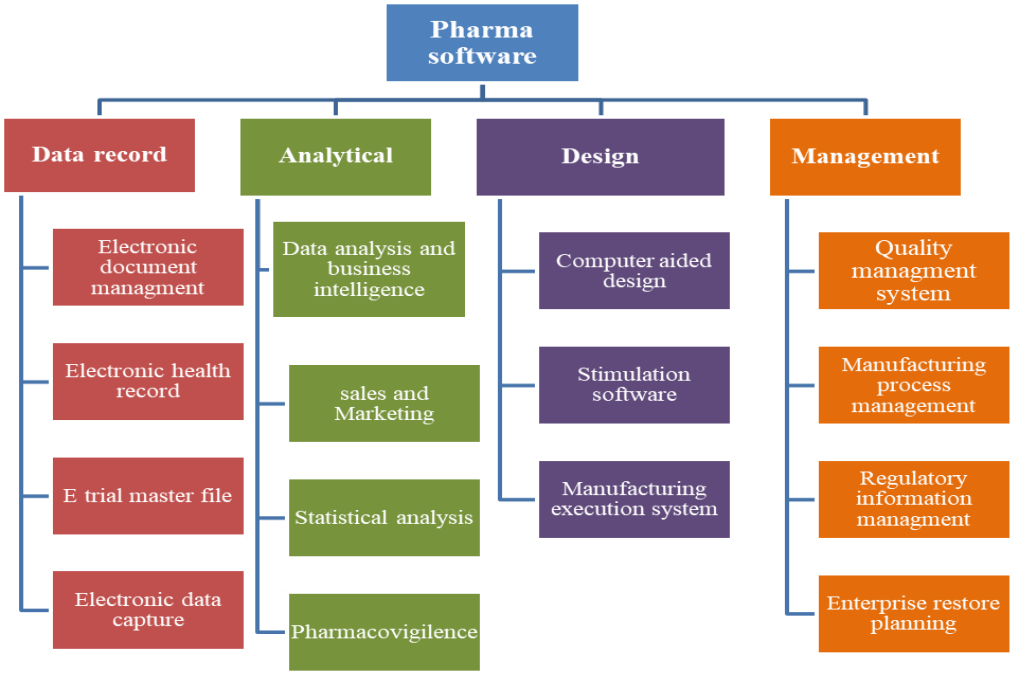
The importance of validation in pharmaceutical software systems cannot be overstated. A U.S. FDA analysis of 3,140 medical device recalls (1992–1998) revealed that 242 were due to software issues, with 192 linked to post-deployment failures. These findings underscored the necessity of robust system testing and quality-centric software engineering practices.

In response, the FDA mandated computerized system validation under the Federal Register (October 7, 1996; effective June 1, 1997), to establish control over software quality. Software validation in medical and pharmaceutical applications is essential to ensure patient safety, data integrity, and compliance with regulatory standards. It plays a pivotal role in the lifecycle of healthcare products, from development to deployment.

Classification of Software in Pharmaceutical Industry:

Types of Software Used in Pharmaceutical Manufacturing

In the pharmaceutical industry, a wide range of software applications are employed to support diverse functions such as drug development, regulatory compliance, manufacturing, and marketing. Each software category addresses specific operational challenges and enhances efficiency, accuracy, and data integrity.



1. Electronic Data Capture (EDC)

EDC software is used in clinical research to collect and manage data from clinical trials electronically, replacing traditional paper-based systems that are time-consuming and error-prone. These systems enable real-time data collection directly from participants, enhancing trial efficiency and data accuracy. Commonly integrated with Clinical Trial Management Systems (CTMS) and Clinical Data Management Systems (CDMS), EDC software improves the quality and speed of clinical trials.

Examples:

- Oracle Health Sciences Clinical
- Medidata CTMS
- BioClinica CTMS
- Veeva Vault CTMS (cloud-based)
- Clinical Conductor CTMS

2. Electronic Document Management Systems (EDMS)

EDMS software manages electronic documents related to drug development and manufacturing, including SOPs, regulatory submissions, and quality documentation. It ensures controlled access, version control, and compliance with regulatory standards.

Examples:

- Veeva Vault
- Documentum
- OpenText
- SharePoint
- MasterControl

3. Pharmacovigilance Software

Pharmacovigilance software is used to monitor and report adverse drug reactions (ADRs) and other safety data. As part of post-marketing surveillance (Phase IV), this software ensures regulatory compliance and patient safety by managing safety-related data effectively.

Examples:

- Oracle Argus
- ArisGlobal Safety
- VigiFlow
- AB Cube
- PVWorks

4. Enterprise Resource Planning (ERP) Systems

ERP systems streamline organizational functions such as inventory control, procurement, financial operations, and supply chain management. They provide a centralized platform for better resource planning and process integration.

Examples:

- SAP
- Oracle ERP
- Microsoft Dynamics
- Infor ERP
- Epicor ERP

5. Computer-Aided Drug Design (CADD) Software

CADD software supports the development and optimization of drug molecules by simulating their interactions with biological targets. It enhances drug efficacy, selectivity, and bioavailability while reducing toxicity.

Examples:

- Schrödinger
- MOE (Molecular Operating Environment)
- Discovery Studio
- OpenEye
- Accelrys

6. Manufacturing Execution Systems (MES)

MES software facilitates real-time monitoring and control of manufacturing operations. It supports batch processing, equipment management, and quality assurance, helping optimize production efficiency.

Examples:

- Siemens Opcenter
- SAP Manufacturing Execution
- Wonderware MES

- ABB Ability Manufacturing Operations Management
- Rockwell Automation FactoryTalk

7. Quality Management Systems (QMS)

QMS software ensures consistent product quality by managing documents, deviations, audits, and changes throughout the product lifecycle. It plays a critical role in meeting compliance and regulatory standards.

Examples:

- MasterControl
- EtQ Reliance
- SAP QM
- AssurX
- Pilgrim Quality Solutions

8. Regulatory Information Management (RIM)

RIM software is used to manage regulatory submissions and ensure compliance with agencies such as the FDA. It facilitates product registration, electronic submissions, and document tracking.

Examples:

- ArisGlobal RIMS
- Veeva Vault RIM
- ISI Regulatory Suite
- Samarind RMS
- Lorenz Docubridge

9. Electronic Trial Master File (eTMF) Software

eTMF software digitally manages documents required for clinical trials. It replaces traditional paper-based TMFs, enhancing accessibility, compliance, and documentation quality.

Examples:

- MasterControl eTMF
- SureClinical eTMF
- Phlexglobal PhlexEview
- TMF Connect

10. Statistical Analysis Software

Used to analyze preclinical and clinical data, statistical software enables researchers to identify patterns, trends, and correlations for data-driven decision-making.

Examples:

- SAS (Statistical Analysis System)
- SPSS
- STATA
- MedCalc

11. Simulation Software

Simulation software models drug behavior and manufacturing processes to optimize design, predict outcomes, and reduce trial-and-error in experimentation.

Examples:

- GastroPlus
- Simcyp
- Arena
- ADAPT
- Virtual Cell
- MATLAB

12. Sales and Marketing Software

These tools manage CRM, market analytics, and sales forecasting. They support targeted marketing strategies and enhance customer engagement.

Examples:

- Veeva CRM
- Salesforce Health Cloud
- Marketo
- Adobe Experience Manager
- SAP Sales Cloud
- QlikView

13. Data Analytics & Business Intelligence (BI) Software

BI software is used to interpret complex datasets from clinical trials, manufacturing, and sales. It supports strategic decision-making through actionable insights.

Examples:

- Tableau
- SAP BusinessObjects
- SAS
- IBM Cognos Analytics
- Microsoft Power BI
- Oracle BI

14. Electronic Health Records (EHR) Software

EHR systems store and manage patient health data, including medical history, test results, and prescriptions. They are also used in clinical research for patient tracking and data collection.

Examples:

- OpenClinica
- Epic
- Cerner
- CareCloud

- Athenahealth

15. Manufacturing Process Management (MPM) Software

MPM software oversees the complete pharmaceutical manufacturing workflow—from raw material sourcing to final product distribution—ensuring efficiency and regulatory compliance.

Examples:

- SAP Manufacturing Execution
- Camstar Medical Device Suite
- MasterControl Manufacturing Excellence
- Apriso MES
- Werum PAS-X (Joshi & Salunkhe, 2020)

Formulation Optimization Using Pharmaceutical Software

Traditional formulation approaches often lack precision and efficiency. To overcome these limitations, the use of Experimental Design (DoE – Design of Experiments) has become vital. Optimization in pharmaceutical formulation aims to derive the most effective composition by analyzing assessment data, choosing optimal materials, and improving dosage forms.

This methodology is applied in the development of advanced drug delivery systems, including sustained-release tablets, liposomes, nanoparticles, and more. The software Design Expert is widely utilized for such optimization tasks—not only in pharmaceuticals but also in other fields like biofuel production (Ramadhani *et al.,* 2017).

A review of 63 articles (2011–2020) focusing on formulation using Design Expert software found 51 that met inclusion criteria, demonstrating the software's broad adoption and critical role in formulation development (BS, R, & N, 2008).

Design Expert

Developed by Stat-Ease in 1996, *Design-Expert* is a statistical software application primarily used to facilitate experimental design and optimization in formulation development. The software supports comprehensive analysis by identifying and evaluating key variables involved in experiments. It offers three major study paths tailored to different experimental design stages: screening**,** characterization, and optimization.

- **Screening** involves the fewest experimental runs and is employed when multiple factors are present but their significance is uncertain. This phase uses two-level designs to identify major effects without considering interactions. It helps narrow down the variables requiring further investigation.
- **Characterization** includes a greater number of experimental runs and is suitable when working with a limited number of variables. This phase focuses on determining the significance of individual factors and their interactions using a two-factor interaction (2FI) model.

- **Optimization** demands the highest number of experimental runs but yields the most detailed information. Once critical factors have been identified, this phase seeks to fine-tune their levels to achieve desired responses.

Each phase can be executed using one of three DOE strategies: factorial/response surface**,** mixture, or combined designs. These methods enable researchers to select the most appropriate approach based on the nature of the experiment and the optimization objectives.

Design of Experiment (DOE) Techniques in Formulation OptimizationVarious statistical designs under the Design of Experiment (DOE) framework are widely used for optimizing pharmaceutical formulations. One of the most common approaches is the Factorial Design, which employs regression equations to analyze the relationship between one or more independent variables (factors) and the response variable. This design helps explore how different experimental conditions influence outcomes and identify potential interactions among variables. The three essential components of factorial design include factors (quantitative like concentration or qualitative like polymer type), levels (specific values assigned to each factor), and effects (changes observed due to varying conditions) (S & C., 2004).

The Central Composite Design (CCD) is a key method within Response Surface Methodology (RSM), particularly effective when both the optimal response and path to optimization are unknown. CCD allows for rotatable designs and supports multiple model types—mean, linear, quadratic, 2FI, and cubic. The selection of the appropriate response model is based on statistical criteria similar to those used in mixture designs, with the desirability function guiding the identification of optimal settings. A desirability value close to 1 indicates an outcome that closely aligns with the desired result (Montgomery, 2019).

Another efficient RSM design is the Box-Behnken Design (BBD), often employed when three independent variables are involved. It is considered more resource-efficient than CCD due to requiring fewer experimental runs while maintaining strong predictive capability for both linear and quadratic models. This makes BBD suitable for precise determination of optimal conditions with minimal resource expenditure (Perincek & Colak, 2013; I, R, & IS, 2022).

Mixture Designs are uniquely suited for experiments in which the independent variables are proportions of components that together make up a constant whole, such as 100% of a formulation by weight. These designs enable optimization by systematically varying the proportions of components while maintaining a fixed total. The optimal formulation is determined by evaluating which mixture yields responses that meet predefined quality parameters across all criteria.

Finally, the Combined Design integrates elements of factorial/RSM and mixture designs into a single experimental strategy. This hybrid approach allows researchers to simultaneously investigate the influence of both compositional variables and process parameters, providing a comprehensive understanding of the system under study.

Drug Delivery Systems and Predictive Accuracy Using Design Expert Software

Design Expert software is not limited to optimizing traditional dosage forms such as tablets, capsules, and emulsions; it has also been widely applied in the development of advanced drug delivery systems. Numerous studies have demonstrated its effectiveness in designing and optimizing novel delivery mechanisms that enhance therapeutic outcomes and patient compliance.

A key performance metric in evaluating the software's predictive capabilities is the Percent Prediction Error (PPE). This statistic measures the accuracy of a model's predictions in relation to actual experimental outcomes. An acceptable prediction error is typically considered to be below 4%. In relevant studies, the PPE for dependent variables has consistently ranged between 2% and 3.5%, thereby confirming the reliability and applicability of the software in pharmaceutical formulation and delivery system optimization (Usman, Ejaz, & Safdar, 2018).

Market Outlook for Pharmaceutical Software Solutions

The global market for pharmaceutical quality management software was valued at approximately USD 1.5 billion in 2022 and is projected to grow at a compound annual growth rate (CAGR) of 12.58% from 2023 to 2030. This significant growth is driven by several factors, including the increasing integration of digital technologies, the rising complexity of pharmaceutical supply chains, and the critical need for regulatory compliance.

Moreover, the escalating costs associated with drug manufacturing have intensified the demand for automated, software-driven solutions to streamline operations and reduce inefficiencies. According to the Congressional Budget Office (2021), the cost of developing a new drug—including expenditures on failed drug candidates—can range from under USD 1 billion to over USD 2 billion, underscoring the need for advanced software systems that enhance productivity and decision-making across the pharmaceutical development lifecycle.

Application of Design Expert Software in Drug Delivery Systems

Design Expert software plays a pivotal role not only in optimizing conventional dosage forms such as tablets, capsules, and emulsions but also in the development of advanced drug delivery systems. A growing body of research demonstrates the software's utility in designing, analyzing, and optimizing complex pharmaceutical delivery methods, enabling more efficient formulation development and process validation.

Prediction Accuracy and Error Analysis

An important metric for evaluating the performance of analytical and predictive models in pharmaceutical research is the Percent Prediction Error. This statistic quantifies how closely a model's predictions align with observed outcomes. Generally, a prediction error below 4% is considered acceptable. In several studies, including those by Usman, Ejaz, and Safdar (2018), prediction error values for dependent variables were found to range between 2% and 3.5%. These results affirm the accuracy and robustness of the model, validating its reliability for assessing formulation outcomes and guiding experimental design.

Market Outlook for Pharmaceutical Quality Management Software

The global market for pharmaceutical quality management software is experiencing substantial growth. As of 2022, it was valued at approximately USD 1.5 billion and is projected to grow at a compound annual growth rate (CAGR) of 12.58% from 2023 to 2030. This expansion is driven by several key factors, including increased integration of digital technologies in pharmaceutical operations, the imperative for regulatory compliance, and the growing complexity of pharmaceutical supply chains. Additionally, the rising costs of drug development, which the Congressional Budget Office (2021) estimates to range from less than USD 1 billion to over USD 2 billion per drug (including failed development costs), further underscore the need for advanced software solutions that enhance operational efficiency and reduce development risks.

Graphical representation of market share of pharmaceutical management software market:

(Pharmaceutical Quality Management Software Market Size, Share & Trends Analysis Report By Application (Data Management, Risk Management), By Deployment Mode (Cloud & Web-based, On-premise), By Region, And Segment Forecasts, 2023 - 2030, 2018 - 2021).

Pharmaceutical Quality Management Software Market Summary (2023–2030)

Parameter	**Details**
Market Size (2023)	USD 1.7 Billion
Forecasted Market Size (2030)	USD 3.9 Billion
CAGR (2023–2030)	12.58%
Base Year	2022
Historical Data	2018–2021

Forecast Period	2023–2030
Quantitative Metrics	Revenue (USD million/billion), CAGR (%)
Report Coverage	Revenue forecast, company ranking, competitive landscape, growth factors
Market Segments	By Application, By Deployment Mode, By Region
Geographic Scope	North America, Europe, Asia Pacific, Latin America, Middle East & Africa
Key Countries	U.S., Canada, UK, Germany, France, India, China, Japan, Brazil, South Africa
Major Companies	MasterControl, AmpleLogic, Qualio, Pilgrim (IQVIA), Sparta, Veeva Systems
Customization Options	Yes (equivalent to 8 analyst working days)
Purchase Flexibility	Custom pricing and regional/segment-specific purchases available

Artificial Intelligence in the Pharmaceutical Industry

The pharmaceutical industry is a vital sector dedicated to preserving human life through constant innovation and the adoption of advanced technologies. This drive for progress is particularly evident during global health emergencies, such as the recent COVID-19 pandemic (Krikorian & Torreele, 2021). Innovation in this field extends across various domains—including production processes, packaging technologies, and patient-centric marketing strategies—supported by intensive research and development efforts (Chavda *et al.,* 2023).

Pharmaceutical advancements include a wide range of formulations, from traditional small molecules to complex biologics, all aimed at addressing unmet clinical needs. A key focus is on enhancing the stability, safety, and efficacy of these compounds. However, high toxicity levels associated with new drug candidates remain a critical challenge, demanding thorough investigation. The overarching goal is to develop pharmaceutical products that are both therapeutically effective and feasible for integration into healthcare systems.

Despite its transformative potential, the pharmaceutical sector faces significant challenges that require accelerated innovation through technology-driven approaches, particularly Artificial Intelligence (AI) (Scannell *et al.,* 2012; Munos, 2009; Mak & Pichika, 2018).

Current Pharmaceutical Challenges and the Role of AI

In recent years, challenges in the pharmaceutical industry have intensified. Beyond the complexities of treating rare diseases, new small-molecule drugs often encounter generic competition, leading to increased economic pressures and the need for costly clinical validation. Consequently, companies are driven to innovate continually. Meanwhile, the biologics sector is

rapidly evolving to address the limitations of traditional small-molecule approaches. The pharmacological activity of small molecules is largely influenced by their molecular structure and chemical reactivity (Dickherber *et al.,* 2015; Colombo *et al.,* 2018).

To meet these evolving demands, AI has emerged as a powerful tool in drug discovery, development, and delivery. It aids in identifying drug targets, optimizing formulations, and predicting therapeutic outcomes. However, while AI significantly enhances data processing capabilities, it does not entirely replace human expertise. The complexity of biological data often necessitates human interpretation, especially when AI-generated outcomes are ambiguous or uncertain.

One notable limitation of AI is algorithmic bias, which can affect prediction accuracy and hypothesis testing. Moreover, AI-driven docking simulations frequently yield inactive or non-viable molecules, underscoring the need for expert validation in drug candidate selection (Cerón-Carrasco, 2022).

Machine Learning Approaches in Pharmaceutical AI

AI applications in pharmaceutical research are commonly built upon machine learning (ML) techniques, which are broadly categorized into:

1. Supervised Learning

Supervised learning involves training algorithms on labelled datasets where both inputs and expected outputs are known. It is widely used in areas such as:

- **Image recognition** (e.g., analyzing microscopy or diagnostic images)
- **Natural language processing** (e.g., analyzing clinical trial records or literature)
- **Predictive modelling** (e.g., forecasting patient responses)

The two primary tasks in supervised learning are:

- **Classification**: Assigning data into categories (e.g., disease diagnosis)
- **Regression**: Predicting continuous outcomes (e.g., drug concentration levels)

This approach enables pharmaceutical researchers to model complex relationships between molecular features and therapeutic outcomes (Sarker, 2022).

2. Unsupervised Learning

In contrast, unsupervised learning deals with unlabelled datasets. Its goal is to uncover hidden patterns or groupings within data, such as clustering similar compounds or patient profiles. It is useful for exploratory data analysis, drug repurposing, and detecting novel associations that may not be immediately apparent through traditional methods (Sarker, 2021).

Applications of Supervised AI Learning in Pharmaceuticals

1. **Drug Discovery and Design**

   Supervised learning systems play a vital role in predicting the activity or characteristics of potential drug candidates. By training on datasets containing known compounds and their corresponding biological activities, these models learn underlying molecular

patterns and relationships. This facilitates the prediction of efficacy, potential side effects, or bioactivity of new molecules, thereby accelerating the drug design and development process (Dara *et al.,* 2022).

2. **Predictive Maintenance and Quality Control**

   In pharmaceutical manufacturing, supervised learning is employed for predictive maintenance and quality assurance. Trained on historical datasets derived from sensor readings, manufacturing parameters, or quality testing results, these models can forecast equipment failures, deviations in product quality, and process anomalies. This leads to proactive maintenance strategies and improved compliance with quality standards (Kavasidis *et al.,* 2023).

3. **Drug Target Identification**

   Supervised learning is useful in identifying therapeutic targets by analysing biological datasets containing genetic, proteomic, or transcriptomic information. These algorithms learn patterns that associate certain biological features with treatment outcomes or disease progression, allowing researchers to pinpoint potential targets for drug action (Bagherian *et al.,* 2021).

4. **Disease Diagnosis and Prognosis**

   Supervised algorithms can assist clinicians by diagnosing diseases or predicting patient outcomes. Trained on labelled datasets containing clinical information, patient demographics, and outcomes, the models can classify patients into disease categories and forecast prognosis or treatment responses, thereby supporting personalised medicine approaches (Kumar *et al.,* 2023).

5. **Adverse Event Detection**

   In the field of pharmacovigilance, supervised models are trained on adverse event data to detect and classify potential drug-related safety signals. These models identify recurring patterns and flag unusual side effects, aiding regulatory bodies and pharmaceutical companies in their safety monitoring efforts (Chapman *et al.,* 2019).

6. **Predictive Modelling for Clinical Trials**

   Historical clinical trial data, including patient characteristics, treatment regimens, and trial outcomes, can be used to train supervised models. These models help predict patient responses, treatment efficacy, and safety issues, ultimately improving trial design and patient recruitment strategies (Elkin & Zhu, 2021).

Applications of Unsupervised AI Learning in Pharmaceuticals

Unsupervised learning algorithms operate without labelled data and aim to uncover hidden structures or patterns within datasets. These approaches are especially useful for exploratory data analysis, clustering, dimensionality reduction, and anomaly detection in pharmaceutical research.

1. **Clustering**

   Clustering algorithms identify natural groupings within datasets by assessing similarities among data points. In pharmaceutical contexts, clustering can be used on gene expression profiles, chemical structures, or patient information to reveal subpopulations, classify compounds, or identify phenotypic patterns. This facilitates target discovery and patient stratification (Karim *et al.,* 2021).

2. **Dimensionality Reduction**

   Techniques like Principal Component Analysis (PCA) and t-Distributed Stochastic Neighbour Embedding (t-SNE) reduce the number of variables in large datasets while preserving essential patterns. This aids in data visualisation, feature selection, and simplification of complex datasets such as gene expression data or drug screening results, enhancing interpretability (Vamathevan *et al.,* 2019).

3. **Anomaly Detection**

   Unsupervised anomaly detection models, such as the Local Outlier Factor (LOF) and Isolation Forest, are adept at identifying rare or unexpected data points. In pharmaceutical applications, this can highlight potential data integrity issues, rare adverse events, or unusual clinical trial outcomes, thereby enhancing safety and quality assurance (Goldstein & Uchida, 2016).

4. **Association Rule Mining**

   Using algorithms like Apriori, association rule mining discovers meaningful relationships or patterns among items in datasets. In the pharmaceutical domain, it can be applied to analyse co-occurrence of drug usage, drug-drug interactions, and common adverse events. This supports decision-making in drug safety and therapy optimisation (Noguchi *et al.,* 2018).

5. **Topic Modeling**

   Topic modelling algorithms such as Latent Dirichlet Allocation (LDA) are used to uncover latent themes in large collections of text. These methods are valuable for analysing research articles, clinical trial documents, or patient reviews to detect trends, emerging topics, or sentiment. This supports competitive intelligence, literature synthesis, and public health analysis (Liu *et al.,* 2016; Zhao *et al.,* 2015).

AI for Drug Discovery

Artificial Intelligence (AI) has significantly revolutionized drug research and development by enhancing efficiency, precision, and speed across various stages of the pharmaceutical pipeline. The key applications of AI in drug discovery include:

1. Target Identification

AI systems are capable of analysing diverse data types—such as genomic, proteomic, and clinical datasets—to identify potential therapeutic targets. By uncovering disease-associated

genes, molecular pathways, and protein interactions, AI facilitates the discovery of biological mechanisms that can be modulated for therapeutic intervention.

2. Virtual Screening

AI enables high-throughput virtual screening of extensive chemical libraries to identify promising drug candidates with strong binding affinities to specific biological targets. Through the simulation of molecular interactions and prediction of binding strengths, AI helps prioritize compounds for experimental testing, thereby saving both time and resources.

3. Structure-Activity Relationship (SAR) Modeling

Machine learning models are adept at establishing correlations between the chemical structures of compounds and their biological activities. This assists researchers in designing molecules with improved pharmacological profiles, including enhanced potency, selectivity, and optimized pharmacokinetic properties.

4. De Novo Drug Design

Advanced AI techniques, such as reinforcement learning and generative models, are capable of proposing novel chemical structures with drug-like characteristics. By leveraging data from chemical libraries and experimental results, AI expands the chemical space and supports the innovation of entirely new therapeutic candidates.

5. Optimization of Drug Candidates

AI algorithms aid in the optimization of lead compounds by evaluating multiple parameters simultaneously—such as efficacy, safety, absorption, distribution, metabolism, and excretion (ADME). This multi-objective analysis supports the refinement of drug candidates to achieve maximum therapeutic benefits while minimizing adverse effects.

6. Drug Repurposing

AI-driven analysis of biomedical data can identify new therapeutic uses for existing approved drugs. By detecting previously unexplored associations between drugs and diseases, AI accelerates the drug discovery timeline and significantly reduces development costs through the repositioning of known medications.

7. Toxicity Prediction

AI can predict the toxicity profiles of chemical entities by examining their structural and physicochemical properties. Machine learning models trained on toxicology datasets can flag potentially harmful compounds early in the drug development process, enabling researchers to prioritize safer candidates for further testing.

In conclusion, AI-powered methodologies are reshaping the landscape of drug discovery and development. These technologies not only expedite the identification and optimization of drug candidates but also reduce research costs and improve overall success rates, thereby enhancing the efficiency and effectiveness of pharmaceutical innovation (Shah, Chavda, & Soniwala, 2023).

AI Tool Application in Dosage Form Design

Artificial Intelligence (AI) is revolutionising pharmaceutical formulation by introducing automation and precision across various stages of dosage form design. AI tools enable more accurate estimations, enhanced data processing, and continuous process refinement. By aggregating data from multiple sources—such as molecular information, patient profiles, and pharmacokinetic parameters—AI can suggest optimal drug delivery mechanisms tailored to individual patient needs.

AI plays a significant role not only in drug discovery but also in drug repurposing by identifying new therapeutic applications for existing compounds. This is particularly relevant in patient-centric formulation design, where patient-specific conditions influence pharmacokinetics and therapeutic outcomes. Modern AI systems analyse complex datasets to support rational drug design, assess drug behaviour, and evaluate pharmacokinetic profiles in silico. These tools enhance the reliability of preclinical models, particularly when simulating drug-membrane interactions, which are often difficult to study experimentally (Balogh, Müller, & Könczöl, 2013).

AI in Drug Delivery

The fusion of AI and big data analytics has led to the emergence of computational pharmaceutics—a discipline that applies multiscale modelling techniques to optimise drug delivery systems. AI-driven models reduce reliance on trial-and-error by predicting drug performance through correlations between physicochemical properties, formulation components, and physiological responses. These tools can anticipate in vitro release profiles, stability, and drug efficacy, helping researchers identify formulation risks at early stages and proactively implement corrective measures. As a result, development timelines are shortened, costs are reduced, and the overall success rate of drug candidates is improved (Lou, Lian, & Hageman, 2021).

AI Applications in Parenteral, Transdermal, and Mucosal Drug Delivery

AI technologies are increasingly used in designing complex dosage forms such as injectables, biologics, and advanced transdermal and mucosal systems. AI can model and predict physicochemical attributes—such as pH, solubility, viscosity, and stability—based on formulation excipients and processing parameters. This improves product homogeneity and reduces batch failure rates.

In parenteral formulations, AI systems can monitor real-time production data, identify process variables affecting product quality, and propose optimisations. This promotes compliance with Good Manufacturing Practice (GMP) standards by supporting traceability and reducing deviations (Mohan, Kamaraj, & Navyaja, 2022).

Machine learning (ML) algorithms are also used to predict in vitro drug release for long-acting injectables (LAIs), ocular, pulmonary, and transdermal systems. Unlike conventional trial-

and-error approaches, AI provides deeper insights through in silico simulations, enabling a rational, data-driven formulation design. Overcoming data limitations and promoting interdisciplinary collaboration will further enhance AI's impact in this domain (Daka & Peer, 2012; Turchin, Masharsky, & Zitnik, 2023; Das, Preuss, & Mazumder, 2016).

Limitations of AI Tools in Pharmaceuticals

Despite their transformative potential, AI tools face several limitations:

- **Lack of Transparency:** The "black-box" nature of many AI models makes it difficult to gain regulatory approval, as the logic behind predictions can be opaque (Kiseleva, Kotzinos, & Hert, 2022; Kelly *et al.,* 2019).
- **Limited Data Availability:** For rare diseases or niche drug products, insufficient data can impair model accuracy and introduce bias.
- **Bias in Training Data:** If training datasets are non-representative or incomplete, resulting models may yield skewed or unreliable predictions, especially in heterogeneous patient populations.
- **Updating Challenges:** AI models are often difficult to retrain or update with new data, limiting adaptability in fast-evolving fields like drug development.
- **Limited Variability Handling:** AI models may generalize to the mean, making them less effective for patients who deviate from average responses (e.g., cancer therapeutics) (Thomas *et al.,* 2021).
- **Interpretability:** Many models produce complex outputs that are hard to understand, limiting their utility in clinical settings.
- **Ethical Issues:** AI systems trained on sensitive health data raise concerns about privacy, consent, and data protection.
- **Biological Complexity:** AI struggles to fully mimic dynamic biological systems due to their intricate feedback loops and multiscale interactions (Eslami *et al.,* 2022).
- **Lack of Clinical Intuition:** Statistical models often miss contextual clinical reasoning, which is vital for patient-specific treatment decisions (Davenport & Kalakota, 2019).
- **Inactive Molecule Prediction:** Molecular docking algorithms may fail to capture conformational flexibility, leading to misclassification of active or inactive compounds (Cerón-Carrasco, 2022).

Emerging Trends in AI for Pharmaceuticals

AI is gaining momentum across the pharmaceutical landscape, with major trends including:

1. **Drug Discovery & Development:** AI accelerates discovery by enabling virtual screening, predictive analytics, and molecular simulations.
2. **Precision Medicine:** AI helps stratify patient populations, forecast treatment responses, and personalise therapies based on multi-omic and clinical data.

3. **Drug Repurposing:** Algorithms identify alternative uses for approved drugs, saving time and resources.
4. **Formulation & Delivery:** AI models predict release kinetics and absorption patterns, optimising dosage forms for targeted therapy.
5. **Clinical Trial Optimisation:** AI improves trial design, patient recruitment, and real-time monitoring.
6. **Regulatory Compliance:** AI supports post-market surveillance, safety signal detection, and adherence to quality standards.
7. **Supply Chain Efficiency:** AI streamlines inventory, production, and distribution processes, ensuring consistent product availability.

Companies such as GNS Healthcare, AstraZeneca, Atomwise, Recursion.Pharmaceuticals, and Insilico Medicine are at the forefront of integrating AI into their drug development pipelines.

A Progressive Perspective

AI is poised to significantly accelerate pharmaceutical innovation in the coming years. Virtual screening tools can scan vast chemical spaces to identify promising leads, while AI-powered pharmacokinetic models help optimise dosing regimens and delivery strategies.

Despite growing adoption, AI applications in real-world therapeutic settings remain limited. Several machine learning approaches—such as k-nearest neighbours, decision trees, random forests, logistic regression, SVMs, ANN, and Feedback System Control (FSC)—have been explored for infectious disease drug delivery but lack clinical validation or mainstream adoption (Pokhriyal, Chavda, & Pathak, 2023; Levin *et al.,* 2018; Chen & Decary, 2020; Sun *et al.,* 2003; Magill *et al.,* 2023).

Nevertheless, as data accessibility improves and interdisciplinary collaboration grows, AI will increasingly bridge the gap between experimental insight and therapeutic application—bringing personalised, efficient, and safe drug solutions to the forefront.

Conclusion:

Software solutions have become indispensable tools in the pharmaceutical industry, transforming traditional operations into streamlined, data-driven processes. Their integration has ushered in a paradigm shift, enhancing productivity, operational efficiency, and accuracy across all stages of the pharmaceutical value chain. From initial drug discovery and clinical trials to regulatory compliance and quality control, software applications now play a pivotal role in ensuring optimized outcomes.

Computational pharmaceutics—fueled by artificial intelligence and expansive datasets—has revolutionized drug delivery systems by offering more efficient, cost-effective, and personalized approaches. These innovations enable the fine-tuning of drug formulations, development of patient-specific therapies, and improved adherence to regulatory standards.

Moreover, they contribute significantly to risk mitigation and decision-making processes throughout drug development.

Ultimately, the adoption of advanced software and AI-driven technologies fosters not only better manufacturing practices but also improved therapeutic efficacy and patient outcomes. The future of pharmaceutical development lies in continued innovation through intelligent, data-centric methodologies.

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ANTIMICROBIAL PROPERTIES OF SOME SIGNIFICANT SPICES: A REVIEW

Mohd Zishan and Uzma Manzoor

Mohd Zishan[1] and Uzma Manzoor[2]

[1]Department of Agricultural Sciences, Sharda University, Greater Noida, India

[2]Department of Agriculture, Galgotias University, Greater Noida, India

Corresponding author E-mail: zishanmohd357@gmail.com, uzmamanzoor52@gmail.com

Abstract:

Spices have been used to preserve food and their medicinal utility since ancient times. These are used to enhance the taste, quality, flavour, aroma and colour of the food. These can be capable as an alternative source of chemical preservatives. The most common spices are garlic, onion, clove, fennel, coriander, turmeric, black cumin, bay leaf, thyme, turmeric, cinnamon, asafoetida, chili powder, ginger and black pepper etc. These possess antimicrobial activity against bacterial and fungal pathogens such as *Staphylococcus aureus, methicillin -resistant S. aureus, Escherichia coli, Enteropathogenic E. coli, Enterococcus faecalis, Pseudomonas aeruginosa, Proteus mirabilis, Bacillus cereus, Bacillus pumilus, Listeria monocytogenes, Salmonella typhimuricum, Shigella dysentriae, Proteus mirabilis, Yersinia enterocolitica, Klebsiella pneumoniae, Streptococcus pneumoniae,* and *Aspergillus niger,* etc. Most spices are very effective against food-borne pathogens. These are very essential in the manufacturing of Ayurvedic, Unani and Homeopathic systems of medicines. Most of the world population depends on botanical formulation for physical, sexual and mental health problems because these are cheap, effective, and easily available and have less or no side effect.

Keywords: Antimicrobial Activity, Food-Borne, Inhibition, Pathogens, Spice.

Introduction:

The spices have been used since ancient times to improve the quality of food, taste, colour and preservation. The spices are essential elements of human food. These are used as preservative, for medicinal purpose and enhancement of flavour [1,2]. These have antimicrobial properties so; therefore, these can be used as an alternative to chemical additives in food [3]. These are the parts of plants and plant substances such as flowers, fruits, seeds, barks, leaves, rhizomes, and stems etc. The common spices are coriander, garlic, onion, nutmeg, cardamom, ginger, mint, thyme, black cumin, clove, turmeric, red chilli, black paper, cinnamon, cumin seeds, fennel, oregano, and asafoetida etc. About 80% population of the world trust on botanical products for their health issues [4]. Currently spices are used in the formulation of medicines such as Ayurveda, Unani and Homeopathy. The alternative of chemical preservatives is highly confidential as a natural substance consequence these substances are tolerable to human health and essential leads for food corporate [5]. The spices have lot of medicinal properties such as anti-inflammatory, antiseptic, antispasmodic, antibacterial, antifungal, antiemetic, analgesic,

aphrodisiac, cardiac stimulant, carminative, refrigerant, appetizer, thermogenic, anthelmintic, digestive, stomachic, use in asthma, cough, bronchitis, liver disorder, skin disorder, dental caries, and vomiting etc. The antimicrobial potential of spices has been reported, explained and used as medicines for many centuries. Several spices have antimicrobial properties such as Cinnamon (*Cinnamomum verum*), Turmeric (*Curcuma longa*), Clove (*Syzygium aromaticum*), Black pepper (*Piper nigrum*) and Ajwain (*Trachyspermum ammi*). Spices comprise antimicrobial phytochemical substances and having medicinal values [6]. They have phytochemicals such as alkaloids, flavonoids, iso-flavonoids, coumarins, tannins, phenolic and glycosides etc. They have high economic worth for their contribution in cosmetics, perfumes, nutritional foodstuff and medicines [7].

Cinnamon (*Cinnamomum verum*) Family: Lauraceae

The antimicrobial potential of cinnamon essential oil was assessed against five foodborne pathogenic microbes such as *Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium, Escherichia coli* and *Candia albicans*. Cinnamon essential oil exhibited potentially effective antimicrobial activity against selected foodborne pathogenic microbes [8]. The antimicrobial activity of crude cinnamon ethanol extract observed against four orofacial pathogenic microbes such as *S. aureus, E. coli, Streptococcus mutans* and *Peptococcus*. These pathogenic microbes were inhibited from ethanol extract. The maximum zone of inhibition has been performed against *S. aureus*, 29mm at the concentration of 30 mg/ml [9]. Ethanol, methanol, acetone and aqueous (hot &cold) cinnamon bark extracts was tested against five pathogenic microbes such as *S. mutans, Staphylococcus aureus, C. albicans, Saccharomyces cerevisiae* and *Lactobacillus acidophilus* that causing dental caries. The antimicrobial activity of methanolic, ethanolic and acetonic extracts of cinnamon bark were stronger than aqueous extracts of cinnamon bark against *S. aureus, S. mutans, C. albicans* and *S. cerevisiae* whereas *L. acidophilus* were resistant towards all the cinnamon bark extracts. The cinnamon bark extract of acetone performed stronger effectivity than aqueous and alcoholic extracts. The maximum zone of inhibition was observed against *C. albicans* 29.30mm [10]. The antimicrobial activity of cinnamon essential oil, monolaurin, nisin and combination of cinnamon essential oil +monolaurin, cinnamon essential oil +nisin, cinnamon essential oil + EDTA was carried. The zone of inhibition of cinnamon essential oil against S. aureus and E. coli measured 28.5±0.6mm and 21.7±0.3mm, respectively. The zone of inhibition with the combination of cinnamon essential oil and nisin against S. aureus and E. coli measured 33.8±0.4mm and 26.5±0.4mm. So, therefore cinnamon essential oil and all above combination performed potentially strong antimicrobial activity against both Gram-negative and Gram-positive bacteria [11]. The cinnamon essential oil was investigated against some spoilage and pathogenic bacteria such as Gram-positive bacteria including *S. aureus, B. cereus* and *Listeria innocua* and Gram-negative bacteria including E. coli, *Salmonella typhi* and

Pseudomonas aeruginosa. The Gram-positive bacteria were more inhibited than Gram negative bacteria by cinnamon essential oil [12].

Turmeric (*Curcuma longa*) Family: Zingiberaceae

The antimicrobial activity of turmeric is due to curcumin, essential oil, alkaloid, turmeric oil, valeric acid and turmerol etc. [13]. In an invitro study of turmeric natural dye was studied against ten bacterial strains by agar well diffusion method. The natural dye showed effective antibacterial activity against *Vibrio cholera, Vibrio alginolyticus, Vibrio harveyi, E. coli, E. faecalis, P. aeruginosa, Sarcina lutea* but natural dye showed no antibacterial activity against three bacterial strains *Vibrio mimicus*, *Plesiomonas shigelloides, Salmonella paratyphi*. The effective zone of inhibition of V. cholera and E. coli measured 10mm to 15mm and 07mm to 15mm respectively [14]. The antibacterial potential of ethanolic extracts of leaves and rhizome of turmeric has been observed by invitro techniques against *B. subtilis, K. pneumonia, S. aureus* and *P. aeruginosa*. The ethanolic turmeric leaves extracts showed essential antibacterial activity on test pathogenic bacteria except *S. aureus* and the maximum zone of inhibition of ethanolic leaves extract and ethanolic rhizome extract was measured for *B. subtilis* 08mm and 7.5mm, respectively at 300mg/ml concentration [15]. The antimicrobial activity of turmeric in different solvents was determined by agar well diffusion for bacteria and food poisoned food technique for fungi. The antimicrobial activity of turmeric extracts was performed against ten food associated bacterial pathogens and eleven moulds. The maximum zone of inhibition of methanolic extract measured against *E. coli* I was 28±0.57mm, the maximum zone of inhibition of ethanolic extract measured against *E. coli* I was 24±0.81mm and the maximum zone of inhibition of aqueous extract examined against *S. aureus* I was 28±0.57mm. The aqueous and methanolic extracts showed no antifungal activity against all tested moulds and the ethanolic extract performed effectivity against four food associated fungi including *Mucor* sp. I, *Mucor* sp. II, *Rhizopus stolonifer* I and *R. stolonifer* II 25%, 25%, 25%and 30%, respectively [16]. In another antimicrobial study of turmeric extract evaluated against endodontic pathogens *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212) and *C. albicans* (NTCC 3736). The aqueous and hydroalcoholic extracts showed effectivity at various concentration such as 25%, 50%,75% and 100% against *S. aureus* and *C. albicans* but in case of *E. faecalis* only aqueous extracts 50% and 75% performed antimicrobial activity. The maximum zone of inhibition measured against *C. albicans* 15.66±0.577mm at 50% concentration was performed by aqueous extract [17].

Clove (*Syzygium aromaticum*) Family: Myrtaceae

The antimicrobial potential of clove extract and eugenol against three pathogenic microbes such as *S. aureus, E. coli* and *C. albicans* have been observed by agar disc diffusion method. The eugenol and non-polar extract of clove showed inhibition against all tested pathogenic microbes. The eugenol performed maximum zone of inhibition against *C. albicans* 12.1 ± 2.2mm when it was applied 57µg/ disk [18]. The ethanolic and methanolic extracts of

clove examined against three foodborne pathogenic bacteria include *S. aureus* (MTCC 2940), *P. aeruginosa* (MTCC2453) and *E. coli* (MTCC 739). The ethanolic and methanolic extracts showed sufficient inhibitory effect against all tested food borne pathogens. The maximum zone of inhibition of ethanolic and methanolic extracts measured against *P. aeruginosa* was 20mm and *S. aureus* was 24mm [19]. The invitro evaluation of antifungal activity of clove essential oil has been observed by agar disc diffusion method using various dilutions (0%,10%,20%,50% and 100%) on the pathogenic fungi. The clove essential oil has shown strong antifungal activity against all pathogenic fungi like *Trichophyton Mentagrophytes, Microsporum canis, Aspergillus flavus* and *C. albicans*. The mean value of zone of inhibition of clove essential oil measured against *T. Mentagrophytes, M. canis, A. flavus* and *C. albicans* were 50mm, 45mm, 30mm and 26mm, respectively at 100% concentration [20]. The ethanol, methanol and acetone clove extracts were used to evaluate antibacterial activity against eight bacterial pathogens including *S. typhimuricum*, *E. coli, S. dysentriae, P. mirabilis, Y. enterocolitica, K. pneumoniae, B. cereus* and *S. pneumoniae*. The effectivity of ethanol, methanol and acetone extracts against all pathogenic bacteria was satisfactory. The maximum zone of inhibition of acetone extract measured against E. coli was 15.83mm at 1000mg/ml concentration [21]. The antibacterial study of clove essential oil determined by agar well diffusion method against two standard strains *S. aureus* (ATCC29213), *P. aeruginosa* (ATCC27853) and four multidrug resistant strains include *S. aureus, P. aeruginosa, E. faecalis* and *Acinetobacter baumannii*. The essential oil showed essential effectivity against all tested bacterial strains. The maximum zone of inhibition measured against *A. baumannii* was 28±2.3mm and *E. faecalis* was 25±2.6mm respectively at higher concentration (10%v/v) [22].

Cardamom (*Elettaria cardamomum*) Family: Zingiberaceae

The antimicrobial evaluation of cardamom extract against three Gram positive bacteria *B. cereus, Bacillus pumilus, S. aureus* and three Gram negative bacterial pathogens *E. coli, S. typhi, P. aeruginosa*. The aqueous, methanolic, ethanolic and liquid nutrient extract have been used to determine antibacterial potential against all tested bacterial pathogens. The aqueous extract showed no sensitivity against *E. coli* and *S. typhi* whereas effective against remaining four bacterial pathogens. The aqueous, ethanolic, methanolic and liquid nutrient extracts revealed antimicrobial activity against all tested bacterial pathogens. The maximum zone of inhibition obtained by the use of nutrient extracts against *E. coli* was 10mm and *B. cereus* was 10mm [23]. The crude methanolic extract of cardamom seeds used to determine the antibacterial potential by well diffusion assay against *L. monocytogenes, E. coli, B. pumilus* and *Enteropathogenic E. coli*. The methanolic crude extracts demonstrated inhibitory effect against all tested bacterial pathogens. The zone of inhibition of methanolic crude extract performed against *Enteropathogenic E. coli* 20.3mm, *B. pumilus* 19.0mm, *L. monocytogenes* 18.5mm and *E. coli* 16.5mm, respectively [24]. The investigation of antimicrobial effectivity of alcoholic seed

extract against pathogenic bacteria *S. aureus*. The different concentration (6%, 18% and 30%) of cardamom extracts was used to determine the antibacterial activity against *S. aureus*. The maximum zone of inhibition of cardamom extract occurred against S. aureus 11mm at 30% concentration [25]. An invitro antimicrobial activity of cardamom seed extracts was investigated by well diffusion technique against more resistant species *S. aureus* and *Proteus mirabilis*. The different extracts including aqueous, methanol and ethanol cardamom extracts used to examine the effectivity against both resistant strains. The maximum zone of inhibition was obtained by methanolic extracts against both bacterial strains *S. aureus* 13.5mm and *P. mirabilis* 11mm at 5000µg/ml concentration [26]. In another study, the antimicrobial potential was investigated using methanol, petroleum ether, aqueous and ethyl acetate against pathogenic bacteria and fungi *C. albicans, A. niger, B. subtilis, P. aeruginosa, E. coli, Staphylococcus epidermis* and *S. aureus* by using agar well diffusion method. All four solvent extracts (aqueous, methanolic, petroleum ether and ethyl acetate) extracts showed sensitivity against all tested pathogenic microbes. The maximum zone of inhibition was obtained by using methanolic extract against bacterial strain *P. aeruginosa* 21.1±0.43mm and the maximum zone of inhibition was occurred by using petroleum ether extract against fungal species *A. niger* was 20.7± 0.52mm [27].

Garlic (*Allium sativum*) Family: Alliaceae

The antimicrobial effectivity of garlic (fresh and heat treated) and applied two types of garlic domestic (kakanj) and the imported (China) against the bacterial strains including *S. aureus, B. subtilis, E. coli, S. enteritidis, methicillin resistant S. aureus* and fungi *C. albicans*. The fresh domestic and fresh imported garlic possess maximum zone of inhibition against *C. albicans* 62mm and 54mm, respectively and heat treated domestic and heat-treated imported garlic showed no zone of inhibition. The fresh domestic garlic possess maximum zone of inhibition against *S. aureus* 31mm and *methicillin resistant S. aureus* 29mm, respectively [28]. The antibacterial potential of crude extract of garlic investigated against two clinical and standard isolate of *S. aureus* and *E. coli*. The maximum zone of inhibitions of garlic crude extracts showed against clinical isolates of S. aureus 24mm and E. coli 19mm at 25mg/ml concentration (per 20ml of media) while the maximum zone of inhibitions against standard isolates of *S. aureus* 28mm and *E. coli* 26mm at 25mg/ml concentration (per 20ml of media). So therefore, the crude extracts revealed essential antibacterial potential against both clinical and standard isolates of *S. aureus* and *E. coli* [29]. The effectivity of garlic extracts examined against three bacterial strains *S. aureus, P. aeruginosa* and *E. coli* by disc diffusion method at different concentrations 20% and 50%, respectively. The zone of inhibition of garlic extracts measured *S. aureus* 10mm and *P. aeruginosa* 9mm at 50% concentration whereas no zone of inhibition obtained against *E. coli* at any concentrations [30]. An invitro study has investigated the effectivity of alcoholic extract of garlic against skin infectious isolate strain *S. aureus* by agar diffusion method at different concentrations. The garlic extracts possessed antibacterial activity

against S. aureus at 10%,20%,40%,60%.80% and 100% concentration. The zone of inhibition of garlic extracts measured against *S. aureus* 23mm at 100% and 9mm at 10% concentration [31]. The invitro evaluation of antibacterial potential of fresh garlic juice against bacterial pathogens *S. aureus, E. coli, P. aeruginosa, K. pneumoniae* and *P. mirabilis* using disc diffusion method. The garlic juice possessed inhibitory effect against above mentioned bacterial pathogens at various concentrations 5%, 10%, 25%, 50%, and 100%. The effective zone of inhibition of garlic juice was measured against *P. mirabilis* 28.08±0.64mm, *E. coli* 26.44±0.72mm, *S. aureus* 26.44±0.57mm, and *K. pneumoniae* 20.68±0.79mm whereas lowest zone of inhibition obtained against *P. auregonosa*12.480.36mm at 100% concentration [32].

Ginger (*Zingiber Officinal*) Family: Zingiberaceae

An invitro study was done to investigate the antimicrobial activity of ethanolic ginger extracts against *S. aureus* and *E. faecalis*. The different dilutions (5µl,10µl&15µl) were used to determine antimicrobial potential of ethanolic ginger extracts against both tested bacterial pathogens. The ethanolic ginger extracts showed good inhibitory effect against both tested bacterial strains *S. aureus* and *E. faecalis* at various dilutions mentioned above. The maximum zone of inhibition of ethanolic ginger extracts obtained against *S. aureus* 23mm and *E. faecalis* 24mm at 15µl dilution [33]. The antimicrobial potential of ginger extract has been investigated against some human pathogenic microbes like S. *aureus, S. faecalis, E. coli, B. subtilis, C. albicans and A. niger* by agar diffusion method. The maximum zone of inhibition of ginger extracts obtained against *A. niger* 20mm and *S. aureus* 19mm while no zone of inhibition occurred against *C. albicans* and *S. faecalis*. The ginger extract showed essential inhibitory effect against *A. niger, E. coli, S. aureus*, and *B. subtilis* except *C. albicans* and *S. faecalis* [34]. The antimicrobial activity of ethanolic and methanolic ginger extract investigated against certain bacterial pathogens *Salmonella* spp., *E. coli, Enterobacter* spp., *Shigella* spp., and *Citrobacter* spp. The methanolic and ethanolic ginger extracts showed inhibitory effect all above bacterial pathogens but ethanolic extract performed very low inhibitory effect against *Enterobacter* spp. The effective zone of inhibition of ethanolic ginger extract obtained against *Citrobacter* spp. 14mm, *Shigella* spp. 12mm, and *Salmonella* spp. 11.1mm and methanolic ginger extract against *Citrobacter* spp. 12mm and *Shigella* spp. 11mm [35]. The antimicrobial potential of dried ginger powder was estimated against *Fusarium oxysporum* f. sp. lycopersici by paper disc diffusion assay. The maximum zone of inhibition of chloroform, ethanol, acetone and petroleum ether ginger extract obtained against *Fusarium oxysporum* f. sp. lycopersici 25.75 ±2.7mm, 16.27 ±1.4mm, 25.00 ± 1.7mm and 20.50 ±1.2mm respectively at 750mg/ml concentration. The antifungal activity of chloroform, ethanol, acetone and petroleum ether ginger extracts showed inhibitory effect against tested pathogenic fungus at various concentration (250mg/ml, 500mg/ml and 750mg/ml) [36].

Conclusion:

The spices have rich source of medicinal properties and health-enhancing bioactive compounds. These bioactive compounds possess antimicrobial activity which prevents food-borne diseases. These can be used as an alternative and preservative of chemical compounds. The chemical preservatives may release toxic elements so therefore spices play an important role in food preservation. It has been reported that several types of bacterial and fungal pathogens have inhibited by the use of spices. These are very effective against oral, respiratory and intestinal pathogenic microbes. Spices have a tremendous ability to inhibit the food-borne pathogens.

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A COMPARATIVE PHARMACOGNOSTIC ANALYSIS OF LEAVES AND STEMS OF *SARPAGANDHA*: *RAUWOLFIA SERPENTINA* VERSUS *RAUWOLFIA TETRAPHYLLA*

Sonal Singh Kushwaha
Department of Dravyaguna Vigyana,
Shri Dhanwantry Ayurvedic College and Hospital, Chandigarh
Corresponding author E-mail: sonal27091999@gmail.com

Abstract:

Sarpagandha, derived from the root of *Rauvolfia serpentina*, is extensively utilized in *Ayurveda* for treating various ailments such as high blood pressure, insomnia, and mental illnesses. However, due to overharvesting and habitat loss, *Rauvolfia serpentina* is critically endangered. To address this, identifying substitutes from related species is crucial. This study evaluates and compares the pharmacological properties of *Rauvolfia serpentina* and *Rauvolfia tetraphylla.* Morphological and microscopic analyses of their leaves and stems reveal distinct features aiding in their identification. *Rauvolfia tetraphylla* exhibits unique characteristics, including whorled leaves and a simple stem structure, while *Rauvolfia serpentina* displays a more complex stem with distinct vascular bundles. This study provides insights essential for conservation and pharmacological research, emphasizing the importance of sustainable utilization and further exploration of these species' medicinal potential.

Keywords: *Sarpagandha*, *Rauwolfia serpentina, Rauwolfia tetraphylla*, Macroscopic, Microscopic

Introduction:

Sarpagandha, derived from the root of *Rauvolfia serpentina*, finds extensive usage in *Ayurveda* for a multitude of ailments. Primarily, it is utilized for treating high blood pressure, insomnia, asthma, acute stomach ache, and painful delivery. Moreover, in *Bhavaprakash Nighantu*, its therapeutic applications extend to addressing mental illnesses such as neuropsychiatric disorders, psychosis, and schizophrenia [1]. Additionally, it is employed in managing snake-bite, insect stings, gastric tumors, general weakness, goiter, hysteria, insanity, lipoma, paraplegia, paratyphoid, piles, pneumonia, splenomegaly, stomach disorders, tonsillitis, traumatic wounds, tuberculosis, and vertigo [2].

Rauvolfia, a genus in the family Apocynaceae, encompasses over 80 species found in tropical climates. Among these, *Rauvolfia serpentina* stands out for its abundance of indole alkaloids. Reserpine, the primary alkaloid, holds significant therapeutic value. Widely recognized for its efficacy, reserpine is utilized in the treatment of hypertension, cardiovascular ailments, and nervous disorders. Moreover, it serves as a sought-after tranquilizing agent, witnessing high demand within modern pharmaceutical industries [3].

Rauvolfia tetraphylla, commonly referred to as "devil pepper," is a woody shrub prevalent in India. Although native to the West Indies, it has become naturalized in South India. This ethnomedicinally significant plant is utilized for various purposes, including treating snakebites, inducing uterine contractions, and aiding in challenging childbirth cases [4].

The herbal drug industry is frequently facing disruptions caused by the unavailability of high-quality raw materials for drug manufacturing. This shortage of plant raw materials significantly impacts the quality of finished herbal products, compelling manufacturers to purchase substandard raw materials at inflated prices. Consequently, many manufacturers are on the verge of shutting down their operations. Moreover, the habitat loss resulting from the export of medicinal plants collected from wild sources exacerbates the situation, leading to irreversible depletion of genetic stocks in many species. *Vriksha Ayurveda* describes various methods to increase seed germination of plants, thus it can greatly help in increasing the population of endangered species like *sarpagandha*, thereby removing the threat of their extinction [5]. In India, the Ministry of Environment and Forests has implemented a ban on the export of 29 species, including certain popularly used drugs in *Ayurvedic* formulations such as *Sarpagandha [6]*.

Rauvolfia serpentina, identified as a critically endangered species, faces significant threats to its survival [7]. Owing to high demand and scarcity, the roots of *Rauvolfia serpentina* are often adulterated with other species.

The herbal drug industry commonly substitutes *Sarpagandha* with the roots of other *Rauvolfia* species. Given the endangered status of *Rauvolfia serpentina*, identifying scientifically validated substitutes from allied or related species holds significant importance. This approach aims to eliminate unauthorized substitution and adulteration, which can adversely affect the quality of herbal preparations. The objective of the present study is to evaluate and compare the pharmacological properties of both species.

Material and Methods:

Collection of the sample

Fresh leaves of *Rauwolfia serpentina* and *Rauwolfia tetraphylla* were collected from the campus of Shri Dhanwantry Ayurvedic College and Hospital, Chandigarh (Herbal Garden) in the month of November 2023. The leaves were washed under running tap water and blotted dry.

Organoleptic examination of the fresh drug:

Table 1: The Organoleptic evaluation of the leaf and stem of the fresh drug *Rauwolfia tetraphylla*

Examination	Leaf	Stem
Auditory	No characteristic sound	No characteristic sound
Tactile	Smooth with fine hairs	Rough with hairs
Visual	Greenish	Greenish
Taste	Bitter, Pungent	Bitter, Pungent
Odour	Characteristic	Characteristic

Table 2: The Organoleptic evaluation of the leaf and stem of the fresh drug *Rauwolfia serpentina*

Examination	Leaf	Stem
Auditory	No characteristic sound	No characteristic sound
Tactile	Smooth with fine hairs	Rough with hairs
Visual	Greenish	Greenish
Taste	Bitter, Pungent	Bitter, Pungent
Odour	Characteristic	Characteristic

Macroscopic and Microscopic evaluation

Macroscopic characters like shape, size, and margins were recorded as per visual observation. For the Micrometric evaluation, the fresh leaves were used. Sections were visualized under Compound microscope after slide preparation by transverse sectioning using dissecting kit.

Results and Discussion:

Leaves of *Rauwolfia tetraphylla*

Macroscopic features:

The leaves of *Rauwolfia tetraphylla*, commonly known as "devil pepper," exhibit distinct morphological characteristics. Typically, these leaves are arranged in whorls of four, hence the species name "*tetraphylla*." Each leaf is elliptical or lanceolate in shape, with a smooth or slightly serrated margin. The leaf surface is glossy green and glabrous, often exhibiting prominent venation. The leaves are borne on slender petioles and are arranged oppositely along the stem. Leaves unique morphology aids in species identification and classification within the *Rauwolfia* genus [8].

Microscopic features:

Microscopic examination of *Rauwolfia tetraphylla* leaves revealed distinct features in surface preparation. The upper epidermis [Fig 1.1] lacked stomata but contained numerous uniseriate multicellular trichomes. Conversely, the lower epidermis exhibited innumerable paracytic stomata and trichomes [Fig 1.3] similar to those on the upper epidermis [Fig 1.4]. Transverse section analysis of the midrib displayed a single layer of upper and lower epidermis [Fig 1.2] with a thin cuticle layer. Beneath the epidermis, 5-7 layers of collenchymatous cells were observed, exhibiting a polygonal shape and turning pink with safranin. Collenchymatous cells were also present above the lower epidermis. Vascular bundles, identified as bicollateral, comprised xylem at the centre with phloem on both sides. Other areas of the midrib contained parenchymatous cells. The mesophyll tissue consisted of upper palisade and lower spongy parenchyma cells, with chlorophyll present throughout.

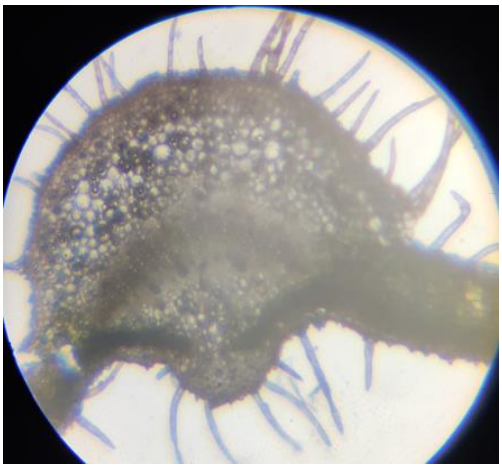
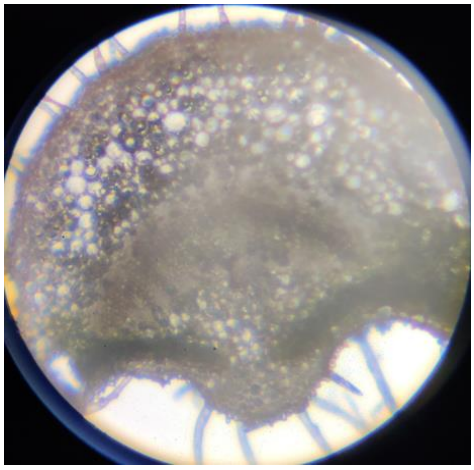


Fig. 1.1



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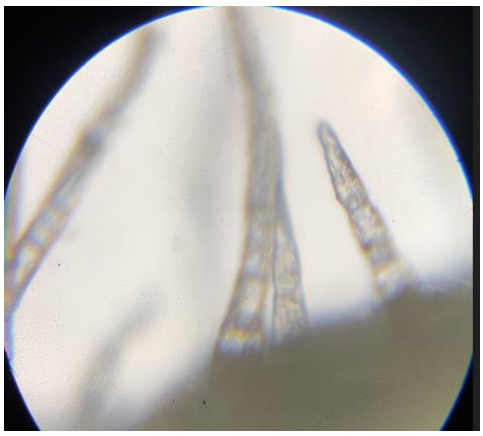

Fig. 1.3

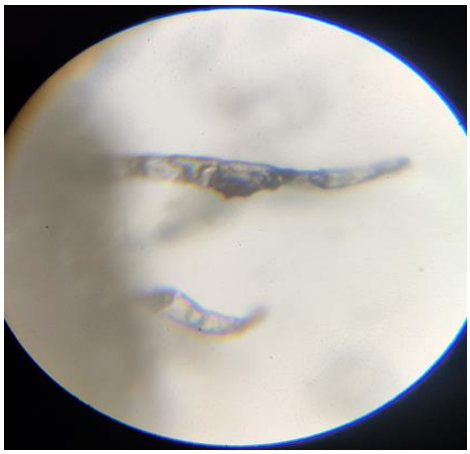

Fig. 1.4

Stem of *Rauwolfia tetraphylla*

Macroscopic features:

The stem of *Rauwolfia tetraphylla* is relatively short and typically exhibits a cylindrical or slightly flattened shape. It is characterized by a smooth texture and may appear green or brown, depending on its maturity. Occasionally, the stem may branch, giving rise to multiple stems from the base of the plant. Additionally, it may bear small leaf scars where leaves were once attached.

Microscopic features:

The stem of *Rauwolfia tetraphylla* exhibits a relatively simple structure. It features a single layer of epidermis [Fig. 1.5] with uniseriate multicellular trichomes. Below the epidermis, there are patches of non-lignified fibres [Fig 1.6] with a yellowish tinge towards the end of the cortex. The cortex consists of 10-12 layers of parenchymatous cells, varying in size and shape from oval to oblong. The vascular bundles [Fig 1.7] are bicollateral, with xylem at the centre and phloem on both sides. In the centre of the stem, there is a large pith [Fig 1.8] filled with parenchymatous cells.

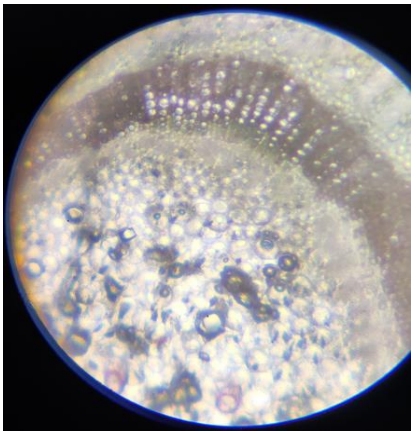

Fig. 1.5

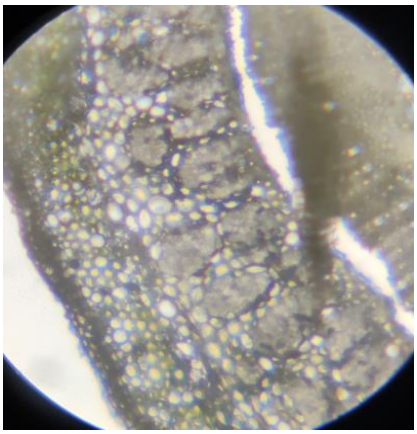

Fig. 1.6

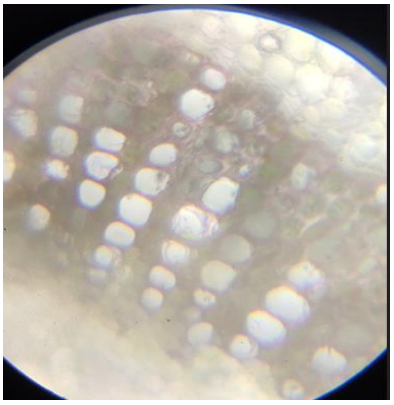

Fig. 1.7

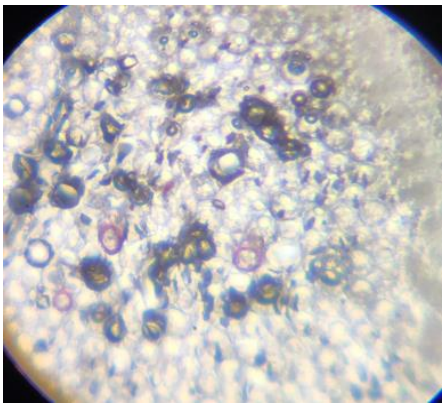

Fig. 1.8

Leaves of *Rauwolfia serpentina*

Macroscopic features

The leaves of *Rauvolfia serpentina* are arranged in whorls of three to four, forming a distinctive pattern along the stem. These leaves are lanceolate in shape, meaning they are long and narrow with pointed tips, measuring approximately 8 to 18 cm in length and 4 to 6 cm in width. One notable characteristic of these leaves is their glabrous nature, meaning they lack any noticeable hairs or trichomes on their surfaces.

The upper surface of the leaves is a vibrant and glossy bright green, giving them a lush appearance. In contrast, the underside of the leaves tends to be paler in coloration, creating a subtle yet distinct contrast between the upper and lower surfaces. This color contrast adds to the visual appeal of the foliage.

Microscopic features

The leaf's epidermis features distinct characteristics on both upper and lower surfaces. The upper epidermis lacks stomata but is adorned with numerous uniseriate, multicellular trichomes. In contrast, the lower epidermis is marked by abundant paracytic stomata and shares similar trichomes with the upper epidermis. In a transverse section [Fig 1.10], the leaf reveals a stratified cork with two to eight alternating zones. These zones consist of one to seven layers of

smaller, radially narrower, suberized, non-lignified cells, alongside one to three layers of larger, radially broader, lignified cells.

Stem of *Rauwolfia serpentina*

Macroscopic features

The stem of *Rauvolfia serpentina* is characterized by its erect and slender nature, often branching as it grows. It typically possesses a bark that ranges in color from light brown to gray, potentially developing a slight corky texture as the plant ages.

Microscopic features

The stem of *Rauwolfia serpentina*, observed under microscopy, reveals rectangular epidermal cells with a thin cuticle, a parenchymatous cortex, and pith [Fig 1.11]. It features numerous glandular uniseriate trichomes. Young stems display four bicollateral vascular bundles [Fig 1.12] and a 2-4 layered vascular cambium producing more secondary xylem. Phellogen generates parenchymatous phelloderm [Fig 1.13]. Xylem contains thick-walled cells with lignin, solitary vessel elements, and scattered sclereids in the pith. Lipid globules and starch grains are evident in older stems. Internal phloem forms a ring beneath the secondary xylem in mature stems.

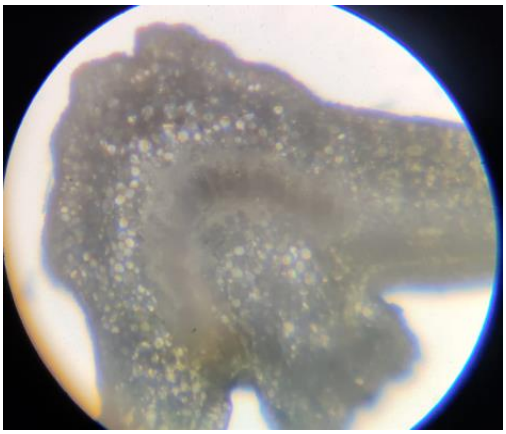

Fig. 1.10

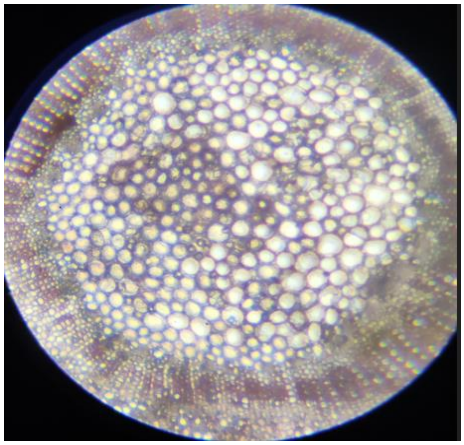

Fig. 1.11

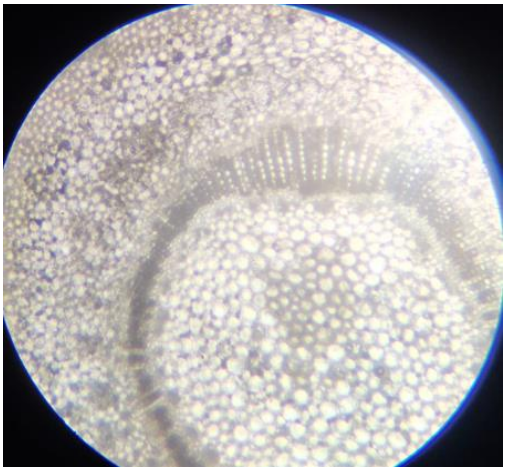

Fig. 1.12

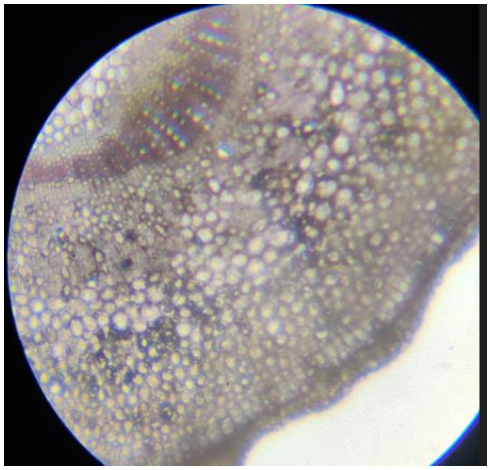

Fig. 1.13

Table 3: Morphological difference between *Rauwolfia serpentina* and *Rauwolfia tetraphylla* leaf

Morphological Characteristics	*Rauvolfia serpentina*	*Rauvolfia tetraphylla*
Leaf Arrangement	Whorled	Opposite
Leaf Size and Shape	Larger, narrower, lanceolate	Smaller, broader
Leaf Venation	Prominent midribs and lateral veins	Venation pattern may differ
Leaf Texture and Surface	Smoother	Rougher or distinct surface features
Leaf Pigmentation	Variable	Variable

Conclusion:

The study conducted aimed to explore and compare the morphological and microscopic characteristics of leaves and stems of *Rauwolfia serpentina* and *Rauwolfia tetraphylla*. The findings revealed distinct features in both species, aiding in their identification and classification within the *Rauwolfia* genus.

Macroscopic examination of *Rauwolfia tetraphylla* leaves highlighted their unique arrangement in whorls of four, elliptical or lanceolate shape, and glossy green surface. Microscopic analysis further unveiled specific traits such as the absence of stomata on the upper epidermis, presence of paracytic stomata on the lower epidermis, and the composition of the midrib layers.

Similarly, *Rauwolfia serpentina* leaves exhibited characteristic features including lanceolate shape, glabrous surface, and distinct venation pattern. Microscopic examination revealed differences in epidermal structure, presence of glandular trichomes, and tissue composition.

The stems of both species also displayed notable differences in their macroscopic and microscopic characteristics. *Rauwolfia tetraphylla* stems featured a simple structure with a single layer of epidermis, while *Rauwolfia serpentina* stems exhibited a more complex arrangement with distinct vascular bundles and trichomes.

Overall, the study provides valuable insights into the morphological and microscopic features of *Rauwolfia serpentina* and *Rauwolfia tetraphylla*, facilitating their accurate identification and potential pharmacological exploration. Such knowledge is essential for conservation efforts, pharmacological research, and the development of herbal medicines derived from these species. Further research into the pharmacological properties and medicinal potential of these plants is warranted to harness their therapeutic benefits effectively.

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ACNE VULGARIS: EXPLORING MULTIFACTORIAL PATHOGENESIS AND THE ROLE OF HERBAL INTERVENTIONS

Aarti S. Zanwar*, Dhanya B Sen, Krupa Joshi and Krishna Suthar
Department of Pharmacy,
Sumandeep Vidyapeeth Deemed to be University, Piparia, Waghodia, Vadodara 391760
*Corresponding author E-mail: aarti.zanwar@gmail.com

Abstract:

Acne vulgaris is a common chronic inflammatory skin disorder affecting adolescents and young adults, causing significant psychosocial effects and reduced self-esteem. This review aims to explore the underlying causes and pathophysiological mechanisms of acne, evaluate current conventional treatment modalities, and highlight emerging evidence on herbal therapies as alternative or complementary treatments. Conventional therapies like topical retinoids, benzoyl peroxide, systemic antibiotics, hormonal agents, and isotretinoin remain the mainstay of treatment, but long-term use is associated with side effects and antibiotic resistance. Herbal remedies like *Melaleuca alternifolia, Camellia sinensis, Aloe barbadensis, Berberis vulgaris, Garcinia mangostana, Glycyrrhiza glabra etc* demonstrate antimicrobial, anti-inflammatory, and antioxidant properties, making them effective in mild to moderate acne with fewer side effects. Further clinical research is needed to validate their efficacy and standardize formulations for widespread clinical use.

Keywords: Acne Vulgaris, Sebum, Cutibacterium Acnes, Herbal Medicine, Skin Disorder

Introduction:

In regions with a high density of sebaceous glands, acne vulgaris, a persistent skin disorder, causes inflammation of the sebaceous glands and hair follicles. It can show up as comedones, papules, pustules, nodules, and cysts, among other kinds of lesions. Because of hormonal changes, it is most prevalent throughout adolescence, but it can continue until maturity.[1] Excessive sebum production, follicular hyperkeratinization, bacterial growth, and inflammation are some of the factors that lead to acne vulgaris. [2] About 9.4% of the world's population is impacted. Key influences include increased fast-food consumption (74.54%), use of cosmetics (32.73%), reduced water intake (29.09%), and poor dietary habits involving soft drinks, junk food, and chocolate. Additional exacerbators include poor sleep patterns and inadequate skin hygiene.

Certain foods and drinks, particularly those with a high glycemic index (e.g., sugary drinks, starchy foods, highly processed foods) and skim milk, seem to affect acne severity. Other factors that may be involved in the development or progression of acne include psychological stress, tobacco smoke, and damaged or unhealthy skin.[3]

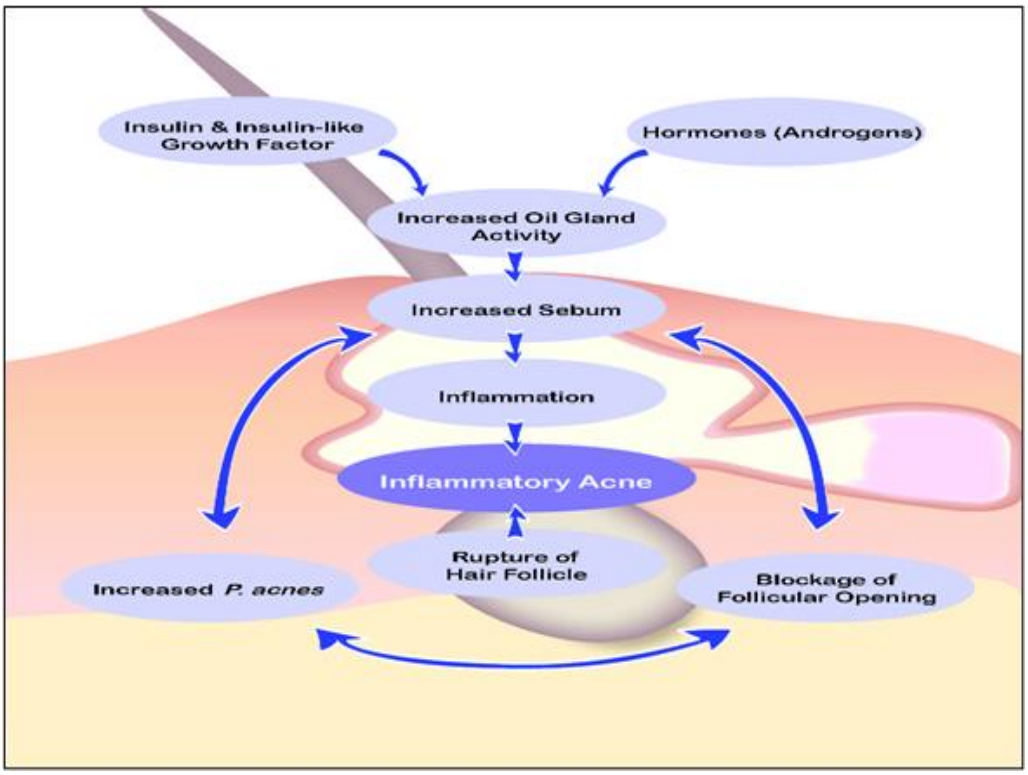

Fig. 1: Diagrammatic representation of the key components and processes involved in Acne

Pathophysiology:

Numerous host variables, including as immunological responses, microbiome dysbiosis, and androgen stimulation, contribute to acne vulgaris. The development of the illness is also influenced by food and genetics. The microcomedones, a tiny, hyperkeratotic plug made of corneocytes, is the main lesion. It develops into inflammatory papules, pustules, and nodules, as well as other acne lesions including blackheads and whiteheads.[4]

When sebum and keratinous particles build up, a micro comedon becomes closed and becomes an open, dark-black comedon. C acnes exacerbate inflammation and forms nodules by causing ruptures, pustules, papules, and inflammation.[5]

The pathophysiology of acne vulgaris involves a complex interplay of factors leading to the formation of acne lesions. Here's a diagrammatic representation of the key components and processes involved in acne.

1. Increased Sebum Production: Androgens stimulate sebaceous glands, leading to excess oil production.

2. Follicular Hyperkeratinization: Abnormal keratinocyte proliferation causes blockages in hair follicles, forming Comedones.

3. Colonization of Cutibacterium acnes: This bacterium thrives in the anaerobic environment created by excess sebum and contributes to inflammation.

4. Inflammatory Response: The presence of C. acnes triggers an immune response, resulting in the release of pro-inflammatory cytokines.

5. Follicular Wall Rupture: The pressure from accumulated sebum and inflammatory mediators leads to rupture, spilling contents into surrounding tissue.

6. Inflammatory Lesions: This process results in various types of lesions, including papules and pustules.

7. Scarring: Chronic inflammation and tissue damage may lead to scarring as a result of connective tissue damage.[6-9]

Treatment of Acne Vulgaris

Acne vulgaris is a prevalent skin condition that necessitates a comprehensive treatment strategy based on its severity. Primary treatment options are categorized by severity.

1. Mild Acne Vulgaris

- **Benzoyl Peroxide:** An effective antibacterial agent that helps reduce inflammation and unclog pores. The product is offered in various formulations and concentrations.
- **Topical Retinoids:** Such as adapalene (Differin) and tretinoin (Retin-A), which prevent follicular plugging and promote cell turnover.
- **Topical Antibiotics:** Erythromycin or clindamycin can be used, but should be combined with benzoyl peroxide to reduce the risk of bacterial resistance.

2. Moderate Acne Vulgaris

- **Combination Therapy:** The recommended treatment involves a combination of benzoyl peroxide and a topical antibiotic like erythromycin or clindamycin.
- **Oral Antibiotics:** Tetracyclines, such as doxycycline or minocycline, are preferred for their effectiveness against inflammatory acne.
- **Topical Retinoids:** Should also be included to enhance treatment efficacy.

3. Severe Acne Vulgaris

- **Oral Isotretinoin:** Considered a first-line option for severe cases, particularly for nodular or cystic acne. It has a high success rate but may have significant side effects.
- **Combination of Oral Antibiotics and Topical Treatments:** This includes oral antibiotics, benzoyl peroxide, and topical retinoids to manage severe inflammatory acne effectively.
- **Hormonal Treatments for Women:** Combined oral contraceptives or spironolactone may be prescribed to address hormonal influences on acne.

4. Alternative Treatments

- **Azelaic Acid:** Effective for both acne and post-inflammatory hyperpigmentation, it has antibacterial properties and can be used in pregnancy.
- **Dapsone Gel:** Particularly useful for inflammatory acne in women.
- **Chemical Peels and Laser Therapy:** These can help reduce scarring and improve skin appearance post-acne treatment. [10,11]

Recent Advances in Nanotechnology for Acne Vulgaris Treatment

Nanotechnology is emerging as a promising approach in the treatment of acne vulgaris, addressing limitations of conventional therapies through innovative drug delivery systems. Recent studies highlight several advancements in this field:

1. Liposomes and Nanocarriers

Recent research emphasizes the use of phospholipid-based nanocarriers such as liposomes, ethosomes, and niosomes to enhance the delivery of anti-acne agents like benzoyl peroxide, adapalene, and tretinoin. These nanocarriers improve drug solubility, stability, and skin penetration while minimizing side effects and enhancing patient compliance. By encapsulating active ingredients, these systems target the sebaceous glands more effectively, leading to improved therapeutic outcomes in acne treatment. [12-14]

2. Gold/Alginate Nanoparticles

A study introduced gold/alginate nanoparticles (GANPs) as a novel treatment for acne vulgaris. These nanoparticles demonstrated significant antibacterial activity against common acne-related bacteria such as Cutibacterium acnes, showcasing a minimum inhibitory concentration that suggests their potential as effective topical agents. This approach offers a promising alternative to traditional antibiotics, which often come with side effects and resistance issues. [15]

2. Enhanced Retinoid Formulations

Advancements in retinoid formulations have also been noted, with novel delivery systems being developed to enhance their efficacy and tolerability. For instance, Tazarotene 0.045% lotion has gained FDA approval, demonstrating improved outcomes through innovative nanotechnology strategies that optimize retinoid delivery while reducing irritation. [16]

5. Photodynamic Therapy and Laser Treatments

Nanotechnology is also being integrated into laser and light therapies for acne management. Techniques utilizing nanoparticles can enhance the effectiveness of photodynamic therapy by improving the targeting of C. acnes and reducing inflammation through controlled light application. [17]

Herbal treatment:

A growing body of clinical and preclinical research supports the use of various herbal drugs for the treatment of acne vulgaris. These herbal remedies often offer anti-inflammatory, antibacterial, and sebum-regulating effects, targeting key factors in acne pathogenesis.

Herbal Drugs and Their Evidence-Based Efficacy

Tea Tree Oil (*Melaleuca alternifolia*)

Multiple clinical trials have shown that tea tree oil is effective in reducing both inflammatory and non-inflammatory acne lesions, with efficacy comparable to standard treatments like benzoyl peroxide and erythromycin, and with fewer side effects. Patient

satisfaction with tea tree oil formulations is generally high, and it is considered safe for topical use.

Tea tree oil, derived from Melaleuca alternifolia leaves, exhibits potent antibacterial, anti-inflammatory, and antioxidant properties, with terpinen-4-ol being its primary active component against acne-causing bacteria. [18]

Clinical Efficacy: Clinical trials show that 5% tea tree oil gel significantly reduces both inflammatory and non-inflammatory acne lesions in individuals with mild to moderate acne. A randomized double-blind trial found that the gel reduced total lesion count by 43.6% and acne severity index by 40.5% over six weeks. Studies comparing the gel with 5% benzoyl peroxide found that the gel matched the effectiveness of benzoyl peroxide in decreasing non-inflammatory acne lesions but had faster results for inflamed ones.

Patient Tolerability: Tea tree oil products are generally well tolerated. Adverse effects, when present, are usually mild and limited to local skin irritation, which resolves without intervention. To reduce the risk of skin sensitization, the European Cosmetic Association advises limiting tea tree oil concentration to a maximum of 1% in cosmetic products.

Formulation and Use: TTO is used in gels, creams, and face washes, typically at concentrations of 5% for acne treatment. Twice-daily application is common in clinical studies. TTO can be incorporated into modern delivery systems like nanogels and liposomes for enhanced efficacy and stability. [19,20]

Green Tea (*Camellia sinensis*)

Topical application of 2% green tea lotion over six weeks has been shown to significantly reduce acne lesions, likely due to its tannins and flavonoids, which have antiseptic and anti-inflammatory properties. Green tea extracts are also associated with high patient satisfaction and are well-tolerated. Green tea is rich in polyphenols, particularly epigallocatechin-3-gallate (EGCG), which provide anti-inflammatory, antioxidant, antibacterial, and sebum-suppressive effects.

Clinical Efficacy

Topical Application: A systematic review and meta-analysis of five randomized controlled trials revealed that topical green tea extract (GTE) significantly decreased both inflammatory acne lesions (average reduction of 11.39 lesions) and non-inflammatory lesions (average reduction of 32.44 lesions) compared to a placebo, with only minor side effects reported. Clinical studies show that topical green tea formulations (such as 2–3% creams or toners) reduce both lesion counts and facial sebum levels, often within 2–4 weeks of use. In direct comparisons, a 3% green tea cream was found to be as effective as 4% benzoyl peroxide for moderate to severe acne, but with fewer and milder side effects.

Safety and Tolerability: Most studies report no significant adverse events with topical green tea use. Occasional mild stinging, pruritus, or irritation may occur, typically resolving without intervention. [21,22]

Aloe Vera (*Aloe barbadensis*)

Aloe vera gel contains bioactive compounds such as polysaccharides (aloe mannan, ace Mannan), amino acids, zinc, and phenolic antioxidants. These components contribute to its:
Anti-inflammatory effects (reducing leukocyte adhesion and TNF-α), antioxidant activity (via glutathione peroxide, superoxide dismutase, phenolic antioxidants), antibacterial properties (helping control acne-causing bacteria), Moisturizing and skin barrier repair functions (enhancing skin flexibility and reducing fragility. [23]

Clinical Efficacy: A recent clinical study demonstrated that a non-drug therapy combining aloe vera gel, ultrasound (to enhance absorption), and a soft mask significantly reduced acne lesions, hyperpigmentation, and improved skin texture and blood circulation. This effect was most pronounced in patients with moderate acne, where the "markedly effective" rate reached over 92% after two months. The remedy exhibited a considerably higher effectiveness rate than that of the control group, with minimal side effects. Aloe vera has also been shown to enhance the anti-acne effects of other herbal agents (e.g., Ocimum oil), and in some studies, combinations outperformed standard treatments like 1% clindamycin.

Adjunct to Conventional Therapy: When combined with conventional medications such as tretinoin, aloe vera cream improved acne outcomes more than tretinoin alone, with better tolerability and fewer side effects [22]

Safety and Tolerability: Aloe vera is generally well-tolerated, with rare reports of skin irritation or allergy. It is suitable for long-term use and for individuals seeking treatments with mild side effects and low risk of antibiotic resistance. [24,25]

Barberry (*Berberis vulgaris*)

Berberis vulgaris, commonly known as barberry, is recognized in traditional medicine for its anti-inflammatory, antibacterial, and antilipo genic (sebum-reducing) properties. These effects are attributed to its bioactive compounds, particularly berberine, which target several key factors in acne pathogenesis

Clinical Evidence: A clinical trial, designed as double-blind, randomized, and placebo-controlled, assessed the efficacy of oral aqueous extract of dried barberry fruit (600 mg daily for 4 weeks) in adolescents with moderate to severe acne vulgaris. The study found a significant reduction in non-inflamed, inflamed, and total acne lesions, as well as in the Michaelson's acne severity score—by approximately 44%—in the barberry group compared to placebo ($p < 0.001$). No notable side effects were reported, indicating that oral barberry extract is safe and well-tolerated for teenagers.

Another placebo-controlled study found that fresh Berberis vulgaris fruit juice was effective against acne lesions in patients with mild-to-moderate disease, further supporting the anti-acne potential of this herb.

Safety and Tolerability: Clinical trials report that both oral and topical barberry preparations are generally safe, with negligible adverse effects compared to conventional treatments.[26-28]

Mangosteen (*Garcinia mangostana*)

Formulations containing mangosteen have demonstrated greater efficacy than clindamycin in reducing acne severity in clinical trials. Garcinia mangostana, commonly known as mangosteen, is rich in xanthones (notably α-mangostin) that exhibit strong antibacterial and anti-inflammatory activities. These properties target key factors in acne pathogenesis, including inhibition of *Cutibacterium acnes* and reduction of skin inflammation. [29]

Clinical and Experimental Evidence

- Topical Formulations: A hydrogel patch containing G. mangostana pericarp extract showed effective antibacterial activity against *C. acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. The patch released active α-mangostin quickly and was considered safe for use as an anti-acne facial mask.
- Oral supplementation: 400 mg of mangosteen rind extract orally led to a greater reduction in acne lesions compared to placebo.
- Topical application: A gel containing 3% mangosteen peel extract showed a significant 47.4% decrease in total acne lesions among Asian female volunteers. [30,31]

Molecular docking studies suggest that mangosteen phytochemicals may inhibit enzymes involved in C. acnes fatty acid biosynthesis and human inflammatory pathways, providing a plausible mechanism for its anti-acne effects.[32]

Safety and Tolerability: Topical and oral mangosteen preparations are generally well-tolerated. Mild skin irritation or delayed allergic reactions are rare but possible, especially in individuals sensitive to mangosteen.

Licorice (*Glycyrrhiza glabra*)

Licorice extract inhibits *P. acnes* growth without inducing bacterial resistance, making it a promising adjunct or alternative to conventional antibiotics. Licorice root contains several active compounds—most notably glycyrrhizin (glycyrrhizic acid), glycyrrhetinic acid, licochalcone A, and glabridin—that contribute to its anti-acne effects. These compounds act through multiple mechanisms:

- Anti-inflammatory**:** Licorice flavonoids and glycyrrhizin acid decrease the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, helping to control the inflammatory response in acne.
- Antimicrobial: Licochalcone A, licochalcone E, and the total extract have demonstrated significant activity against acne-causing bacteria including *Propionibacterium*

acnes (now *Cutibacterium acnes*), *Staphylococcus aureus*, and *Staphylococcus epidermidis*.

- Sebum regulation: Licorice compounds can inhibit lipid synthesis and balance oil production, which is critical in acne management.
- Anti-androgenic: Glycyrrhizin and licochalcone A can reduce androgen activity, which may help decrease sebum production and follicular blockage.
- Antioxidant and skin barrier support: The extract is rich in antioxidants, supports collagen synthesis, and helps repair the skin barrier, making it suitable for sensitive and acne-prone skin. [33]

Clinical and Experimental Evidence

Animal and In Vitro Studies: Licorice flavonoids gel significantly reduced epidermal hyperkeratosis and inflammatory markers (TNF-α, IL-8) in acne models, indicating improvement in both the pathological and inflammatory aspects of acne. In vitro studies confirm that *G. glabra* extract has notable antibacterial effects against *P. acnes* and other skin pathogens relevant to acne.

Clinical Use: Licorice extract and its derivatives are used in topical formulations for their soothing, oil-balancing, and skin-brightening benefits, with a good safety profile and minimal risk of irritation.

Safety and Tolerability: Licorice extract is widely regarded as safe for topical application and poses minimal risk of adverse effects. [34-35]

Conclusion:

Acne vulgaris is a multifactorial disorder requiring individualized treatment strategies. Herbal treatments show potential as safer alternatives or adjuncts to conventional therapy with their anti-inflammatory, antibacterial, antioxidant, and sebum-regulating properties, plant-based remedies like tea tree oil, green tea extracts, and aloe vera offer a more holistic and potentially safer approach. However, more standardized clinical trials are needed to validate their efficacy and safety. Integrating herbal therapies into acne management could enhance patient outcomes, especially in individuals seeking natural or well-tolerated treatment options.

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NANOPARTICLES IN DENTISTRY – APPLICATIONS, BENEFITS, RISKS, AND FUTURE DIRECTIONS

Tamanna Chhabra* and Lavi Shah
Department of Prosthodontics and Crown and Bridge,
K M Shah Dental College and Hospital,
Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara-391760, Gujarat
*Corresponding author E-mail: drtamannachhabra@gmail.com

Abstract:

Nanoparticles—materials smaller than 100 nanometers—are transforming the way dentistry is practiced. Their unique abilities, such as fighting harmful bacteria, blending seamlessly with tissues, and improving the strength of dental materials, have given rise to *nanodentistry*. Today, these particles are already enhancing treatments by making fillings stronger, improving root canal disinfection, supporting gum and bone healing, and creating longer-lasting prosthetics and implants.

A newer breakthrough is the introduction of *nanorobotics*—tiny robots built from or powered by nanoparticles, like magnetic or gold-based particles. These nanorobots can travel through microscopic spaces, deliver medicines exactly where they are needed, clean away biofilms, and may one day repair tissues at the molecular level. Such innovations could make dental care more precise, less invasive, and more comfortable for patients.

Despite these exciting advances, challenges remain. Some nanoparticles may pose risks, such as toxicity, allergic reactions, or environmental concerns, and strict testing and regulations are essential before they can be widely used.

This chapter explores how nanoparticles and nanorobotics are being used in dentistry today, their benefits, safety concerns, and how they are shaping the future of more personalized, sustainable, and effective dental care.

Keywords: Nanoparticles, Nanodentistry, Nanorobotics, Restorative Dentistry, Silver Nanoparticles, Titanium Dioxide, Hydroxyapatite, Drug Delivery Systems

Introduction:

Nanotechnology, the science of manipulating materials at sizes between 1 and 100 nanometers, is transforming how dentistry is practiced. At this nanoscale, materials behave differently than they do in bulk, gaining a much larger surface area, enhanced reactivity, and improved ability to integrate with biological tissues.[1] These unique traits have paved the way for *nanodentistry*, where nanoparticles (NPs) are incorporated into fillings, crowns, implants, drug delivery systems, and regenerative therapies to make them stronger, more effective, and longer-lasting. Such applications not only improve the durability and esthetics of dental restorations but also enhance antimicrobial activity and promote faster healing, improving overall patient comfort and outcomes.[2]

One of the most exciting frontiers in this field is *nanorobotics*. These nanoscale robotic systems, often constructed from or powered by functionalized nanoparticles such as gold or magnetic materials, can navigate microscopic spaces in the oral cavity. Guided by magnetic fields, light, or programmed biochemical triggers, nanorobots are capable of delivering therapeutic agents directly to infected or damaged tissues, breaking down biofilms, and—potentially in the near future—repairing enamel microcracks or aiding tissue regeneration. When combined with artificial intelligence (AI) and biomimetic propulsion mechanisms, nanorobotics offers the potential for highly precise, minimally invasive, and efficient dental treatments tailored to individual needs.[3]

Despite these breakthroughs, important concerns remain. Some nanoparticles exhibit dose-dependent cytotoxicity, potential allergenicity, or pose environmental risks if not carefully managed. Current research focuses on creating biodegradable and biocompatible formulations and establishing clinical protocols that ensure patient and ecological safety. With continued innovation and regulation, nanotechnology—especially when integrated with robotics—is expected to make dental care more personalized, sustainable, and effective, marking a major leap toward the future of *smart dentistry*.

Classification of Nanoparticles in Dentistry

Nanoparticles used in dentistry can be grouped based on their chemical composition or origin and each type caters to specific therapeutic or material needs.[4] The most widely researched and applied nanoparticles fall into the categories listed in Table 1.

Table 1: Common nanoparticles used in dentistry and their main characteristics[4-6]

Nanoparticle Type	Examples	Key Functions
Metallic	Silver (AgNPs), Gold (AuNPs), Titanium dioxide (TiO_2), Zinc oxide (ZnO)	Antibacterial, implant coatings, restoration additives
Ceramic	Nano-hydroxyapatite (n-HA), bioactive glass	Remineralization, bone graft scaffolds
Polymeric	Chitosan, PLGA	Controlled drug release, scaffold matrices
Carbon-based	Graphene oxide, carbon nanotubes	Mechanical reinforcement, conductivity

Metallic nanoparticles like silver and zinc oxide exhibit potent antimicrobial properties by disrupting bacterial membranes and inhibiting biofilms, making them invaluable in preventing dental infections. Titanium dioxide nanoparticles serve as coatings to improve implant osseointegration and offer photocatalytic properties useful in antimicrobial therapy.

Ceramic nanoparticles such as nano-hydroxyapatite closely mimic the mineral phase of natural enamel and bone, facilitating remineralization and serving as scaffolds in bone regeneration.

Polymeric nanoparticles like chitosan and PLGA are biocompatible carriers for controlled drug release and act as matrices in tissue engineering.

Carbon-based nanoparticles reinforce mechanical properties, enhance electrical conductivity, and are explored in biosensor applications.

Clinical Applications and Indications

Nanoparticles have revolutionized various branches of dentistry by enhancing material properties, improving treatment outcomes, and enabling innovative therapeutic and diagnostic approaches. Their use extends across restorative dentistry, endodontics, periodontics, prosthodontics, implants, and preventive care, making nanodentistry a vital component of modern clinical practice.

Restorative Dentistry

In restorative procedures, nanoparticles enhance the performance of resin-based dental materials by increasing mechanical strength, minimizing polymerization shrinkage, and improving wear resistance. Nano-fillers also replicate enamel's natural optical properties, providing superior polishability and esthetics.[7]

- **Nano-hydroxyapatite (n-HA):** Promotes remineralization of early carious lesions by replenishing calcium and phosphate ions, allowing for minimally invasive, structure-preserving treatments.[8]
- **Silver (AgNPs) and Zinc Oxide (ZnO):** Incorporated into composites to provide long-lasting antimicrobial activity, effectively reducing bacterial colonization and secondary caries at restoration margins.[9]

Endodontics

Successful root canal therapy relies on optimal disinfection and sealing, where nanoparticles enhance effectiveness:

- **Silver and Chitosan Nanoparticles:** Penetrate dentinal tubules more efficiently than conventional irrigants, improving bacterial elimination.[10]
- **Nano-modified Sealers:** Offer superior flow and antimicrobial properties, ensuring a tighter seal and minimizing reinfection risks.[11]
- **Bioceramic Scaffolds with Nano-fillers:** Support pulp tissue regeneration and root development, particularly in immature teeth, advancing regenerative endodontics.[12]

Periodontics

Nanoparticles aid in both infection control and regeneration in periodontal therapy:

- **Chitosan and ZnO-based Localized Gels:** Reduce periodontal pathogens and enhance clinical attachment levels.[13]
- **Graphene Oxide and TiO_2-enriched Membranes:** Improve guided tissue regeneration by enhancing cell adhesion and promoting healing.[14]

Prosthodontics and Implants

Nanoparticles improve the durability and biological performance of prosthetic and implant materials:

- **AgNPs and ZnO in Denture Bases:** Prevent fungal colonization, improving oral hygiene and comfort.[15]
- **TiO_2-coated Implants:** Enhance osseointegration by increasing bone-to-implant contact and reducing peri-implant inflammation.[16]
- **Silica and Zirconia Nanoparticles:** Strengthen provisional crowns and luting cements by improving their fracture resistance.[3,17]

Preventive and Diagnostic Applications

Nanoparticles are increasingly found in preventive and diagnostic products:

- **Nano-hydroxyapatite and Silver Nanoparticles in Toothpaste:** Reinforce enamel and reduce biofilm formation, aiding caries prevention.[6,18]
- **Nanoparticle-based Biosensors:** Detect pH changes and disease biomarkers for non-invasive, real-time monitoring of early oral diseases.[19]

Advantages of Nanoparticles

Chart 1: Key Advantages of Nanoparticles in Dentistry (Compiled from sources)[6-11]

Benefit	Description
Antibacterial Activity	Nanoparticles such as silver (AgNPs) and zinc oxide (ZnO) disrupt bacterial cell membranes, inhibit biofilm formation, and reduce microbial colonization on dental materials, thus helping prevent infections and recurrent caries.
Mechanical Reinforcement	Incorporation of nanoparticles enhances the mechanical properties of dental restoratives and prosthetics, increasing strength, elasticity, wear resistance, and fracture toughness, thereby improving longevity.
Improved Esthetics	Nano-sized fillers improve the polishability, gloss retention, and translucency of restorative materials, closely mimicking natural enamel's optical properties for superior color matching and appearance.
Regenerative Potential	Specific nanoparticles stimulate biological processes such as osteogenesis (bone formation), angiogenesis (new blood vessel growth), and pulp cell differentiation, facilitating periodontal and endodontic tissue regeneration.
Targeted Drug Delivery	Polymeric and other nanoparticles enable controlled, localized drug release directly at the treatment site, increasing therapeutic efficiency while minimizing systemic side effects.
Biomimicry	Nanoparticles like nano-hydroxyapatite and bioactive glass closely resemble natural enamel and bone minerals, promoting remineralization and supporting tissue repair in a biomimetic manner.
Improved Adhesion	Nanoparticles improve the bonding strength between dental materials and tooth structures, leading to more durable restorations and reduced microleakage.

Limitations and Contraindications

While nanoparticles offer numerous benefits in dentistry, their application is not without limitations, and certain contraindications must be carefully considered to ensure patient safety.

Cytotoxic and Genotoxic Effects

Several studies have raised concerns regarding the cytotoxicity of some nanoparticles, especially metallic types like silver (AgNPs) and zinc oxide (ZnO). At elevated concentrations, these particles have been shown to cause cell damage in oral epithelial tissues, dental pulp cells, and fibroblasts, potentially impairing normal cellular functions.[20,21] Long-term exposure to these nanoparticles may also interfere with DNA repair mechanisms and mitochondrial activity, which could lead to genotoxic effects and cellular dysfunction over time.[22] These findings highlight the importance of dose control and thorough biocompatibility testing before clinical use.

Allergic Reactions

Hypersensitivity reactions represent another challenge in nanodentistry. Metal-based nanoparticles containing silver, gold, or nickel may trigger allergic responses in susceptible individuals, manifesting as localized inflammation or systemic reactions.[23] Additionally, chitosan nanoparticles, commonly derived from shellfish, pose a risk for allergic reactions in patients with shellfish allergies, necessitating caution in patient selection and pre-treatment screening.

Data Deficiency and Environmental Concerns

Most current knowledge about the safety and effectiveness of dental nanoparticles comes from in vitro studies, with limited long-term clinical or in vivo data available.[24] This gap underscores the need for comprehensive clinical trials to assess their real-world impact. Moreover, environmental risks arise from the improper disposal of nanoparticle-containing dental products. These materials can enter aquatic ecosystems and drinking water supplies, potentially causing ecological disruption and raising public health concerns.[25] Implementing stringent disposal protocols and environmental regulations is essential to mitigate such risks.

Trends and Future Directions

Nanodentistry is rapidly evolving, driven by advances in nanoparticle technologies aimed at making dental treatments more precise, functional, and sustainable. One of the most promising trends is the development of smart or responsive nanoparticles. These particles can sense and react to specific triggers within the oral environment. For instance**,** pH-sensitive nanoparticles can release antimicrobial agents only in acidic plaque zones, selectively targeting harmful bacteria while leaving healthy oral flora unaffected. Similarly, photocatalytic titanium dioxide (TiO_2) nanoparticles can be activated by light to provide on-demand disinfection, reducing the need for systemic antibiotics and minimizing potential resistance issues.[26]

Another key advancement lies in the adoption of green synthesis techniques. Instead of relying on toxic chemicals, researchers are using natural plant extracts, enzymes, and biodegradable polymers to produce biocompatible and environmentally friendly nanoparticles.

This approach not only minimizes ecological harm but also supports safer, more sustainable dental materials and treatments.[26]

Looking toward the future, nanorobotics and nanoscale engineering hold transformative potential. Microscopic nanorobots may soon be able to perform highly targeted tasks such as real-time plaque removal, pathogen-triggered drug delivery, and even the autonomous repair of microfractures in enamel or implant materials. These innovations could redefine dental care, making it more personalized, minimally invasive, and efficient.[26]

As research advances, the integration of smart, sustainable, and robotic nanoparticles is expected to significantly improve treatment outcomes, fostering better oral health while reducing both environmental and systemic risks.

Case Study in Practice

A 3-month controlled trial involving 60 pediatric patients using nano-hydroxyapatite toothpaste showed significantly higher enamel remineralization (measured using DIAGNOdent and SEM) compared to controls using standard fluoride paste.[8]

Table 2: Comparative Benefits of Nano-Enhanced Toothpastes[8]

Parameter	Nano-hydroxyapatite Paste	Conventional Fluoride Paste
Caries Arrest Rate	86%	63%
Lesion Mineral Gain	High	Moderate
Patient Acceptance	High	High
Plaque Inhibition	Good	Moderate

Robotics in Nanoparticles

The integration of robotics with nanotechnology has given rise to the field of *nanorobotics*, where nanoscale robots and devices are designed using nanoparticles to perform highly precise tasks in medical, industrial, and environmental applications. These nanorobots, often built from or powered by functionalized nanoparticles such as gold, silica, or magnetic materials, can navigate microscopic environments and execute controlled actions at the cellular or molecular level. In medicine, robotics-driven nanoparticles are revolutionizing targeted drug delivery by acting as intelligent carriers that can autonomously travel through the bloodstream, detect diseased cells, and release therapeutic agents only at specific sites, minimizing systemic side effects. Magnetic and light-responsive nanoparticles, when integrated into nanorobots, allow remote guidance and activation using external fields or stimuli, offering exceptional control in minimally invasive treatments like cancer therapy, gene editing, or microsurgeries. Beyond healthcare, robotics in nanoparticles also plays a critical role in environmental applications, such as detecting and neutralizing toxins or pollutants in water systems through self-propelled nanoswimmers. The autonomous movement of these systems is achieved using catalytic reactions, magnetic propulsion, or ultrasound-driven mechanisms, enabling them to operate without external fuel in complex environments. Moreover, advances in artificial intelligence (AI) and microfabrication have enabled the development of smart nanorobots that can process real-

time data, adapt to changing biological conditions, and even communicate with other nanodevices, enhancing their precision and functionality. These breakthroughs are further supported by the use of biomimetic designs, where nanorobots mimic natural systems like bacteria or sperm cells to improve locomotion and energy efficiency. Despite their vast potential, challenges such as biocompatibility, large-scale manufacturing, energy sourcing, and regulatory hurdles remain significant barriers to their widespread clinical and industrial adoption. However, with rapid progress in materials science, robotics, and AI, nanoparticles integrated into robotic systems are expected to play a transformative role in personalized medicine, smart diagnostics, and environmental sustainability. This synergy between robotics and nanoparticles is steering science toward a future where autonomous, intelligent nanosystems can seamlessly operate inside the human body or the natural environment, performing tasks once thought impossible, from repairing tissues at a molecular level to purifying contaminated ecosystems with unprecedented efficiency.

Summary

Nanoparticles have become a cornerstone in advancing modern dentistry, thanks to their ability to enhance antibacterial activity, improve the mechanical properties of materials, and promote tissue regeneration. Their use spans nearly every discipline, including restorative dentistry, endodontics, periodontics, and prosthodontics, where they help strengthen materials, reduce bacterial colonization, and stimulate healing. Beyond these benefits, the field is rapidly advancing toward *robotics-enabled nanosystems*, representing the next major leap for dental innovation.

Nanorobots, designed from or powered by functionalized nanoparticles such as magnetic or gold-based materials, can autonomously navigate oral environments, targeting diseased tissues, delivering drugs with pinpoint accuracy, and potentially repairing enamel or soft tissue at the microscopic level.Guided by magnetic fields, light, or biochemical signals—and enhanced by artificial intelligence (AI)—these nanosystems promise highly personalized, minimally invasive treatments that reduce systemic drug exposure and speed up recovery.

Despite the promise, several challenges remain. Some nanoparticles, particularly metallic types, can cause cytotoxic effects or trigger allergic responses if not carefully controlled. Environmental safety also remains a concern, as nanoparticle-containing materials may impact ecosystems if improperly disposed of. However, new approaches such as *green synthesis*—which uses plant-based or biodegradable methods—and the development of biodegradable robotic systems are helping to address these issues.

As clinical studies and regulatory standards evolve, and as AI-driven nanorobotics integrates with digital dentistry, these technologies are poised to transform oral healthcare. The combination of nanoparticles and robotics will deliver dental treatments that are not only more effective but also safer, eco-friendly, and tailored to each patient's unique needs—heralding a new era of *smart, sustainable dentistry*.

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PHYSIOTHERAPY APPROACHES IN DEGENERATIVE CEREBELLAR ATAXIA: A NARRATIVE REVIEW

Rucha Agnihotri[1] and Vibhuti Tiwari[2]

[1]Department of Neurophysiotherapy

Dhaneshwari College of Physiotherapy, Aurangabad, Maharashtra

[2]Department of Cardiorespiratory Physiotherapy,

MGM Institute of Physiotherapy, Aurangabad, Maharashtra

Corresponding author E-mail: ruchagnihotri11@gmail.com, tiwari98vibhuti@gmail.com

Abstract:

Degenerative cerebellar ataxia (DCA) is a group of progressive neurodegenerative disorders characterized by impairments in coordination, balance, speech, and gait, due to dysfunction of the cerebellum and its connections. DCA encompasses both inherited forms, including spinocerebellar ataxias (SCAs), and sporadic variants, such as the cerebellar subtype of multiple system atrophy (MSA-C). Although physiotherapy has emerged as a promising intervention, there is a lack of standardized, ataxia-specific rehabilitation protocols. Current literature, including recent meta-analyses and systematic reviews, shows modest improvements in ataxia severity and functional performance with interventions such as balance training, aerobic exercise, virtual reality, and cueing techniques. Yet, limitations such as inadequate sample sizes, brief follow-up durations, and methodological inconsistencies restrict the broader applicability of current findings. Furthermore, high heterogeneity in intervention types and outcomes, along with underutilization of technological tools like wearables and telerehabilitation, represents critical research and practice gaps. This narrative review aims to summarize existing physiotherapy interventions for DCA, evaluate evidence from clinical studies, highlight challenges in real-world practice, and offer recommendations for personalized, technology-integrated rehabilitation strategies. Emphasis is placed on the importance of early intervention, standardized outcome measures, and caregiver engagement to enhance therapy adherence and functional gains. The review concludes with a call for multi-center trials and the development of core outcome sets to inform future evidence-based clinical guidelines and bridge the gap between research and practice in cerebellar ataxia rehabilitation.

Keywords: Cerebellar Ataxia, Neurorehabilitation, Balance Training, Physiotherapy, Virtual Reality, Motor Learning

Introduction:

1. **Background on Degenerative Cerebellar Ataxia (DCA) -**

Degenerative cerebellar ataxia (DCA) refers to a heterogeneous group of progressive neurological disorders that affect the cerebellum and its afferent and efferent pathways, leading to impaired motor coordination, postural control, and gait stability.[1,2] These disorders include

hereditary forms—such as spinocerebellar ataxias (SCAs), Friedreich's ataxia, and ataxia telangiectasia—and sporadic types like multiple system atrophy-cerebellar type (MSA-C), idiopathic late-onset cerebellar ataxia (ILOCA), and sporadic adult-onset ataxia (SAOA).[3],[4] The global prevalence of hereditary ataxias varies by region and population, while sporadic forms often remain underdiagnosed due to overlapping clinical presentations and the lack of universal diagnostic criteria.[1],[4] Degeneration of cerebellar neurons, particularly Purkinje cells, results in hallmark symptoms such as limb and trunk ataxia, dysmetria, dysarthria, dysdiadochokinesia, gaze-evoked nystagmus, and an unsteady, wide-based gait.[5],[6] As the disease advances, individuals often experience growing difficulty with daily tasks, a heightened risk of falling, and eventual dependence on others, which can contribute to social withdrawal.[7],[8]

2. Pathophysiology and Clinical Manifestations -

Beyond coordinating voluntary movement, the cerebellum also plays critical roles in motor learning, spatial awareness, postural control, and anticipatory movement regulation. Its damage disrupts sensorimotor integration, feedforward processing, and adaptation to environmental demands. In SCAs, genetic mutations such as CAG trinucleotide repeat expansions lead to cerebellar and spinal degeneration, while in MSA-C, widespread neurodegeneration affects the cerebellum, basal ganglia, and autonomic nervous system.[3],[10] Clinically, patients with DCA present with ataxic gait, reduced joint position sense, poor postural adjustments, speech disturbances, and difficulty executing fine motor tasks.[26],[11] As the disease progresses, symptoms extend to involve autonomic dysfunction, spasticity, and cognitive decline in certain subtypes. Even with progress in diagnostic tools like imaging and genetic analysis, current treatment strategies remain largely supportive in nature.[4], [12]

3. The Role and Relevance of Physiotherapy in DCA -

Given the limited effectiveness of pharmacological interventions in modifying disease progression, physiotherapy has become the mainstay of non-pharmacological management in DCA.[13], [14] Physiotherapeutic interventions aim to maintain or improve motor performance, reduce fall risk, and prolong functional independence through task-specific, evidence-informed strategies. The cerebellum retains some capacity for neuroplastic adaptation, which forms the foundation for physiotherapy approaches aimed at maximizing functional compensation.[9],[15] Studies have consistently shown that targeted physiotherapy—particularly when intensive, repetitive, and task-specific—can lead to measurable improvements in gait, balance, coordination, and trunk control, even in chronic or advanced stages of disease.[6],[16] Ilg *et al.* (2009, 2010) and Miyai *et al.* (2012) demonstrated that intensive coordinative and balance training programs produced durable benefits in ataxic gait and motor precision.[6],[17],[18] A wide variety of physiotherapeutic modalities have been explored for this population: •

Physiotherapeutic Modalities in DCA:

1. **Balance and Postural Control Training:** Foundational in all DCA programs; helps reduce fall risk.[16], [19]
2. **Coordinative and Motor Re-learning Exercises:** Including Frenkel exercises and dual-task activities.[18], [20]
3. **Aerobic and Endurance Training:** Enhances cardiovascular fitness, stamina, and fatigue management.[21]
4. **Virtual Reality (VR) and Exergaming:** Increases engagement and provides real-time feedback to improve motor learning.[22]
5. **Motor Imagery (MI):** Especially useful when physical activity is limited due to fatigue or disease severity.[23]
6. **Rhythmic Auditory Stimulation (RAS):** Helps improve gait timing and cadence.[24]
7. **Sensory Substitution and Biofeedback:** Such as electrotactile tongue biofeedback for balance.[25]
8. **Telerehabilitation:** Remote physiotherapy improves accessibility, especially in low-resource areas.[26]

Moreover, meta-analyses and systematic reviews have confirmed the moderate-to-high effectiveness of physiotherapy in reducing ataxia severity and improving mobility-related outcomes.[13], [27], [28] However, rehabilitation for DCA remains underutilized in many settings due to lack of awareness, standardized protocols, and clinician training.[29], [30] There is also a need for wider adoption of innovative technologies such as wearable sensors, motion tracking, and tele-supervised programs.[31], [32]

4. Aim of the Narrative Review –

Despite emerging evidence, rehabilitation in cerebellar ataxia is not uniformly practiced due to variability in study design, intervention methods, and clinical settings. This narrative review aims to synthesize current evidence on physiotherapy-based interventions in degenerative cerebellar ataxia. It seeks to evaluate established and emerging modalities, discuss their clinical impact, identify barriers to implementation, and propose practical recommendations for future research and clinical application. Emphasis is placed on early intervention, personalization of rehabilitation programs, and the integration of technology to maximize functional outcomes in patients with cerebellar ataxia.

Methodology

A narrative synthesis approach was adopted to explore the range and impact of physiotherapy interventions in individuals with degenerative cerebellar ataxia. The review was conducted following a structured and systematic literature retrieval process while maintaining the flexibility necessary for narrative analysis.

Electronic searches were carried out in databases including PubMed**,** Scopus, and Google Scholar to identify relevant peer-reviewed literature published between January 2000 and March 2025. The search strategy incorporated combinations of the following keywords: "degenerative cerebellar ataxia," "spinocerebellar ataxia," "physiotherapy," "rehabilitation," "motor learning," "balance training," "virtual reality," "telerehabilitation," "motor imagery," "rhythmic auditory stimulation," and "neurorehabilitation."

Studies were included based on the following criteria:

1. Involved participants diagnosed with DCA or related hereditary/sporadic cerebellar ataxias.
2. Evaluated the impact of physiotherapy, neurorehabilitation, or motor-based interventions either alone or in combination with assistive technologies.
3. Were published in English in peer-reviewed journals.
4. Included randomized controlled trials (RCTs), systematic reviews, meta-analyses, or high-quality observational studies.

The exclusion criteria comprised:

- Non-peer-reviewed publications, including editorials, opinion pieces, and single-patient case reports.
- Conference proceedings without full-text availability.
- Studies not involving physiotherapeutic or rehabilitative interventions.
- Non-English literature.

A total of 60 articles were initially screened, from which 38 were deemed eligible based on relevance to physiotherapeutic management in DCA. The data extraction focused on study objectives, sample characteristics, types of interventions, outcome measures (e.g., SARA, BBS, and 10MWT), follow-up duration, and reported efficacy.

Given the variability in intervention types, durations, and outcomes across studies, meta-analysis was not conducted. Instead, a qualitative synthesis was carried out to identify patterns in evidence, gaps in implementation, and implications for clinical practice. The review was framed in alignment with the aims of narrative reviews — to generate insight, integrate findings across disciplines, and suggest future directions for clinical innovation and research standardization.

Flowchart: Literature Screening and Selection Process

Records identified through database searching
(PubMed, Scopus, Google Scholar)
|
▼
Total articles retrieved (n = 60)
|
▼
Titles and abstracts screened for relevance
|
▼
Records excluded based on title/abstract (n = 14)
|
▼
Full-text articles assessed for eligibility (n = 46)
|
▼
Full-text articles excluded based on inclusion criteria (n = 10)
|
▼
Studies included in the narrative review (n = 36)

Current Physiotherapy Approaches in Cerebellar Ataxia

Current Physiotherapy Approaches in Cerebellar Ataxia

Physiotherapy remains central to the management of Degenerative Cerebellar Ataxia (DCA), offering multiple targeted strategies aimed at improving balance, gait, coordination, and overall functional capacity. A wide range of conventional and novel interventions have been explored, with varying degrees of evidence supporting their clinical application. The following sections outline ten commonly studied physiotherapy approaches for cerebellar ataxia, based on current literature.

1. Balance and Postural Control Training: Balance training is a primary focus of physiotherapy in DCA, given the significant fall risk and postural instability associated with cerebellar dysfunction. Structured postural control interventions have shown measurable outcomes in reducing falls and enhancing equilibrium. Evidence suggests significant improvements in balance and dynamic gait stability following coordinative exercises aimed at trunk and limb control.[16] These exercises often include weight shifting, dynamic stability tasks, and proprioceptive training on unstable surfaces. Functional gains in static and dynamic balance

have also been observed after targeted trunk stabilization protocols, which aim to enhance core engagement and trunk rigidity essential for maintaining upright posture during complex motor activities.[19]

2. Coordinative and Task-Specific Training: Task-specific coordinative exercises have been extensively investigated for their potential to retrain impaired motor control in DCA. Interventions such as repeated reaching tasks, sequencing exercises, and joint-specific coordination drills have shown improvements in movement accuracy and fluidity. These exercises reinforce motor planning and execution pathways by providing consistent sensory feedback. Individualized coordinative training has been found to improve upper and lower limb coordination, with benefits persisting over time due to the reinforcement of neuromotor control and learned motor patterns.[18], [17] These findings highlight the effectiveness of task-specific approaches in enhancing motor precision and overall voluntary motor performance.

3. Aerobic and Endurance Conditioning: Aerobic training addresses fatigue, deconditioning, and gait inefficiencies in cerebellar ataxia. Interventions like treadmill walking and home-based cycling have shown that moderate aerobic conditioning can improve endurance, walking capacity, and overall quality of life by promoting cardiovascular fitness and muscular efficiency. Improvements in walking speed and cardiovascular endurance have also been reported following structured aerobic programs, which contribute to better energy utilization and delayed onset of fatigue during daily functional tasks.[21] This also supports psychological well-being by enhancing patient engagement and motivation.

4. Virtual Reality and Exergaming: Technological tools such as virtual reality (VR) and exergaming offer interactive and engaging methods to promote motor learning. These platforms provide real-time feedback and sensory stimulation beneficial for motor adaptation, improving error detection and correction mechanisms in individuals with cerebellar deficits. VR-based motor training games have been shown to enhance both postural control and limb coordination by immersing the individual in dynamic, multi-sensory environments that encourage repetition and variability of movement patterns.[22]

5. Motor Imagery and Cognitive Strategies: Motor imagery (MI)—the mental simulation of movement without actual execution—has been used to activate motor networks and promote neuroplasticity. It is particularly useful for individuals unable to perform physical tasks consistently due to fatigue or advanced disease. MI reinforces motor planning and execution circuits and may serve as a supportive strategy to enhance motor learning, especially when used in conjunction with physical practice.[23] This method taps into the brain's internal modeling system, which helps refine motor commands even in the absence of overt movement.

6. Rhythmic Auditory Stimulation (RAS): RAS involves the use of rhythmic auditory cues to support gait timing and coordination. It has been shown to reorganize motor output by improving cadence and gait symmetry, based on principles of auditory-motor coupling.[24] Patients learn to

synchronize their steps with external auditory cues such as metronomes or music, facilitating smoother and more controlled gait patterns. This technique is especially beneficial in enhancing temporal regularity and step predictability.

7. Biofeedback and Sensory Substitution: Biofeedback mechanisms and sensory substitution devices help compensate for proprioceptive and postural deficits in cerebellar ataxia. These tools provide visual, auditory, or tactile input to substitute for impaired internal sensory feedback. Electrotactile tongue stimulation, for instance, has demonstrated improvements in balance, especially in individuals with impaired proprioception.[25] By stimulating alternate sensory pathways, patients are better able to perceive body position in space and make necessary postural adjustments.

8. Telerehabilitation and Remote Physiotherapy: Telerehabilitation platforms deliver consistent physiotherapy care remotely, ensuring continuity and adherence to therapy plans, especially in resource-limited or geographically isolated settings. This approach is feasible and acceptable among patients with cerebellar symptoms and helps overcome logistical and geographical barriers that often limit in-person visits.[26] Virtual supervision and remote monitoring enable therapists to provide timely feedback and adjust interventions as needed, maintaining therapeutic gains over time.

9. Gaze Stability and Oculomotor Rehabilitation: Gaze stability and oculomotor control impairments, such as saccadic dysmetria and oscillopsia, are common in cerebellar disorders. Physiotherapy-based gaze stabilization exercises have shown improvements in head movement coordination and postural control by targeting vestibulo-ocular reflex mechanisms.[15], [16] These exercises involve controlled head and eye movements that reinforce visual fixation and reduce symptoms of dizziness and instability. Vestibular rehabilitation techniques have also demonstrated effectiveness in improving visual fixation and reducing the impact of oculomotor disturbances on daily activities.

10. Coordination and Dysmetria-Oriented Training: Dysmetria disrupts amplitude control during voluntary movements, resulting in over- or undershooting of intended targets. Repetitive, visually guided, and task-specific training improves limb incoordination by reinforcing accurate movement trajectories and motor calibration. Coordination training enhances movement smoothness and accuracy, contributing to functional gains even in progressive cases.[18], [17] The incorporation of visual cues, graded resistance, and manual guidance helps optimize neuromuscular control and reduce compensatory patterns.

Physiotherapy remains central to the management of Degenerative Cerebellar Ataxia (DCA), offering multiple targeted strategies aimed at improving balance, gait, coordination, and overall functional capacity. A wide range of conventional and novel interventions have been explored, with varying degrees of evidence supporting their clinical application. The following

sections outline ten commonly studied physiotherapy approaches for cerebellar ataxia, based on current literature.

Clinical Summary of Physiotherapy Modalities in Cerebellar Ataxia: Study Types, Outcomes, and Findings:

No.	Intervention Type	Author, Year	Study Type	Outcome Measures	Key Results
1	Balance and Postural Control Training	Nardone *et al.* (2014)	RCT	BBS, TUG, Dynamic Gait Index	Improved static and dynamic balance; reduced fall risk
2	Coordinative and Task-Specific Training	Ilg *et al.* (2009, 2010)	RCT, Longitudinal study	SARA, ICARS, BBS	Sustained motor and trunk coordination improvements
3	Aerobic and Endurance Conditioning	Milne *et al.* (2017)	Systematic Review	6MWT, VO_2max, SARA	Enhanced endurance, gait capacity, and cardiovascular fitness
4	Virtual Reality and Exergaming	Ilg *et al.* (2012)	Controlled Trial	BBS, SARA, ICARS	Improved limb coordination and postural control
5	Motor Imagery and Cognitive Strategies	Hétu *et al.* (2013)	Meta-analysis	fMRI activation, Coordination tests	Activated motor circuits, improved motor planning
6	Rhythmic Auditory Stimulation (RAS)	Thaut & Abiru (2010)	Review	Cadence, Gait speed, Stride length	Better gait symmetry, cadence, and timing
7	Biofeedback and Sensory Substitution	Cakrt *et al.* (2012)	RCT	BBS, Center of Pressure (COP)	Improved balance with electrotactile tongue feedback
8	Telerehabilitation and Remote Physiotherapy	Mitchell *et al.* (2019)	Implementation Model	Adherence rates, Function scores	Increased accessibility and patient compliance
9	Gaze Stability and Oculomotor Rehab	Mitoma & Manto (2016), Nardone *et al.* (2014)	Clinical Review, RCT	Visual Fixation, Head movement tests	Improved gaze stabilization and visual focus
10	Coordination and Dysmetria Training	Ilg *et al.* (2009, 2010)	Longitudinal RCT	SARA, Target accuracy	Improved movement accuracy, reduced dysmetria

Challenges and Barriers to Physiotherapy Implementation in Degenerative Cerebellar Ataxia

1. Lack of Awareness and Training: Many physiotherapists receive minimal education on ataxia-specific rehabilitation techniques during their training.[7]
2. Limited Access to Technology: Advanced tools like virtual reality, biofeedback systems, and tele-rehabilitation platforms remain costly and inaccessible, particularly in low- and middle-income countries (LMICs).[24]
3. Patient-Related Barriers: Issues such as fatigue, fear of falling, poor balance confidence, and low motivation hinder active participation in rehabilitation programs.[25]
4. Caregiver Dependence: Successful execution of home-based rehabilitation often depends on caregivers, whose availability, understanding, or training may be limited.[31]
5. Inconsistent Outcome Measures: A wide variety of tools (e.g., SARA, BBS, 10MWT) are used to assess treatment outcomes, making inter-study comparison difficult.[2]
6. Short-Term Focus in Existing Literature: Most studies evaluate only immediate effects, with limited long-term follow-up, making it unclear if benefits are sustained.[13]
7. Heterogeneity of DCA Subtypes: Subtypes like SCA1, SCA3, or Friedreich's Ataxia have diverse clinical profiles, requiring highly individualized treatment approaches.[6]
8. Lack of High-Quality Randomized Controlled Trials (RCTs): Many available studies are case series or pilot trials with small sample sizes and lack control groups.[9]
9. Poor Integration into Multidisciplinary Teams: Physiotherapy is often not coordinated with neurologists, occupational therapists, or speech therapists, leading to fragmented and less effective care.[7]
10. Limited Research on Pediatric and Early-Onset Ataxias: Current evidence mainly targets adult populations, leaving gaps in pediatric-specific rehabilitation protocols.[36]
11. Variability in Intensity and Duration of Therapy: There is no standardized guideline on optimal frequency or duration of therapy sessions, complicating program design and patient adherence.[26]
12. Psychological and Emotional Barriers: Depression and anxiety, common in DCA, can reduce motivation and therapy engagement.[2]
13. Language and Cultural Inappropriateness of Assessment Tools: Many outcome tools lack linguistic or cultural validation in non-Western settings, limiting their utility in LMICs.
14. Lack of Follow-Up and Continuity of Care: Structured post-discharge support or continued rehabilitation services are often lacking, resulting in functional decline over time.[13]
15. Financial and Insurance Barriers: Physiotherapy services may not be covered by insurance in many regions, placing a financial burden on patients and limiting access.[28]
16. Absence of Ataxia-Specific Clinical Guidelines: There is a scarcity of consensus-based physiotherapy protocols specific to cerebellar ataxias, leading to inconsistent practice.[27]

Recommendations for Research and Practice:

To strengthen the evidence base and clinical effectiveness of physiotherapy interventions for Degenerative Cerebellar Ataxia (DCA), a series of research and practice-driven strategies are recommended:

1. **Development of Core Outcome Sets**: Current literature lacks consistency in outcome reporting, using varied scales such as the Scale for the Assessment and Rating of Ataxia (SARA), Berg Balance Scale (BBS), and Timed Up and Go (TUG). Establishing a core set of functional and quality-of-life outcomes would improve the comparability of studies and facilitate meta-analyses and systematic reviews.[2]
2. **Longitudinal, Multi-Center Trials**: Most available evidence derives from small, short-term, and often single-center trials. To determine the durability of physiotherapy effects and their impact on long-term quality of life, future research should focus on well-designed, multicenter randomized controlled trials (RCTs) with extended follow-up durations.[6],[9]
3. **Integration of Technology into Clinical Practice**: Incorporating virtual reality (VR), electrotactile feedback (e.g., tongue biofeedback), and wearable motion sensors into therapy has shown promising results in improving balance and coordination in ataxia patients. However, scalable, affordable versions of these technologies should be developed and validated for routine use, especially in low-resource settings.[5],[24]
4. **Specialized Training Modules for Physiotherapists**: Physiotherapists often lack structured training on ataxia-specific rehabilitation strategies. Developing continuing education programs, certifications, and skill-building workshops would enhance clinical preparedness. These modules should incorporate evidence-based techniques such as balance training, Frenkel exercises, and cueing strategies.[7],[3]
5. **Patient and Caregiver Education:** Empowering patients and caregivers through educational resources about the nature of cerebellar ataxia and the importance of sustained physiotherapy may improve motivation and adherence. Community-based programs and digital platforms can serve as means to increase disease awareness and promote participation.[31]
6. **Tele-rehabilitation and Hybrid Models:** The COVID-19 pandemic highlighted the value of remote rehabilitation. Developing evidence-based tele-rehabilitation protocols supported by regular virtual assessments and therapist feedback can help maintain therapy continuity. Hybrid models combining in-clinic and at-home rehabilitation may be especially useful for patients in rural or underserved areas.[14]
7. **Personalized and Stage-Specific Protocols**: As the clinical manifestations of DCA vary across individuals and progress over time, rehabilitation strategies should be tailored to the patient's motor and cognitive status, comorbidities, and psychosocial context. Early-

stage interventions might focus on motor control and balance retraining, while later-stage approaches may emphasize fall prevention and caregiver support.[17],[18]

Conclusion:

Physiotherapy remains central to the non-pharmacological management of degenerative cerebellar ataxia. Balance training, aerobic conditioning, VR, MI, RAS, and home-based care models have shown promising outcomes in improving motor performance and quality of life. However, real-world implementation is challenged by limited access, training, and outcome standardization. To overcome these barriers, future efforts must emphasize clinical education, integration of digital tools, and global research collaborations aimed at building scalable, patient-centered rehabilitation systems for individuals with cerebellar ataxia.

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COVID-19 VACCINATION IN INDIA: THE JOURNEY FROM HESITANCY TO IMMUNITY, CHALLENGES, STRATEGIES AND FUTURE DIRECTIONS

Tejas B. Chaudhari[1], Anjali S. Patil[1], Leena P. Shirsath[1], Laxmikant R. Zawar[2], Dipak E. Chavan[3] and Sandip P. Patil*[1]

[1]Department of Microbiology and Biotechnology,
R. C. Patel Arts, Commerce and Science College, Shirpur-425405, India
[2]Department of Pharmaceutics,
H. R. Patel Institute of Pharmaceutical Education and Research, Shirpur-425405, India
[3]Department of Computer and Data Science,
R. C. Patel Arts, Commerce and Science College, Shirpur-425405, India
*Corresponding author E-mail: patilsandip3@gmail.com

Abstract:

This comprehensive review explores the development, distribution and impact of COVID-19 vaccines in India. It examines the major vaccines administered, including Covishield and Covaxin, detailing their efficacy, safety profiles and the logistics of their rollout. The highlights of this review include India's vaccination strategy, encompassing production, procurement and the challenges faced that includes vaccine hesitancy, misinformation and supply chain issues. The role of governmental policies, international collaborations and the contributions of India's pharmaceutical industry are also discussed. It also sheds light on the nation's capacity for large-scale vaccine production. Additionally, the review assesses the public health outcomes and socio-economic implications of the vaccination drive, with a particular focus on its role in mitigating the spread of the virus and reducing severe cases and mortality rates. By reviewing data from various studies and official reports, the present study provides a critical understanding of India's efforts in combating the COVID-19 pandemic through vaccination. It also highlights lessons learned, offering valuable insights into future pandemic preparedness, global health security and strategies for rapid vaccine deployment in large and diverse populations.

Keywords: COVID-19; Covishield; Covaxin; Immunity; Pandemic

1. Introduction:

India's COVID-19 vaccination program was launched on January 16, 2021 that aimed to protect the population by vaccinating as many people as possible. Initially, healthcare workers and frontline staff were prioritized, followed by the elderly and those with medical conditions. India used vaccines like Covishield and Covaxin to help the body fight against virus. Despite logistical challenges like maintaining proper storage temperatures and ensuring fair distribution, the government used technology and public figures to promote vaccination [1]. While millions were vaccinated, some hesitancy remained. India also aided other countries, showcasing the

value of global collaboration during the pandemic. According to Indonesia's Ministry of Health (2020), COVID-19, caused by SARS-CoV-2, is a zoonotic disease that spreads between animals and humans. In January 2020, WHO declared it a Public Health Emergency of International Concern, later declaring it as a pandemic in March. COVID-19 affects people of all ages and spreads mainly through inhalation or contact with droplets [2]. With a 2-14 day incubation period, it often causes mild symptoms in adults [3]. Infected children may be asymptomatic or experience symptoms like fever, respiratory issues and gastrointestinal problems. Most recover quickly, though lung abnormalities may persist for months [4]. Vaccination is vital for managing and overcoming pandemics like COVID-19. It helps prevent virus spread by creating collective immunity, making it harder for the virus to transmit between people. Vaccines prepare the immune system to fight the virus, preventing severe symptoms, hospitalization and death, especially in high-risk groups like the elderly. By reducing severe cases, vaccination eases pressure on healthcare systems. It also interrupts virus transmission, limiting opportunities for replication and mutation. On a global scale, widespread vaccination is key to controlling the pandemic and returning to normal life.

2. Types of Vaccines Introduced in India

2.1 Covaxin

Bharat Biotech developed Covaxin in collaboration with the Indian Council of Medical Research (ICMR) is an inactivated virus-based vaccine targeting SARS-CoV-2. Codenamed as BBV152, the vaccine is administered intramuscularly and was approved by the Drug Controller General of India (DCGI) in June 2020 following Phase I and II trials. Phase III trials showed a 78% efficacy rate, with earlier phases revealing stronger immune responses over time [5]. Covaxin uses inactivated Vero cell technology, a proven method used in many vaccines and remains stable at 2-8°C. It offers 63% efficacy in asymptomatic cases, 78% in mild to severe cases and 65% against the Delta variant [6]. Notably, it has a 93% efficacy rate against severe COVID-19 cases and has shown effectiveness against Alpha, Beta and Delta variants [7]. The vaccine is suitable for all age groups, including children and has mild side effects such as injection site pain, fever and headaches. Covaxin is considered a safer option for those sensitive to vaccines with rare blood clotting risks [8]. Emergency approval has been granted in countries like Venezuela, Iran and Nepal, expanding its global reach [9].

2.1.1 Mode of Action

Covaxin operates through a traditional approach of utilizing an inactivated form of the SARS-CoV-2 virus. When administered, Covaxin introduces this inactivated virus into the body, prompting the immune system to recognize it as foreign. In response, the immune system generates antibodies and activates T-cells to combat the perceived threat. This process establishes immune memory, enabling the body to swiftly recognize and neutralize the live virus in the future, thus conferring protection against COVID-19.

2.1.2 Clinical Trial Data on Efficacy and Safety

Bharat Biotech concludes that the final analysis of Covaxin phase III clinical trial efficacy was 77.8%, effective against symptomatic COVID-19, 93.4% effective against severe symptomatic COVID-19, efficacy against asymptomatic COVID-19 63.6%, efficacy data demonstrates 65.2% protection against the SARS-CoV-2 Delta variant (B.1.617.2) [10]. The study evaluates efficacy of Covaxin in preventing breakthrough SARS-CoV-2 infections among healthcare workers (HCWs) at AIIMS Bhubaneswar. Covaxin was created by the Indian Council of Medical Research (ICMR) and Bharat Biotech, uses the Whole-Virion inactivated Vero cell-derived platform technology [11]. Covaxin was initially approved for emergency use in January 2021, demonstrated an 81% efficacy rate in interim analyses during Phase 3 trials. The antibodies were found to neutralize several SARS-CoV-2 variants. However, the study found that vaccinated healthcare workers are less effective at preventing breakthrough infections. Clinical Trial of Covaxin, developed by ICMR and Bharat Biotech, shows 81% efficacy. Several factors contribute to the observed low efficacy. First, the study emphasizes the presence of mutant strains, particularly those classified as 'Variants of Concern' by the World Health Organization. These variants pose global health risks and may affect the efficacy of vaccine. While in-vitro studies suggested that Covaxin-induced antibodies could neutralize beta and delta variants, their real-world effectiveness is uncertain [12]. Finally, the study suggests that factors such as mutant strains and evolving virus dynamics may influence the efficacy of Covaxin in preventing breakthrough infections among healthcare workers.

2.2 Covishield

Covishield developed by the Serum Institute of India in collaboration with AstraZeneca-Oxford, is a promising solution for combating the COVID-19 pandemic [13,14]. This recombinant vaccine employs a chimpanzee adenovirus vector that encodes the SARS-CoV-2 S glycoprotein [15]. Covishield is licensed in both the United Kingdom and India, uses genetically engineered and weakened adenovirus strains to ensure safety while triggering an immune response. Each dose contains 5×10^{10} ChAdOx1-S virus particles and essential ingredients [16]. The vaccination regimen consists of two intramuscular doses of Covishield, which are recommended to be given 4-6 weeks apart [17]. Notably, its storage temperature of 2-8°C makes it easier to distribute and access. The approval of Covishield and formulation highlight its potential as a critical tool in global COVID-19 vaccination efforts, bringing hope for a better future.

2.2.1 Mode of Action

Covishield employs a different strategy known as viral vector technology. This vaccine utilizes a harmless chimpanzee adenovirus vector that carries genetic material encoding the spike protein of SARS-CoV-2. Upon vaccination, the vector enters human cells and instructs them to produce the spike protein. Subsequently, the immune system identifies this spike protein as

foreign and mounts an immune response, generating antibodies and activating T-cells. Similar to Covaxin, this immune response establishes memory, preparing the body to efficiently respond to future encounters with the live virus, thereby providing protection against COVID-19.

2.2.2 Clinical Trial Data on Efficacy and Safety

The COVID-19 pandemic highlighted the urgent need for vaccines to protect healthcare workers (HCWs) and the public. The World Health Organization (WHO) declared COVID-19 a pandemic in March 2020, emphasizing the millions of cases and deaths globally. Vaccine development became a priority to prevent infection and reduce the risks HCWs face, such as illness and stress. WHO accelerated vaccine timelines, cutting the usual development process from years to months [18]. One of the promising vaccines is Covishield, developed by the Serum Institute of India with AstraZeneca/Oxford University. It demonstrated strong results in clinical trials and was approved for emergency use. Studies are assessing its safety, immunogenicity and efficacy in high-risk populations like HCWs. India's vaccination campaign began in January 2021, starting with healthcare and frontline workers and later expanding to the elderly and those with health risks [19]. Covishield, used in 88% of India's vaccinations, has shown 67% efficacy in preventing symptomatic COVID-19 and nearly 100% efficacy in preventing severe infection and hospitalization after the second dose. Real-world studies are essential to confirm its effectiveness in broader populations beyond clinical trials.

2.2.3 Comparison between Covishield and Covaxin

The rapid development and deployment of COVID-19 vaccines in India have been crucial in combating the pandemic, offering hope during challenging times. The country's vaccination campaign relies mainly on two vaccines: Covishield (ChAdOx nCoV-19) and Covaxin (BBV152) [20]. Interim analyses of clinical trials revealed promising efficacy rates, with Covishield showing 70.4% efficacy and Covaxin 77.8% against symptomatic COVID-19 [21]. Despite the emergence of the delta variant during India's second wave, both vaccines continued to provide sufficient protection, with Covishield and Covaxin demonstrating 67.0% and 65.2% efficacy, respectively, against the variant. Real-world data show that vaccinated individuals who experience breakthrough infections have lower mortality rates than unvaccinated individuals. A retrospective analysis comparing Covishield and Covaxin recipients in hospitals found no significant difference in severe outcomes, such as mortality, need for mechanical ventilation, or length of hospital stay. Both vaccines have proven effective in reducing severe COVID-19 outcomes, but ongoing monitoring is essential to assess their effectiveness in diverse populations and against new variants [22].

3. Challenges and Solutions

3.1 Overcoming Vaccine Hesitancy

Vaccine hesitancy is a complex challenge in meeting immunization targets, as seen in the Malappuram district of Kerala, India. While religion has a minor impact on vaccination

resistance, factors such as distrust between careers and health workers, negative experiences with vaccines and misinformation spread via social media all play a significant role. Despite support of religious leaders for vaccination efforts, reported side effects, concerns about vaccine safety and procedures and myths about vaccines fuel parental skepticism. Herd immunity can only be achieved through vaccination programs if there is enough population coverage, which is dependent on people's willingness to accept vaccines. However, vaccine hesitancy presents a significant challenge to achieving this goal [23]. Recent surveys conducted in Europe and around the world revealed varying levels of vaccine acceptance. In Europe, a sizable majority expressed a willingness to receive the COVID-19 vaccine, with only a minority expressing reservations or refusal [24]. In contrast, a global survey found that attitudes towards vaccination in India were mixed, with a significant proportion unsure or disagreeing. The decisions of individuals to accept, delay, or refuse vaccination are influenced by a variety of factors, including history, socioeconomic status, culture and politics. Understanding and addressing these determinants is critical for effective vaccination campaigns and herd immunity against infectious diseases such as COVID-19 [25]. To overcome vaccine hesitancy, tailored educational materials for healthcare workers, as well as social science-informed strategies, must be implemented, considering the contextualized social realities that influence vaccination decisions [26].

3.2 Managing Logistics and Distribution Issues

The administration of vaccines, especially during the COVID-19 pandemic, requires strict adherence to cold chain logistics to maintain their potency and effectiveness [27]. Proper storage, monitoring and timely use are critical to preserving vaccine immunogenicity and minimizing waste. Cold chain management demands robust infrastructure and precise coordination, whether in standard or deep-freeze conditions [28]. However, vaccine production, transportation and distribution often face challenges due to insufficient cold chain equipment, leading to deviations from quality standards during shipment [29]. Supply-chain managers play a key role in maintaining vaccine integrity and adapting to changing storage needs. Critical aspects of vaccine distribution include securing approvals, identifying transportation corridors and assessing government and NGO capacities. The cold chain involves multiple stakeholders, such as senders, transportation companies and ground handling agents, all working together to ensure safe delivery [30]. Any disruptions can jeopardize vaccine efficacy. Organizations like the International Air Transport Association (IATA) help transport vaccines, with trained personnel managing refrigeration during flights. On arrival, local transport companies provide refrigerated trucks and customs clearance is facilitated by officials [31]. Cold chain challenges are prevalent in countries like Ethiopia, highlighting the need for improvements. Promising innovations, such as solar-powered cold storage, demonstrate the potential for ensuring vaccine integrity, particularly in remote areas [32].

3.3 Increasing Vaccine Coverage

From February 4 to March 2, 2021, it is surveyed nursing homes with low (<35%), medium (40%-60%) and high (>75%) staff vaccination coverage to gather data on facility strategies to promote vaccination. Cases were respondents with medium to high vaccination coverage, while controls had low coverage. Use of logistic regression modeling, which was adjusted for county and nursing homes characteristics, to identify strategies associated with facility-level vaccination coverage. The primary goal of this study was to evaluate vaccination rates among residents and staff in response to COVID-19 vaccination efforts. This was accomplished by analyzing information from residents' electronic medical records and facility logs for staff. The study used mixed-effects generalized linear models with a binomial distribution to compare vaccination outcomes across study arms, using an intent-to-treat approach [33]. Specifically, the study sought to determine whether there were differences in vaccination rates between the arms [34].

4. Public Health Impact

4.1 Reduced COVID-19 Incidence

The SARS-CoV-2 pandemic has posed unprecedented challenges to global public health and economies, with over 239 million cases and nearly 4.9 million deaths as of October 2021 [35]. To tackle this crisis, a range of containment and mitigation strategies have been implemented to slow transmission, ease the burden on healthcare systems and protect vulnerable populations [36]. These strategies include vaccination campaigns and non-pharmaceutical interventions like lockdowns, social distancing and mask mandates [37]. While vaccination has saved lives, its effectiveness in preventing transmission remains uncertain, especially with emerging variants. Public health measures will continue to be essential until herd immunity is achieved, particularly in regions with low vaccination rates [38]. Non-pharmaceutical interventions, crucial for managing respiratory infections, face challenges in their long-term sustainability due to the high transmissibility of SARS-CoV-2 [39]. Though universal lockdowns have helped reduce mortality, they are not a viable long-term solution, highlighting the need for tailored strategies that balance disease control with societal needs [40]. The effectiveness of these interventions varies significantly across regions, complicating efforts to determine best practices. A systematic review and meta-analysis were conducted to evaluate the impact of public health measures on reducing COVID-19 transmission, incidence and mortality, offering evidence to guide future policy decisions and pandemic responses [41].

4.2 Impact on Severe Cases and Hospitalizations

Data from the client-patient record system (CPRS) included demographic details such as age, gender, presenting symptoms, duration of symptoms, comorbidities, anthropometric measurements, blood pressure, baseline oxygen saturation (SpO_2) and lab test results. According to the AD Association's Diabetes Care (2020), laboratory evaluations covered glycated

hemoglobin (HbA1c), cell counts, liver enzymes (ALT and AST), renal and thyroid function tests and inflammatory markers such as C-reactive protein (CRP), IL-6, ferritin, LDH, D-dimer and procalcitonin. Patients with diabetes (HbA1c ≥ 6.5%) were classified as diabetic, while those needing insulin without meeting diabetes criteria were considered new-onset hyperglycemic [42]. COVID-19 severity was assessed using the WHO ordinal scale, categorizing patients by oxygen and ventilator needs. Mild cases were in categories 3 (no oxygen therapy) and 4 (oxygen by mask or nasal prongs), while severe cases were in categories 5 (non-invasive ventilation), 6 (intubation), 7 (ventilation plus organ support) and 8 (death). The highest severity score during hospitalization was used to assess outcomes [43].

4.3 Economic and Societal Implications

The COVID-19 pandemic triggered an unprecedented global crisis, causing the worst economic downturn in nearly a century, surpassing the 2008 financial crisis [44]. Lockdowns and self-isolation measures halted businesses, resulting in widespread job losses, reduced economic activity and negative GDP growth [45]. The International Monetary Fund (IMF) predicted a -3.5% global economic contraction in 2020, with major economies like the U.S., U.K. and Japan facing significant declines. Governments responded with aid programs, increasing public spending and debt, raising concerns about long-term fiscal sustainability [46]. The pandemic devastated the global labor market, with millions of jobs lost, particularly in the service sector, which relies on physical presence. This disproportionately affected women and younger people, exacerbating existing inequalities. To recover, it is critical to address these disparities with targeted support for affected industries and vulnerable populations, ensuring a more balanced and inclusive economic recovery [47].

5. Future Directions

Many countries are considering booster doses of the COVID-19 vaccine in response to emerging variants and concerns about waning protection [48]. Despite proven vaccine efficacy, global vaccination rates vary and vaccine hesitancy remains a challenge due to distrust and demographic factors [49]. Research among American healthcare workers has highlighted worries about vaccine effectiveness against new strains and the need for boosters. Cross-sectional studies from China, Italy and Jordan reveal varying acceptance rates; for instance, individuals in Jordan expressed concerns about booster effectiveness and timing, while older, healthier people in Naples were more willing to accept boosters when informed by official sources. In China, income and education levels influenced acceptance, underscoring the need for health literacy initiatives, particularly in developing nations [50].

Conclusions:

COVID-19 vaccination campaign in India is a major public health effort marked by achievements and challenges. Despite logistical issues, vaccine hesitancy and socio-economic disparities, the campaign has successfully immunized a large portion of the population. Key

strategies included using digital platforms for registration, increasing domestic vaccine production and running public awareness campaigns. While millions of doses have been administered, ongoing efforts are needed to tackle vaccine hesitancy and ensure equitable access. In conclusion, India's COVID-19 vaccination campaign offers valuable lessons in public health strategy, crisis management and the importance of resilience and adaptability in the face of a global pandemic. As the country moves forward, these insights will be crucial in strengthening its healthcare infrastructure and ensuring the health and well-being of its population.

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HARNESSING PROBIOTICS, PREBIOTICS AND SYMBIOTICS: TARGETING THE HUMAN MICROBIOME FOR CHRONIC DISEASE MANAGEMENT

Aditi Vohra
Department of Home Science,
Kurukshetra University, Kurukshetra, Haryana, India
Corresponding author E-mail: aditikap09@gmail.com

Abstract:

The human microbiome, particularly the gut microbiota, plays a pivotal role in maintaining physiological homeostasis and regulating various health outcomes. It is critically involved in immune function, nutrient metabolism and the synthesis of bioactive compounds. Disruptions to this delicate microbial ecosystem referred to as dysbiosis, have been associated with numerous chronic diseases, including metabolic disorders, autoimmune conditions and gastrointestinal ailments. Increasing evidence suggests that modulating the microbiome through probiotics, prebiotics and synbiotics can offer therapeutic benefits by restoring microbial balance and supporting host health. Probiotics are live beneficial microorganisms that enhance immune responses and competitively inhibit pathogenic microbes, while prebiotics serve as fermentable substrates that selectively stimulate the growth of beneficial bacteria. Synbiotics, a synergistic combination of both, provide enhanced stability and functionality in microbiome-targeted therapies. Recent advancements, such as organ-on-chip technology and single-bacterium omics, are revolutionizing microbiome research and expanding therapeutic potential. Moreover, clinical trials have demonstrated that specific probiotic strains like *Lactobacillus* and *Bifidobacterium*, along with prebiotics such as fructo-oligosaccharides and galacto-oligosaccharides, can significantly influence outcomes in conditions like obesity, type 2 diabetes, inflammatory bowel disease and Clostridium difficile infections. Looking ahead, personalized microbiome therapies based on individual microbial profiles may improve disease management and reduce reliance on antibiotics. However, standardization, regulatory frameworks, and long-term efficacy data are required for their full clinical integration. This paper explores the multifaceted roles of probiotics, prebiotics and synbiotics in chronic disease management through targeted microbiome modulation, while addressing key advancements, disease-specific outcomes and future directions in this rapidly evolving field.

Keywords: Probiotics, Prebiotics, Symbiotics, Human Microbiome, Gut Health, Chronic Disease

Introduction:

The rising prevalence of chronic noncommunicable diseases (NCDs) including diabetes, cardiovascular diseases, cancer, and chronic respiratory conditions poses a major public health

challenge globally. In India, diabetes and cardiovascular diseases are among the leading causes of death, with cancer cases steadily increasing (Kopparam & Chatterjee, 2024).

Recent research highlights the gut microbiota as a key player in systemic health. This complex ecosystem influences metabolic functions, immune regulation, nutrient absorption, and the production of short-chain fatty acids (SCFAs), which aid in glucose and lipid metabolism (Du, 2023; Vargas *et al.,* 2025).

Disruptions in gut microbial balance, or dysbiosis, are linked to increased intestinal permeability, chronic inflammation, and immune dysregulation—factors that contribute to obesity, type 2 diabetes, cardiovascular disorders, and gastrointestinal diseases (Tsai *et al.,* 2019; Ekka *et al.,* 2024; Vargas *et al.,* 2025).

To address this, microbiome-modulating agents such as probiotics (beneficial live microbes), prebiotics (non-digestible substrates that fuel beneficial microbes), and synbiotics (a combination of both) have gained attention for their therapeutic potential (Tsai *et al.,* 2019; Sheetal *et al.,* 2024).

This paper explores the link between gut microbiota and NCDs, emphasizing how microbiome-targeted interventions can restore microbial balance and support disease prevention and management (Kopparam & Chatterjee, 2024; Du, 2023; Sheetal *et al.,* 2024). While promising, their efficacy is influenced by factors like strain type, dosage, and host response, underscoring the need for standardized clinical protocols and personalized approaches (Sheetal *et al.,* 2024; Tsai *et al.,* 2019).

The Human Microbiome and Its Health Implications

The table 1 summarizes the multifaceted roles of the human gut microbiome in health maintenance and disease development. It outlines the microbiome's composition, key physiological functions, and its role in maintaining homeostasis versus the harmful effects of dysbiosis. The table also highlights its impact on immune regulation, metabolism, the gut-brain axis and nutrient synthesis.

Probiotics in Disease Management

Probiotics—live microorganisms that provide health benefits when consumed in adequate amounts—have emerged as key agents in chronic disease management. Common strains include *Lactobacillus* and *Bifidobacterium*, used widely in therapeutic formulations (Alzanbaqi *et al.,* 2024). Table 2 outlines the link between gut microbiota and chronic diseases.

Prebiotics and Their Therapeutic Potential

The table 3 discusses the therapeutic potential of prebiotics, which are non-digestible food components that promote the growth of beneficial gut bacteria. It outlines their classification, types (like inulin, FOS, GOS), and mechanisms such as SCFA production. Prebiotics help modulate immunity, improve gut flora, and show benefits in metabolic syndrome,

cardiovascular, and bone health. Their synergistic use with probiotics (as synbiotics) is an emerging area of research.

Table 1: Overview of the Human Gut Microbiome and Its Health Implications

Aspect	Key Points	References
Composition & Function	Composed of bacteria, fungi, viruses, and parasites; bacteria are most abundant. Functions in digestion, detoxification, immune modulation, and pathogen defense. Microbiome evolves with age, diet, genetics, and environment.	Božić *et al.,* 2024; Ekka *et al.,* 2024
Homeostasis vs. Dysbiosis	Homeostasis: stable, diverse community. Dysbiosis: reduced diversity, rise in harmful species. Triggers include diet, antibiotics, and environmental changes. Associated with IBD, metabolic, and neurodegenerative diseases.	Das & Nair, 2019; Božić *et al.,* 2024; Farooqui, 2021
Immune Regulation	Microbiota influence immune maturation and cytokine production. Dysbiosis can cause immune dysregulation, leading to autoimmune and inflammatory conditions.	Maurya *et al.,* 2024; Li, 2023
Metabolic Function	Produces short-chain fatty acids, regulates energy balance, and impacts metabolic health. Dysbiosis linked to obesity and diabetes.	Ekka *et al.,* 2024
Gut-Brain Axis	Maintains communication with the CNS, influencing mood and behaviour. Dysbiosis linked to depression, anxiety, and neurological disorders.	Li, 2025; Maurya *et al.,* 2024
Nutrient Synthesis & Absorption	Synthesizes vitamins (B, K), enhances mineral absorption. Dysbiosis may impair these functions, leading to malnutrition.	Farooqui, 2021; Das & Nair, 2019
Therapeutic Potential	Probiotics, prebiotics, and fecal microbiota transplant are promising interventions. Personalized microbiome-based therapies are under development.	Li, 2025; Ekka *et al.,* 2024

Table 2: Gut Microbiota and Chronic Diseases

Aspect	Key Details	References
Composition and Function	Comprises bacteria, fungi, viruses, and parasites (bacteria being most abundant); evolves with age and influenced by genetics, diet, environment.	Božić *et al.,* 2024; Gașpar *et al.,* 2024; Ekka *et al.,* 2024
	Aids in digestion, detoxification, immune modulation, and pathogen defense.	Bhattarai & Janaswamy, (2022).
Homeostasis vs. Dysbiosis	Homeostasis = stable, diverse microbial community; Dysbiosis = imbalance with reduced diversity and overgrowth of harmful microbes.	Das & Nair, 2019; Božić *et al.,* 2024; Ekka *et al.,* 2024
	Dysbiosis linked to inflammatory, metabolic, and neurodegenerative disorders; triggered by diet, antibiotics, and environmental changes.	Farooqui, 2021
Immune Regulation	Regulates immune development and cytokine production; influences immune cell maturation.	Maurya *et al.,* 2024
	Dysbiosis can cause immune dysfunction, leading to autoimmune and inflammatory diseases.	Maurya *et al.,* 2024; Li, 2023
Metabolism	Microbes produce metabolites (e.g., SCFAs) impacting energy regulation and metabolic health. Dysbiosis linked to obesity and diabetes.	Ekka *et al.,* 2024; Bhattarai & Janaswamy, (2022).
Gut-Brain Axis	Facilitates two-way signalling between gut and brain, influencing mood, cognition, and behaviour.	Li, 2025
	Dysbiosis associated with psychiatric disorders such as depression and anxiety.	Maurya *et al.,* 2024; Li, 2023
Nutrient Synthesis & Absorption	Supports synthesis of B and K vitamins and improves mineral absorption.	Farooqui, 2021
	Dysbiosis may impair nutrient bioavailability, increasing risk of malnutrition.	Das & Nair, 2019
Therapeutic Approaches	Emerging interventions include probiotics, prebiotics, and fecal microbiota transplantation; potential for personalized therapies based on microbiome profiling.	Li, 2025; Ekka *et al.,* 2024

Table 3: Overview of Prebiotics and Their Therapeutic Potential

Component	Details	References
Definition & Classification	Prebiotics are non-digestible food ingredients that selectively stimulate beneficial gut microorganisms. They resist digestion in the upper GI tract and are fermented by gut microbiota.	Mukherjee (2022); Davani-Davari *et al.* (2019)
Composition	Composed mainly of oligosaccharides, peptides, and dietary fibers.	Mukherjee (2022)
Types	Inulin, FOS, GOS, XOS, lactulose, resistant starch, polyphenols. Found in natural foods or industrially produced.	Davani-Davari *et al.* (2019); Yoo *et al.* (2024)
Mechanisms of Action	Fermented by gut bacteria into SCFAs (butyrate, acetate, propionate); promote growth of Bifidobacterium and Lactobacillus; support gut barrier and immunity.	Singarayar *et al.* (2024); Mukherjee (2022)
Immune Modulation & Gut Flora	Enhance immune function via SCFA production, immune cell maturation, cytokine modulation, and improved gut barrier function.	Yan (2022)
Clinical Benefits	- Metabolic syndrome: improved glycemic control, reduced inflammation - Cardiovascular health: lipid regulation, lower BP - Bone health: better calcium absorption, improved density	Davani-Davari *et al.* (2019)
Emerging Areas	Symbiotic interactions (prebiotics + probiotics) offer synergistic benefits; industrial production for functional food use.	Yoo *et al.* (2024); Davani-Davari *et al.* (2019)

7. Symbiotics: A Synergistic Strategy

Symbiotics, also known as synbiotics, refer to formulations that combine probiotics and prebiotics to produce synergistic or complementary health benefits. These combinations aim to enhance the viability and functionality of beneficial microorganisms while simultaneously improving host health. The classification into complementary and synergistic synbiotics is key to understanding their efficacy. Complementary synbiotics pair components that function independently, whereas synergistic synbiotics are designed for interactions that amplify mutual effects (Manoyan *et al.,* 2024).

Complementary vs. Synergistic Synbiotics

- **Complementary Synbiotics** include combinations in which both probiotic and prebiotic components independently benefit the host. The prebiotic may support the survival or activity of the probiotic, but the outcomes are generally additive rather than interactive (Manoyan *et al.,* 2024).
- **Synergistic Synbiotics** are formulated so that the prebiotic specifically supports the probiotic, enhancing its growth or metabolic activity. For instance, a formulation containing **Lactobacilli strains and Antigen** prebiotic demonstrated enhanced bactericidal and anti-adhesive effects against *Salmonella enteritidis*, showcasing a true synergistic interaction (Manoyan *et al.,* 2024).

Examples of Effective Combinations

- **Lactobacilli + Antigen Prebiotic**: This combination has shown strong antimicrobial activity against multidrug-resistant *Salmonella enteritidis*, suggesting its preventive potential in infectious diseases (Manoyan *et al.,* 2024).
- **Mixed-Chain Length Oligosaccharides + Multiple Microbial Strains**: A paediatric intervention using this combination led to a significant increase in weekly bowel movements among constipated children, highlighting its utility in gut motility improvement (Tierney *et al.,* 2022).

Superior Efficacy Over Individual Components

- **Digestive Health**: Studies reveal that synbiotics outperform probiotics alone in managing gastrointestinal conditions. For example, combining herbal therapy with probiotics led to better outcomes in paediatric functional constipation than using probiotics in isolation (Tierney *et al.,* 2022).
- **Immune Function and Gut Flora Balance**: Symbiotic-enriched yogurt increased the populations of **bifidobacteria and lactobacilli** in the gut, reduced pathogenic microorganisms, and boosted immune responses across age groups (Mofid *et al.,* 2020).

Clinical Applications

- **Paediatrics**: Synbiotics have shown potential in treating various gastrointestinal disorders in children, although more robust, standardized randomized controlled trials (RCTs) are necessary to substantiate their benefits ((Tierney *et al.,* 2022).
- **Geriatrics**: In elderly populations, synbiotics yogurt has been associated with enhanced immune function and a reduction in illness duration, indicating benefits for aging immunity (Mofid *et al.,* 2020).
- **Gastrointestinal Disorders**: Synbiotics have been effectively employed in managing conditions like irritable bowel syndrome (IBS) and nonalcoholic fatty liver disease (NAFLD), showing promising results in symptom alleviation and gut microbiota modulation (Mofid *et al.,* 2020).

Reflections

While the clinical potential of synbiotics is substantial, inconsistencies in study design, formulations and population groups present barriers to widespread adoption. Standardization of clinical protocols and mechanistic studies are essential for translating these benefits into reliable therapeutic options.

8. Microbiome-Driven Therapeutic Applications in Chronic Diseases

Modulating the gut microbiome offers promising therapeutic avenues for managing chronic conditions such as inflammatory bowel disease (IBD), metabolic syndrome, autoimmune disorders, and neurological diseases. These effects are mediated via immune regulation, metabolic modulation, and inflammation control. Recent trends focus on personalized probiotics and microbiome-targeted therapies to harness these interactions (Jacob & Mathew, 2023).

Inflammatory Bowel Diseases (IBD)

IBD, including Crohn's disease and ulcerative colitis, is marked by dysbiosis—reduced microbial diversity and altered composition (e.g., decreased Firmicutes, increased Proteobacteria) (Jacob & Mathew, 2023). Interventions such as probiotics, prebiotics, antibiotics, and fecal microbiota transplantation (FMT) aim to restore balance and reduce intestinal inflammation (Jacob & Mathew, 2023).

Metabolic Syndrome

Gut dysbiosis is strongly associated with metabolic disorders like obesity and type 2 diabetes, promoting chronic inflammation and metabolic disruption. Probiotic and dietary strategies have shown potential to modulate gut flora and improve metabolic markers, though mechanistic clarity is still evolving (Zhang *et al.,* 2024).

Autoimmune Conditions

In diseases like rheumatoid arthritis (RA), altered gut microbiota may worsen symptoms through mechanisms like molecular mimicry and increased intestinal permeability. Probiotics help alleviate RA symptoms by reducing inflammation and producing beneficial metabolites (Balasundaram *et al.,* 2024).

Neurological Conditions

The gut-brain axis connects microbiome health with neurological outcomes. Dysbiosis has been linked to disorders such as Parkinson's disease and depression via neuroinflammatory pathways and altered neurotransmitter levels. Probiotics and FMT show promise as adjunct treatments in depression, with potential broader neurological applications (Zhang *et al.,* 2024).

Personalized Probiotics and Emerging Trends

Next-generation probiotics (NGPs) and targeted microbial interventions are increasingly tailored to individual microbiome profiles (Tiwari *et al.,* 2024). Advances in metagenomics and microbiome analytics are fuelling precision medicine approaches to chronic disease management (Yaqub *et al.,* 2025).

Final Insights

Although microbiome-based therapies hold significant potential, challenges remain in elucidating mechanisms, optimizing dosing, and standardizing protocols. Variability in study designs and individual microbiome profiles complicate generalizability. Long-term safety and efficacy of interventions like Fecal Microbiota Transplantation (FMT) and Next-Generation Probiotics (NGPs) also demand further investigation before widespread clinical adoption.

9. Challenges and Future Directions

Microbiome research has significantly advanced our understanding of its role in human health, yet several challenges remain—particularly in addressing individual variability, regulatory and safety concerns, and the need for technological innovation and integration with nutrigenomics and precision medicine (Mousa & Ali, 2024; Yaqub *et al.,* 2025).

Individual Variability in Microbiome Composition

The human gut microbiome varies greatly across individuals due to factors like genetics, diet, and lifestyle, making it difficult to design universal therapies (Mousa & Ali, 2024; Yaqub *et al.,* 2025). Personalized interventions, guided by sequencing and metabolomics, are crucial to ensure efficacy (Mousa & Ali, 2024).

Regulatory and Safety Considerations

Current regulatory frameworks for microbiome-based therapies are still evolving. Safety remains a major concern due to potential long-term impacts on the host microbiome. Thorough assessment and monitoring are essential for clinical application (Tiwari *et al.,* 2024).

Technological Advancements in Strain Engineering and Delivery

Synthetic biology and bioinformatics have enabled the development of next-generation probiotics (NGPs) with improved stability and targeted functionality. However, challenges such as maintaining microbial diversity and precise control persist, especially in non-lab settings. Advancements in delivery systems are also crucial to enhance therapeutic viability and outcomes (Tiwari *et al.,* 2024).

Integration with Nutrigenomics and Precision Medicine

Combining microbiome science with nutrigenomics and precision medicine supports individualized treatment strategies, particularly in conditions like IBD (Mousa & Ali, 2024; Yaqub *et al.,* 2025). Microbiome profiling and metagenomic analysis aid in crafting tailored nutritional and therapeutic interventions (Yaqub *et al.,* 2025).

Way Forward

While microbiome-based therapies show immense promise, addressing individual differences, regulatory frameworks and safety concerns is critical. The synergy between microbiome science, precision medicine and nutrigenomics offers a transformative path toward personalized healthcare. Continued research, technological innovation and cautious clinical integration will be key to unlocking the full potential of microbiome interventions.

Conclusion and Implication

Probiotics, prebiotics and synbiotics hold substantial promise in microbiome-targeted therapies for the prevention and management of chronic diseases. These agents modulate the gut microbiota an essential regulator of immune, metabolic, and neurological processes demonstrating therapeutic effects in conditions such as chronic constipation, neurodegenerative disorders, inflammatory bowel disease (IBD), obesity and chronic obstructive pulmonary disease (COPD). (Serkova *et al.,* 2024; Yadav *et al.,* 2022).

Key Roles:

- **Probiotics** are live microorganisms that improve host immunity and gut health. For example, *Bifidobacterium lactis* has enhanced stool frequency and microbiota composition in constipated individuals (Serkova *et al.,* 2024; Yadav *et al.,* 2022).
- **Prebiotics** such as inulin selectively promote beneficial gut bacteria, aiding mineral absorption and immune modulation (Serkova *et al.,* 2024; Yadav *et al.,* 2022).
- **Synbiotics** combine both to synergistically support gut health and clinical outcomes by improving probiotic viability and function (Serkova *et al.,* 2024; Yadav *et al.,* 2022).

Research Priorities:

- An interdisciplinary approach involving microbiology, immunology, nutrition, and clinical medicine is vital to optimize microbiome therapies (Sheetal *et al.,* 2024).
- Technological tools like multi-omics sequencing and AI will deepen our understanding of host–microbe interactions and identify novel targets (Sheetal *et al.,* 2024).

Therapeutic & Preventive Benefits:

Microbiome modulation has shown efficacy in alleviating disease symptoms, improving metabolic parameters, and enhancing immune responses across various chronic illnesses (Boyajian *et al.,* 2024). Moreover, maintaining microbial balance may help prevent disease onset, offering a proactive strategy for health promotion (Boyajian *et al.,* 2024; Serkova *et al.,* 2024).

Despite its potential, challenges such as microbiome data variability, safety concerns, and limited long-term evidence call for rigorous clinical trials. A collaborative framework among researchers, clinicians, and policymakers is essential to translate microbiome-based interventions into safe, effective, and sustainable clinical practices.

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UPLC: A HIGHLY EFFICIENT QUANTITATIVE TECHNIQUE FOR SEPARATING ANTIRETROVIRAL DRUGS IN COMBINED PHARMACEUTICAL DOSAGE FORMULATIONS

Pralhad Rege*, Avinash Jagdale and Amol Kulkarni
Department of Chemistry, St. Xavier's College, Mumbai
*Corresponding author E-mail: pralhad1806@gmail.com

Abstract: [1-7]

In present study, a successful attempt has been made to develop RP-UPLC method for the simultaneous determination of Emtricitabine and Tenofovir disoproxil fumarate from combined pharmaceutical drug formulation. Chromatographic separation of Emtricitabine and Tenofovir disoproxil fumarate was achieved with gradient elution on Waters Acquity UPLC BEH C18; 150 mm length x 2.1 mm ID, 1.7 µm particle size with mobile phase A- Buffer pH 2.0 in Water and mobile phase B- Methanol at a wavelength 260 nm. The method was validated in the terms of its linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. Linearity of the method was found to be in the concentration range of 100 to 300 µg/mL and150-450 µg/mL for both Emtricitabine and Tenofovir disoproxil fumarate respectively with correlation coefficient greater than 0.999 for both the analytes. The total eluting time for the both components is less than three minutes. Proposed method was found to be simple, precise, novel .rapid and accurate and can be successfully applied for routine quality control analysis and simultaneous determination of Lamivudine and Tenofovir disoproxil fumarate in combined pharmaceutical drug formulations.

Keywords: RP-UPLC, Emtricitabine, Tenofovir disoproxil fumarate, Pharmaceutical Dosage Formulations and Validation

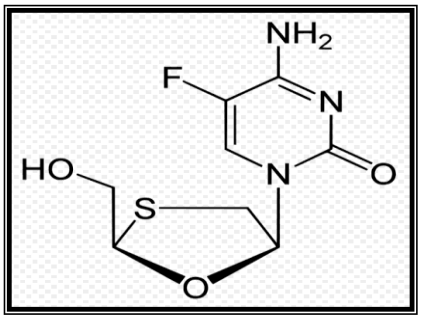
Abbreviations: EMT- Emtricitabine, TDF- Tenofovir Disoproxil Fumarate

Introduction: [8]

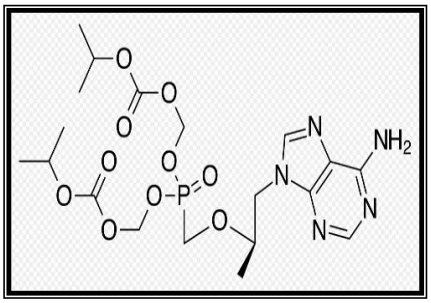
In the topical countries like India, the major problems of health arise due to improper lifestyle, unhealthy environmental conditions, unhygienic and substandard food. Infections caused by the microorganisms like, fungi, protozoa, virus are the most common. In many cases, drugs with two active ingredients are prescribed to the patients to have an added advantage. Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) indicated for the treatment of HIV infection in adults or combined with tenofovir disoproxil for the prevention of HIV-1 infection in high risk adolescents and adults. Emtricitabine is a cytidine analogue. The drug works by inhibiting HIV reverse transcriptase, preventing transcription of HIV RNA to DNA.

Tenofovir disoproxil fumarate, is a pro-drug, fumaric acid salt form of tenofovir, a nucleoside reverse transcriptase inhibitor analog of adenosine Tenofovir disoproxil fumarate is prescribed to treat HIV and chronic hepatitis B virus (HBV) in adults.

Structure:



Emtricitabine



Tenofovir disoproxil fumarate

UPLC Method Development:

Optimization of Chromatographic Condition:

Before developing any Chromatographic method, one must review the nature of the sample and goals of the separation. The sample related information that needs to be known prior to UPLC method development is: Sample solubility and number of components present, Nature of the sample, Chemical structures (functionality of the components), Molecular weight of components and Concentration range of the components in the sample of the interest.

The various parameters that were considered in the development process are:

Mode of separation, Selection of stationary phase, Selection of mobile phase, Selection of detection method (Detector used), Method validation

Mode of separation:

In the present research work, a reverse phase mode of the separation was employed taking in to account the polar nature of Lamivudine and Tenofovir disoproxil fumarate, their solubility in methanol and water. Therefore, a Reverse Phase mode of separation was chosen for simultaneous determination of Lamivudine and Tenofovir disoproxil fumarate using UPLC.

Selection of Stationary phase/ Chromatographic Column:

The column is the heart of UPLC separation process. The availability of stable, high-performance column is essential in developing a rugged, reproducible UPLC method.

The column is selected depending on the nature of the solute and the information about the sample. The number of theoretical plates (N) is an important characteristic of a column.

N- Defines the ability of the column to produce sharp, narrow peaks for achieving good resolution. In the method development, peak shape is equally important. Columns that provide symmetrical peaks are always preferred. In the present research work, Acquity UPLC BEH C18; 150 X 2.1 mm, 1.7 µm (Make- Waters) was selected for the analysis.

Selection of Mobile phase:

In Liquid chromatography, the solute retention is governed by partition coefficient of the solute, which depends on the interactions of the solute with the stationary and the mobile phase. For a given stationary phase, the partition coefficient of a solute will depend upon the mobile phase. The nature and the composition of which must be judiciously selected to get an appropriate and required solute retention.

Solvent polarity is the key word in chromatographic separations, since the polarity of mobile phase decides the retention time. Polar mobile phases give rise to high solute retention in normal phase and low solute retention in reverse phase liquid chromatography. The choice of the mobile phase for a given separation constitutes a very important stage in producing a good separation in UPLC. Methanol and acetonitrile are the most popular solvents in UPLC, both are water miscible, have comparatively low viscosity, low surface tension and readily available in pure form hence they mostly constitute the mobile phase. In the present research work, the best resolution was obtained with gradient elution using the Mobile Phases i.e. Mobile phase A- Buffer pH 2.0 and Mobile phase B- Methanol.

Material and Methods:

Chemicals and Reagents:

Standard Cefixime and Cloxacillin were obtained from local pharmaceutical company with claimed purity above 99.0%. All the solutions were prepared in double distilled water. All the necessary reagents used i.e. water and methanol (HPLC grade). Mobile phase was filtered using 0.45µm syringe filter made by Millipore whereas; Whatman's filter paper No.41 (purchased from local market) was used in the preparation of sample solution

Apparatus and Chromatographic Conditions:

Instruments:

UPLC: Waters Acquity UPLC H-class is an Ultra-performance liquid chromatographic system with a quaternary, high-pressure mixing pump inline vacuum degassing and PDA Detector with Chromeleon software.

Chromatographic Mode	**Gradient**
Column	Waters Acquity UPLC BEH C18; 150 mm length x 2.1 mm ID, 1.7 µm particle size
Wavelength	260 nm
Column oven temperature	45 °C
Autosampler temperature	25 °C
Injection Volume	5.0 µl
Flow rate	0.5 ml/min
Buffer pH 2.0	Weigh and dissolve 2.16 g of 1-octane sulphonic acid sodium salt in 1000 ml of purified water. Add 1 ml of Triethylamine and mix well. Adjust pH to 2.0 with orthophosphoric acid.
Mobile Phase	Mobile phase A- Buffer pH 2.0 Mobile phase B- Methanol
Diluent	Water: Methanol (50:50 v/v)

Solution Preparation:

a) Preparation of 200 µg/mL and 300 µg/mL solution of standard for Emtricitabine and Tenofovir disoproxil fumarate [EMT + TDF]

Weighed accurately 10 mg of Emtricitabine standard and 15 mg Tenofovir disoproxil fumarate standard transfer it into a 50 ml standard flask, added 35 ml of diluent and sonicate to dissolve. Allowed it to cool at room temperature, mixed well and made up to the volume with diluent to obtain 200 µg/mL of Emtricitabine and 300 µg/mL of Tenofovir disoproxil fumarate. This solution was used as working concentration of Emtricitabine and Tenofovir disoproxil fumarate and used as 'Standard'.

b) Preparation of sample solution for Emtricitabine and Tenofovir disoproxil fumarate [EMT + TDF]

Commercial brand containing of Emtricitabine and Tenofovir disoproxil fumarate in combination was procured. Each brand contained a label claim of 200 mg of Emtricitabine and 300 mg of Tenofovir disoproxil fumarate per tablet.

Ten tablets were weighed and powdered for the analysis. The powder (1015 mg) equivalent to 200 mg of Emtricitabine and 300 mg of Tenofovir disoproxil fumarate was accurately weighed, transferred into 100 ml standard flask; added 70 ml of diluent and sonicate to dissolve. Allowed it to cool at room temperature, mixed well and the mixture was sonicated for 30 mins, finally volume of the solution was made up to 100 mL with diluent (Stock solution). The solution was filtered through 0.45 µm membrane filter paper and 2 mL of stock solution was diluted to 20 mL with the diluent to obtain a solution containing 200 µg/mL of Emtricitabine and 300 µg/mL of Tenofovir disoproxil fumarate. This solution was used as working concentration of Emtricitabine and Tenofovir disoproxil fumarate and used as 'Sample'.

The validation parameters studied for the simultaneous determination Emtricitabine and Tenofovir disoproxil fumarate are as mentioned below:

Analytical Method Validation: [9-10]

System Suitability:

System suitability test is used to verify that the system has adequate reproducibility for the analysis to be carried out. It also verifies the proper functioning of the operating system. The test was carried out by injecting 5 µL of the standard solution containing 200µg/mL of EMT and 300µg/mL of TDF i.e. [at their working concentration] into stabilized chromatographic system, under optimized chromatographic conditions **(Table 1).**

Specificity:

Specificity is the ability of the method to assert the presence of the analyte unequivocally in the presence of other components that are present. To show that the other constituents present in the sample formulation do not interfere with the retention times of Emtricitabine and Tenofovir disoproxil fumarate. The peaks corresponding to EMT and TDF in the sample solution

were identified by comparing with the resulting chromatograms of the sample, with that of standard Lamivudine and Tenofovir disoproxil fumarate (Table 1).

Limit Of Detection [LOD] and Limit of Quantification [LOQ]:

Limit of detection [LOD] is the lowest concentration of the analyte that can be detected under the operational conditions of the method. Limit of quantification [LOQ] is defined as lowest concentration of the analyte that can be determined with acceptable precision and accuracy, under the operational conditions of the method. Standard deviation of responses (σ) and slope (S) was used to establish LOD (LOD = σ/S X 3.3) and LOQ (LOQ = σ/S X 10), respectively. LOD and LOQ for Emtricitabine were 2.7 µg/mL and 8.3 µg/mL for Tenofovir disoproxil fumarate were found to be 7.0 µg/mL and 21.3 µg/mL respectively is given in Table 1

Linearity and Range:

The linearity for Lamivudine and Tenofovir disoproxil fumarate was observed simultaneously by addition of standard solution. The linear working range for EMT was found between 100 to 300 µg/mL and for TDF it was found between 150 to 450 µg/mL.

The calibration curves were constructed with concentration (C) against peak area. The slope, intercept, regression equation and correlation coefficient for the EMT and TDF was obtained is given in Table 1 and Figure 1-3.

Intraday and Interday Precision:

The intra-day and inter-day precision was used to study the variability of the method. It was checked by recording the chromatograms of sample solutions of EMT and TDF at working level i.e. 100% both at intra-day (six times within 24 hour) and inter-day (six times during 3 days intervals) to check the precision. The mean % RSD for intra-day and inter-day precision was found to be less than 1.0% for both EMT and TDF. Result of intra and inter day precision studies are given in Table 1.

Assay

The developed chromatographic method was used for simultaneous determination of Lamivudine and Tenofovir disoproxil fumarate from commercial brand of formulation. The sample solutions were analysed by the developed method described above. Chromatograms were recorded under the optimum experimental conditions. Resulting peak area of Emtricitabine and Tenofovir disoproxil were measured and the amount of Emtricitabine and Tenofovir disoproxil fumarate calculated using already constructed calibration graph. Result of assay studies are given in Table 2.

Calculation formula for determination of % Assay content is detailed below;

$$\% \text{ Assay} = \frac{\text{Peak Area of Sample X Weight of Std X Sample Dil. Factor X Potency of Std}}{\text{Avg. Peak Area of Std X Std Dil. Factor X Weight of Sample X 100 X Avg. Weight X Label claim}} \text{ X } 100$$

Robustness:

The robustness of the method was examined by the consistency of peak height and peak shape with the deliberately small changes in the experimental parameter. It is a measure of its capacity to retain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was performed by intentionally modifying the chromatographic conditions such as composition of mobile phase, change in flow rate and change in oven temperature. The chromatographic parameters of each analyte such as retention time, tailing factor, resolution and theoretical plates were measured at each changed condition. In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following two factors were selected for change: Change in the pH of buffer for mobile phase A by ± 0.2 of the original flow in the proposed analytical method i.e., from pH 3.0 to 2.8 and 3.2. Change in column oven temperature by ± 2°C of original Temperature, i.e., change in oven temperature from 40 °C to 38°C and 40 °C to 42°C. One factor at the time was changed to estimate the effect. The working concentration solution of both the drugs was applied onto the column. A number of replicate analyses (n = 3) were conducted for evaluation of each change of factors. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-UPLC method developed is robust.

Accuracy (Recovery):

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the method of varying weight of sample for sample preparation. A weight of sample was varied at different concentrations of preanalyzed sample solutions and analyzed by proposed method. The percentage recovery was determined at different levels i.e. from 50% to 150% level. The results of recovery analysis for Lamivudine and Tenofovir disoproxil fumarate are shown in Table 3.

Result and Duscussion:

In the present work conditions were optimized for development and validation of a simple and accurate HPLC method for simultaneous quantification of Emtricitabine and Tenofovir disoproxil fumarate in combined pharmaceutical drug formulation. Method development was right from optimization of the condition and parameters i.e., selection of system, column, mobile phase, different composition of mobile phases have been tried. During optimizing the method, Methanol and Acetonitrile were choices as organic solvents. The cost of acetonitrile favored to choose methanol as solvent for further studies. The chromatographic conditions were optimized by using 1-octane sulphonic acid sodium salt, triethylamine and orthophosphoric acid as a buffer for mobile phase preparation. After a series of screening experiments, it was concluded that gradient elution using buffer pH 2.0 and methanol gave better peak shapes and resolution, finally mobile phase A- Buffer pH 2.0 and mobile phase B-

Methanol is the most appropriate composition because both the components were eluted with good resolution and good peak shape. Under the described experimental conditions, sharp peaks that belong to EMT and TEN were obtained with gradient elution at retention time of 0.61 min and 2.54 min respectively (Figure 1). The developed chromatographic method was validated using ICH guidelines. A new chromatographic method has been developed and subsequently validated for the simultaneous quantification of Emtricitabine and Tenofovir disoproxil fumarate from a combined drug formulation. The advantages of this method for analytical purposes lie in the rapid determination, its cost effectiveness, easy preparation of the sample, good reproducibility.

Table 1: Method validation parameters for the determination of Emtricitabine and Tenofovir disoproxil fumarate

Parameters	Values	
	Emtricitabine	**Tenofovir disoproxil fumarate**
System suitability Theoretical Plates- Tailing Factor-	 More than 5175 1.0	 More than 58626 1.0
Linearity range (µg/mL)	100 to 300 µg/mL	150 to 450 µg/mL
Slope (m) [a)]	3308.4	3051.6
Intercept(c) [a)]	3291	13104
Correlation coefficient (R^2)	1.000	1.000
LOD (µg/mL)	2.7 µg/mL	7.0 µg/mL
LOQ (µg/mL)	8.3 µg/mL	21.3 µg/mL
Intraday precision (n=6)	0.2%	0.1%
Interday precision (n=6)	0.2%	0.2%
Assay	98.8% to 99.0%	102.6% to 103.0%
Recovery	99.1% to 101.5%	98.1% to 102.0%

Sample Details

Sample name: ***Anti-retroviral tablet formulation containing Emtricitabine and Tenofovir disoproxil fumarate***

Brand Name: TENOF EM (HETERO HEALTH CARE LTD)

Batch No.: 2011566

Excipients: q. s.

Table 2: Result of Assay studies of *Emtricitabine and Tenofovir disoproxil fumarate*

Brand name	TENOF EM **(HETRO HEALTH CARE LTD)**	
	Emtricitabine	**Tenofovir disoproxil fumarate**
Labelled claim (mg)	200 mg	300 mg
Drug found in (mg)	201.5 mg	299.4 mg
% RSD (n=6)	0.85	0.56
% Assay	100.75 %	99.8 %

Table 3: Results of Recovery studies of *Emtricitabine and Tenofovir disoproxil fumarate*

Analyte	**Level**	**RSD (%) (n = 6)**	**Recovery (%)**	
			Minimum	**Maximum**
Emtricitabine	50%	0.2	101.1	101.5
	100%	0.1	100.2	100.4
	150%	0.0	99.1	99.1
Range			**99.1**	**101.5**
Tenofovir disoproxil fumarate	50%	0.4	101.1	102.0
	100%	0.2	99.2	99.6
	150%	0.1	98.1	98.3
Range			**98.1**	**102.0**

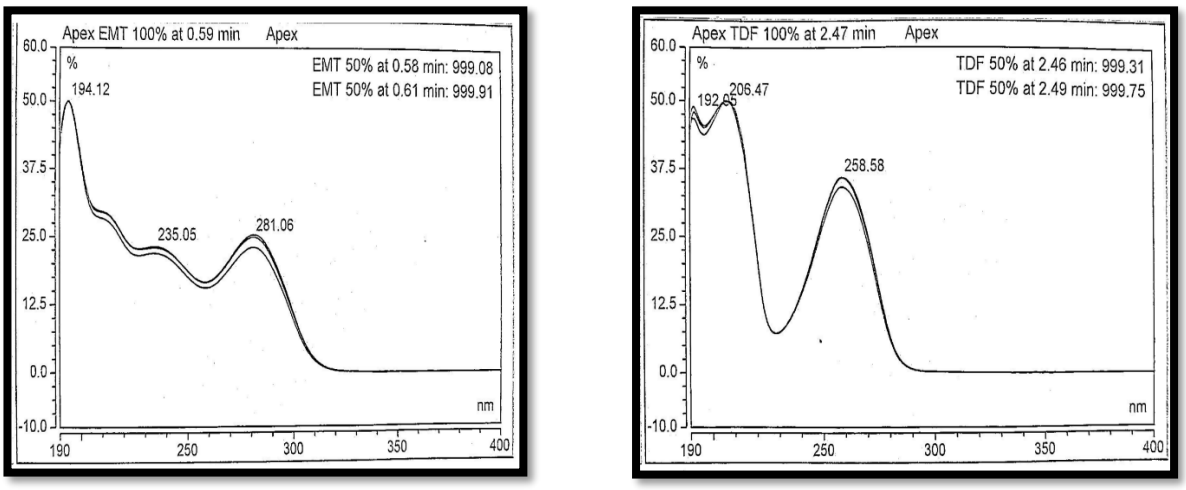


Fig. 1: UV spectrum of Emtricitabine and Tenofovir disoproxil fumarate

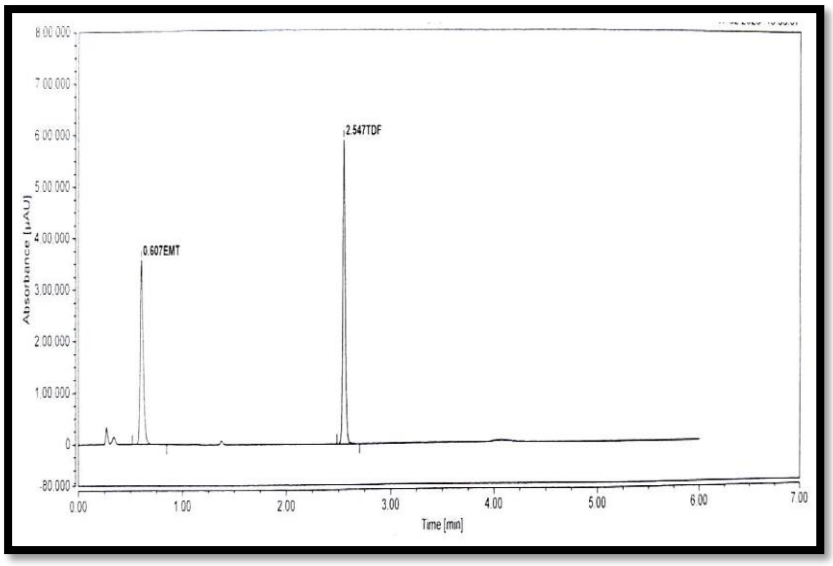
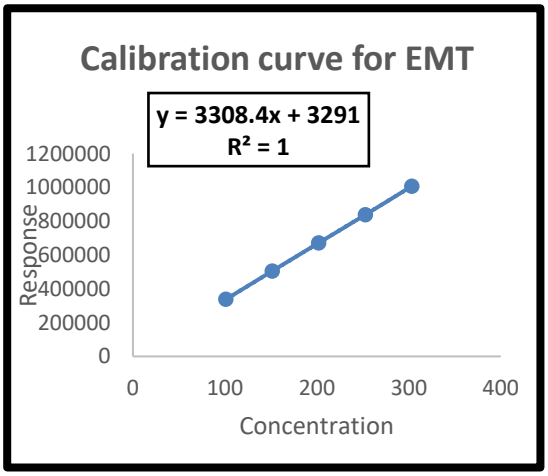


Fig. 2: UPLC Chromatogram for Standard Emtricitabine and Tenofovir disoproxil fumarate respectively



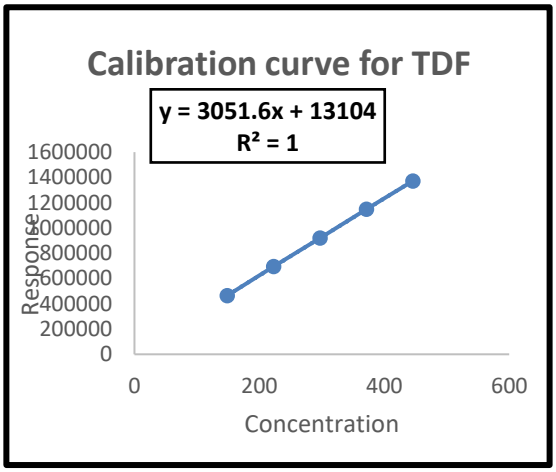


Fig. 3: Linear working range for EMT. **Fig. 4: Linear working range for LAM.**

Y-axis – Peak Area **X-axis**- Concentration of Drug in µg/mL

Conclusion:

In addition to above mentioned points, the proposed method is found to be more simple, economic, accurate and practical. Thus, presented method can be recommended for simultaneous determination of Emtricitabine and Tenofovir disoproxil fumarate in routine quality control analysis in combined drug formulations.

Acknowledgement:

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BRYOPHYLLUM PINNATUM: A FASCINATING MEDICINAL PLANT

Jharna Medhi and Niladry Sekhar Ghosh*
Faculty of Pharmaceutical Science,
Assam down town University, Guwahati, Assam-781026
Corresponding author E-mail: ghoshniladry@gmail.com

Abstract:
Plants are an invaluable source of medicine, offering easy extraction, cost-effectiveness, and minimal side effects. According to the World Health Organization (WHO), medicinal plants are expected to become the primary source of drugs in the future. The bioactive compounds found in these plants can be used therapeutically or serve as precursors for the synthesis of pharmaceutical drugs. Therefore, studying these plants is essential to evaluate their safety and efficacy. One such plant is *Bryophyllum pinnatum*, commonly known as Panfuti, Life Plant, Love Plant, Air Plant, Good Luck Plant, or Resurrection Plant. This succulent herb is widely distributed in hot and humid regions, often found near human dwellings, along roadsides, and in abandoned fields and farms. In traditional medicine, the leaves of *Bryophyllum pinnatum* are known for their significant therapeutic properties and are used for both internal and external treatments. The plant contains various phytochemicals, including alkaloids, flavonoids, glycosides, steroids, bufadienolides, and organic acids. The primary aim of this paper is to provide a comprehensive overview of the pharmacological activities of this important medicinal herb.

Keywords: *Bryophyllum pinnatum*, Air Plant, Crassulenscent, Pharmacological, Bufadienolide, Morphology, Phytochemistry, Traditional Uses

Introduction:

Bryophyllum pinnatum, also known as the oken plant, is a fascinating medicinal plant that has been used for centuries in traditional medicine. This plant is an environmental weed that belongs to the Crassulaceae family and can grow up to one meter tall. Its leaves are opposite, smooth, and have 3-5 deeply notched, fleshy leaflets, making them distinguishable from other plants. The indigenous system of medicine recognizes it as a valuable medicinal plant that can be used both internally and externally. The leaves are highly effective in treating a wide range of human disorders, including hypertension, diabetes mellitus, bruises, wounds, boils, burns, sloughing ulcers, ophthalmia, corn, diarrhea, dysentery, vomiting, abscesses, insect bites, arthritis, rheumatism, joint pains, headaches, antifungal, antibacterial, body pains, and acute inflammation. Moreover, the leaves are also renowned for their efficacy in treating lymphadenitis and ear disease. *Bryophyllum pinnatum* is a plant with a rich history and a wide range of therapeutic properties. The name *Bryophyllum pinnatum* comes from ancient Greek, where "bryo" means "to sprout out," and "phyllon" means "leaf." This plant contains a plethora of secondary metabolites, including alkaloids, flavonoids, tannins, glycosides, and phenolic

compounds, which are renowned for their medicinal properties. Pharmacological studies have unequivocally demonstrated that this plant has many effects on the body, including but not limited to anti-cancer, antioxidant, immunomodulating, antibacterial, anthelmintic, antiprotozoal, neurological, anti-inflammatory, analgesic, diuretic, antiurolithic, nephroprotective, hepatoprotective, anti-peptic ulcer, hypotensive, antidiabetic, wound healing, and other effects. The unique combination of phytochemicals in this plant makes it an incredibly powerful and versatile traditional medicine that has been used for centuries to treat a wide range of ailments. [1]

Synonyms:

Bryophyllum calycinum Salisb, Kalanchoepinnata (Lam.) Pers, Cotyledon pinnata Lam., Sedum madagascaricumClus.[1]

Plant Profile: [2] [3]

Kingdom : Plantae – Plants
Sub kingdom : Tracheobionta – Vascular plants
Division **:** Spermatophyta
Subdivision **:** Magnoliophyta – Flowering plants
Class **:** Magnoliopsida – Dicotyledons
Subclass **:** Rosidae
Order **:** Rosales
Family **:**Crassulaceae
Genus **:** Bryophyllum
Species **:** Bryophyllumpinnatumkurz

Vernacular names[4] [5]

Sanskrit: Parnabeeja, Asthibhaksha
English: Air plant
Hindi: Zakhmhaiyat, Pathharchoor
Kannada: Gandukalinga, Kadubasale
Malayalam: Elamarunga
Tamil: Malaikalli, Ranakalli
Telugu: Ranapala
Marathi: Gayamari
Bengali: Koppatha, Patharkuchi.

Geographical Indication:[6]

It is perennial herb growing widely and used in Folkoric medicine in tropical Africa, Tropical America, India, China, Australia, Asia, New Zealand, Philippines. The plant thrives throughout India, particularly in Bengal and Uttarakhand, where the climate is hot and humid.

Family Features[7] [8]

The Crassulaceae family, also known as the plant of crassula tribe, comprises a whopping 25 genera and 450 species! These plants are typically herbs or under shrubs, with fleshy and succulent stems and branches. The leaves exhibit either alternate or opposite arrangement, are often simple, and occasionally pinnately split. Notably, they do not possess stipules. The flowers often exhibit cymose inflorescence, are hermaphrodite or occasionally unisexual, and display consistent symmetry. The calyx is completely separate and consists of 4-5 divisions or segments, whereas the petals are equal in number to the sepals and alternate with the single-petaled corolla. The number of carpels is equal to the number of petals, and they are positioned opposite to the petals. At the base of each carpel, there is a hypogynous gland or scale. And let's not forget the fruits, which are follicles and membranous with few seeds.

Plant Description:[9]

This herb stands tall at a height of 0.3-1.2m and is comprised of several distinct parts, which illustrated as bellows.

- ***Stems:*** The herb stems are definitely obtusely four-angled. The older stems are undoubtedly light-colored while the younger stems are strikingly reddish, speckled with white.
- ***Leaves:*** The plant has fresh dark green leaves with scalloped edges trimmed in red. The leaf blade is pinnately compound and has 3-5 leaflets. The petiolules are 2-4 cm long, and the erect leaflet blades are elliptic with a crenate margin. The inflorescences are terminal and paniculate, and the leaves frequently generate buds that become new plants.
- ***Flowers:*** The flowers grow in large, spreading panicles with stout branches and slender pedicles. They are mainly bell-shaped, droop downwards, and are arranged in branched clusters at the tip of the stem. The flowers are yellowish-green or pale green, with prominent sepals. They are mainly produced during winter and spring.
- ***Fruits and Seeds:*** The fruits are undoubtedly enclosed in a persistent papery calyx and corolla, and the seeds are unequivocally small, smooth, and oblong-ellipsoid in shape

Macroscopy [10]

Bryophyllum pinnatum is an exquisite hairless herb that can reach up to 1.2 meters in height. The twigs are squarely four-sided, with mature ones brilliantly colored and the newer ones spotted with snowy roseate. The leaves are highly adaptable and opposite, with the lower leaves usually simple or compound while the upper ones are 3-5/7 foliate with lengthy petioles.

The petioles are connected by an edge near the stem. The flowers are oval/elliptic with a crenate/notched border and are suspended in large panicles with opposing branches and thin pedicels. The sepals are strikingly red-striped, green at the base, and pale green at the top. The petals are a striking shade of red or pink, puffy and octagonal at the base with triangular lobes. The filaments are green at the base and pinkish-red below the dark, hastate-shaped anthers. The flowers are green in color. The fruit is encased in a persistent wispy calyx and corolla, and the seeds are small, oblong-ellipsoid, and flat.

Microscopy [11]

1. The minuscule entity clearly exhibits a slender layer on the abaxial surface and a curved contour on the adaxial surface. The epidermal film on the upper side possesses a sharp and distinct appearance with slight, yet less prominent compartments. The stranded tissue in the midrib comprises of parenchymatous cells that are round, pointed, and compressed.
2. This vascular strand is a compact and semicircular band that houses a dense parallel arrangement of xylem and phloem.
3. The xylem is a sharply tapered structure with thin walls, while the vascular bundles are arranged in an upright and parallel plane. The lamina is notably smooth, and the mesophyll is distinctly divided into palisade and spongy parenchyma. The stomata are of the anisocytic type, and they are notably well-formed.
4. The presence of coiled vessels is evident in the green segment, with no trichomes found on either the abaxial or adaxial cross.

Chemical Constituent

- Isocitric acid & citric acid
- Bufadienolides like bryotoxin A, B, and C
- Phenols, Phenylpropanoids, and Flavanoids: Syringic acid, caffeic acid, 4- hydroxy-3-methoxy-cinnamic acid, 4-hydroxybenzoic acid, p-hydroxycinnamic acid, paracoumaric acid, ferulic acid, protocatechuic acid, phosphoenolpyruvate, protocatechuic acid
- Triterpenoids and Steroids: α-amyrin, αamyrinacetate, β-amyrin, β-amyrinacetate, bryophollenone, bryophollone, taraxerol, pseudo taraxasterol, 18-α-oleanane, friedelin, glutinol[12]
- The leaves contain essential vitamins and minerals such as ascorbic acid, riboflavin, thiamine, niacin, calcium, zinc, and phosphorous
- A GC-MS analysis of the ethanol extract from *Bryophyllum pinnatum* leaves showed the presence of compounds such as butyrolactone, 3,4-Epoxytetrahydrothiophene-1,1-dioxide, 1-Octen-3-ol, Benzaldehyde, Oleic acid, Octadecanoic acid, and n-Hexadecanoic acid.[13]

- The stem of Bryophyllum pinnatum contains active constituents such as alkaloids, flavonoids, saponins, tannins, phytate, phenols, calcium, magnesium, phosphorus, sodium, and potassium.[14]

Ayurvedic Properties[15]

Rasa: Kashaya, Amla

Guna: Laghu

Virya: Sheeta

Vipaka: Madhura

Doshaghnata: Vatakaphahara

Karma: Ashmarighna, Vranaropaka, Mootrala, Shonitasthapana, Raktastambaka, Grahi**.**

Rogaghnata: Ashmari, Atisara, Raktasrava, Visuchika.

Traditional Uses:[16] [17] [18] [19] [20] [21]

- *Bryophyllum pinnatum's* leaves and bark possess potent medicinal properties and have been extensively used in traditional medicine to treat multiple ailments including diarrhea, vomiting, earache, burns, abscesses, gastric ulcers, insect bites, and lithiasis. Their bitterness, astringency, analgesic, and carminative properties are widely recognized.
- The plant has many medicinal uses. It is used to treat leg swelling and its leaves are made into a powder for use as a wound dressing, called 'Jakhmehayat'. In Southeastern Nigeria, the herb is used to help with the delivery of the placenta after a baby is born.
- The juice from fresh leaves is used to treat various ailments including smallpox, otitis, cough, asthma, palpitations, headache, convulsion, and general weakness.
- Leaf juice is used to treat bronchial issues, dysentery, jaundice and gout.
- In traditional medicine, the leaves of the plant have been used for their antifungal, potent antihistamine, and anti-allergic properties.
- This is also applied on the bodies of young children when they are ill
- It is commonly used in folk medicine to treat hypertension, kidney stones, pulmonary infections, and rheumatoid arthritis.
- The plant is useful in treating various conditions such as epilepsy, hemorrhoids, and skin ailments like cuts, wounds, and discolorations.
- *Byophyllum pinnatum* has various medicinal properties such as refrigerant, emollient, mucilaginous, hemostatic, vulnerary, depurative, constipating, anodyne, disinfectant and antitonic. The plant also exhibits hepato protective activity and helps in maintaining vascular integrity.
- Bryophyllum has the potential to lower fever, as well as provide anti-inflammatory and muscle relaxation effects.

- The anti-inflammatory effects of this substance are partly due to their ability to modulate and suppress the immune system.
- In the Indian state of Odisha, the Basampatri plant is traditionally used to treat the condition of flatulence.
- People in Poojapura, Kerala, use crushed leaves externally to treat burns.
- Warm, matured leaves are applied to wounds in West Bengal and Andhra Pradesh.
- In Konkan, the juice of the leaves is used with ghee to treat dysentery. Two teaspoons of the juice are administered for renal calculi.
- In Chota Nagpur, the leaf juice is steamed with ghee/garlic for coughs. Leaves are treated with palm oil and used externally for sore eyes.

Pharmacological Action: [22] [23] [24] [25] [26]

- ***Wound healing work:* -**In a study conducted on Albino rats, *Bryophyllum pinnatum* leaf extracts were administered orally at a dose of 400 mg/kg for 10 consecutive days in the form of petroleum ether, water, and alcohol to determine their effectiveness in wound healing and reconstruction of dead space wounds. The three extracts were combined and compared to a control group. The results showed a significant increase in wound healing ability and reduced drainage in the group treated with the combined extracts. The injury was monitored for 21 days until eschar formation.
- ***Nephroprotective Activity:* -**After being induced with nephrotoxicity by gentamicin, mice were given K. Pinnata liquid discharge which resulted in a nephroprotective effect. The adverse effects caused by gentamicin such as glomerular and peritubular congestion, epithelial desquamation, blood clots, accumulation of inflammatory cells, and kidney cell necrosis were significantly reduced upon the administration of the discharge.
- ***Anti- Diabetic activity:*** The study found that *Bryophyllum pinnatum* aqueous leaf extract has anti-diabetic properties in rats with diabetes induced by a glucose dose of 3g/kg. The extract was tested in four different doses (200mg/kg, 400mg/kg, 800mg/kg, and 800mg/kg in combination with glibenclamide 2mg/kg). The 200mg/kg dose of the extract showed a significant decrease in blood sugar levels compared to the other doses. However, the combination of 800mg/kg extract with glibenclamide 2mg/kg was the most effective and efficient in reducing blood sugar levels compared to the use of 200mg/kg and the other single doses**.**
- ***Anti-inflammatory activity:* -**Studies have found that the leaves of *Bryophyllum Pinnatum* contain anti-inflammatory properties that can be extracted using animal ether, chloroform, acetone, methanol, aqueous component, alkaloidal fraction, flavonoids fraction, phenol and phenolic acid, and alkaloidal anhydride. When administered orally at a dose of 500 mg/kg once a day for two days, the extract was effective in reducing paw edema in mice induced with formaldehyde. In particular, the methanolic fraction showed

significant or less significant reduction in edema at the early stages of prevention as compared to the conventional medicine Indomethacin.

- ***Anti-bacterial activity: -***The problem of antibiotic resistance among bacteria in synthetic medicine is growing day by day. Therefore, it is important to look for new and safe antimicrobials, particularly from natural sources such as plants. One such plant is *Bryophyllum pinnatum*, whose leaves have been found effective in inhibiting the growth of bacteria. The plant is also used for treating typhoid fever and other viral infections caused by bacteria such as *Saureus, E. coli, B. subtilis, P. autogiros, K. aerogenes, K. pneumoniae*, and *S. Typhi*.
- ***Anticonvulsant effect:*** The combination of CH2Cl2 and CH3OH has been shown to effectively reduce the incidence of seizures caused by pentylenetetrazol, strychnine sulphate, and thiosemicarbazide. Additionally, this combination has been found to increase the duration of seizures while reducing the duration of convulsions caused by three different vibrating agents. These findings suggest that CH2Cl2 and CH3OH may have potential as therapeutic agents in the treatment of seizure disorders.
- ***Effectsonrenal system:*** The liquid extract obtained from leaves has demonstrated potent nephroprotective activity against gentamicin-induced nephrotoxicity in mice. More specifically, the hydroalcoholic extract derived from this plant has been identified to possess vital diuretic and antiurolithic properties, which have been efficacious upon oral or intra peritoneal administration in mice. These findings suggest the potential of this extract as a therapeutic agent to counteract nephrotoxicity and urolithiasis.
- ***Neuropharmacological activity:*** The study sought to examine the effects of aqueous leaf extracts *of Bryophyllum pinnatum* on specific neuropharmacological activities in mice. The extract was given at doses of 50, 100, and 200 mg/kg. The findings demonstrated that the extract induced a substantial reduction in exploratory activity, with the extent of the decline being depending on the dosage. Furthermore, it exhibited a noticeable sedative impact, as indicated by the decrease in overt behavior and prolonged duration of sleep induced by pentobarbitone. In addition, the extract postponed the initiation of seizures caused by strychnine and picrotoxin, with a greater degree of safeguarding found in picrotoxin-induced convulsions. The extract also reduced the mortality rate resulting from convulsions induced by picrotoxin, with an LD50 value of 641mg/kg. All of these results suggest that the extract has a suppressive impact on the central nervous system.
- ***Protein profiling:*** Proteins from the leaves of *Bryophyllum pinnatum* were extracted using a Phosphate Extraction Buffer at a specific pH. The proteins were separated using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The resulting bands on the gel were excised and digested with trypsin. The digested proteins were then subjected to Liquid Chromatography-Tandem Mass Spectrometry

(LC/MS/MS) for identification. The MS/MS data revealed the presence of Proteinase K. This protein is commercially used to digest unwanted proteins like keratin.

- ***Cytotoxicity of testis:*** The study discovered that the ethanolic extract derived from the leaf of *Bryophyllum pinnatum* had a deleterious impact on the cells of the rat testis when given orally for a duration of 8 weeks, using two distinct dosages: 100 mg/kg and 200 mg/kg. When administered at a dosage of 100 mg/kg, the seminiferous tubules exhibited a reduction in size and the epithelium displayed gaps or voids. An elevated dosage of 200 mg/kg led to a more pronounced augmentation in intracellular gaps between the germinal epithelium and a decrease in spermatozoa when compared to the control group, which exhibited typical histological characteristics of the testes.
- ***Uterine Contractility:*** In A study was done on 14 women to compare the tocolytic effects of *Bryophyllum pinnatum* with fenoterol. *Bryophyllum pinnatum* suppressed spontaneous contractions in a dose-dependent manner, enhanced contraction frequency by 91%, and reduced oxytocin-induced contractions by 20%. Fenoterol shown a 50% reduction in contractions. A separate investigation including 67 pairs of expectant mothers experiencing premature labor revealed that B. pinnatum is equally as efficacious as beta-agonists in extending the duration of pregnancy and gestational age at childbirth. However, B. pinnatum is notably better tolerated, resulting in fewer negative effects and improved results for both newborns and their health conditions.

Conclusion:

Bryophyllum pinnatum is a well-known herb, used worldwide. Studies have confirmed the ethanobotanical use of Bryophyllum Pinnatum and supported the therapeutic utility of the plant in various disorders. Current research on *Bryophyllum pinnatum* is rooted in evidence-based findings related to its pharmacognostical, phytochemical, and pharmacological properties. This plant boasts numerous active pharmacological compounds that contribute to its therapeutic benefits, which have been upheld by studies and utilized in traditional medicine practices. Nonetheless, further in-depth research is needed to validate its safety and efficacy in modern medicine**.**

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About Editors

Prof. (Dr.) M. S. Kadam, born on 10 January 1976, completed her M.Sc. in Zoology (Fishery Science) from Shri Shivaji Mahavidyalaya, Parbhani in 1998 and was awarded a Ph.D. in January 2006. She is presently working as a Professor in the P.G. Department of Zoology at Yeshwant Mahavidyalaya, Nanded (M.S.), India. She has successfully completed one minor research project and has published more than 115 research papers in reputed national and international journals in the fields of Limnology and Fishery Science. Dr. Kadam has actively participated in over 98 conferences, presenting her research work. She has received seven prestigious awards at national and international levels for her outstanding research contributions. She is a recognized P.G. teacher and research guide at S.R.T.M. University, Nanded. A Fellow of the Society of Life Sciences (F.S.L.Sc.) and IAZ UP, she is also a life member of many scientific bodies and has authored five academic books.

Dr. Mandar Subhash Gaikwad is an Assistant Professor in the Department of Chemistry at Yeshwantrao Chavan Mahavidyalaya, Tuljapur (Maharashtra), with over 10 years of teaching experience. He serves as a Board of Studies (BoS) member for M.Sc. and B.Sc. Analytical Chemistry at Rajashri Shahu College, Latur. Dr. Gaikwad has published four research papers in reputed international journals and authored one international book. He has actively participated in over 80 academic events, including national and international conferences, symposia, webinars, and workshops through oral and poster presentations. His academic involvement reflects his commitment to research and continuous learning in the field of chemistry. He is also a lifetime member of the Society for Polymer Science (India), further underscoring his engagement with the scientific community and his interest in advancing polymer and analytical chemistry education and research.

Dr. Yogita Gulabrao Bodkhe is Head and Associate Professor in Chemistry at Guru Nanak College of Science, Ballarpur, affiliated with Gondwana University, Gadchiroli. She has 15 years of teaching experience at UG and PG levels and is a recognized Ph.D. guide in Chemistry at Gondwana University. She has guided over 12 PG dissertation students and coordinated several state and national-level workshops. Dr. Bodkhe has published more than 17 research papers in reputed, referred, and UGC-listed journals and contributed chapters to e-books. She has also presented 10 research papers at various conferences. As a moderator and paper setter, she is actively involved with Gondwana University and J. B. College of Science, Wardha. She is a lifetime member of ACT and NUTA and has completed more than seven Faculty Development Programs (FDPs), demonstrating her commitment to academic excellence and growth.

Dr. Pollobi Duara completed her Ph.D. in Zoology from Gauhati University in 2018 and is currently working as an Assistant Professor in the Department of Zoology at Majuli College, Assam. She has published ten research papers in national and international journals and twenty abstracts in various conference proceedings. Dr. Duara has presented her work at 14 international conferences and 25 national seminars. She has participated in nine workshops/Faculty Development Programs and two online training programs and has successfully completed a one-month MOOC on statistical analysis. She has published book chapters and organized two national webinars as Convenor. Her research interest focuses on pollinating insects. She has been selected as a Fellow of the Science Frontier Research Council by the Open Association of Research Society, USA. Additionally, she holds a Bachelor's degree in Music from Lucknow Sangeet Vidyapith.

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