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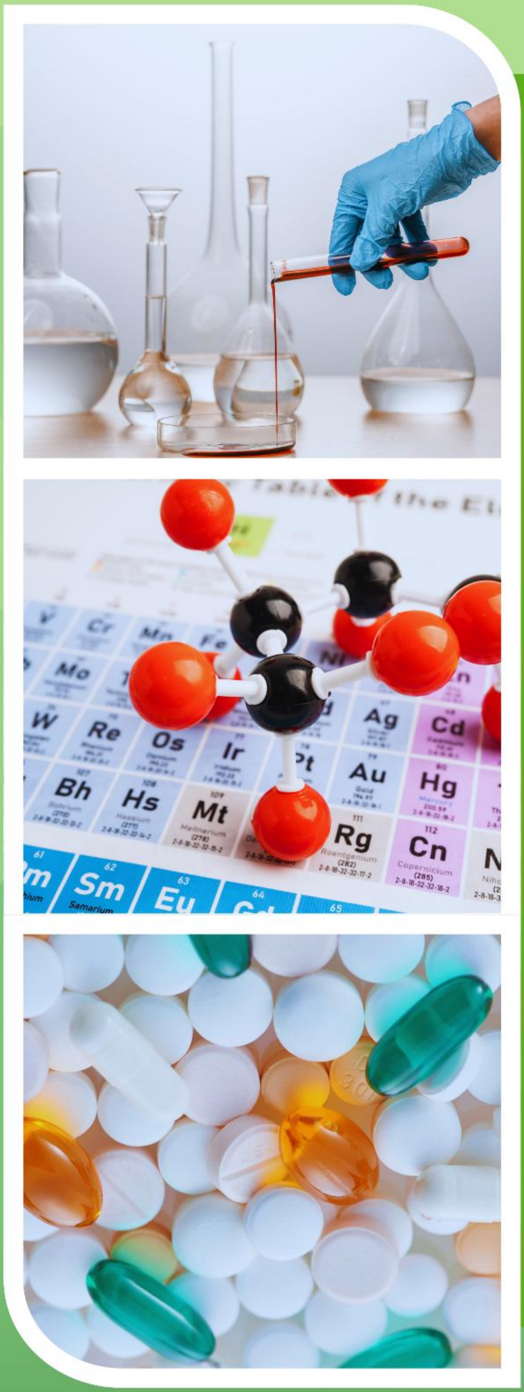

Recent Progress in Chemical, Biological and Pharmaceutical Sciences

Editors:
Dr. Ravindra R. Mahajan
Dr. Uttam B. Chougale
Dr. Pranjali T. Maskar
Dr. Pravin N. Muli

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Editors

Dr. Ravindra R. Mahajan

Department of Chemical Engineering,

National Institute of Technology,

Warangal, Telangana

Dr. Uttam B. Chougale

Department of Chemistry,

Karmaveer Hire College,

Gargoti, Kolhapur, M.S.

Dr. Pranjali T. Maskar

B. K. Birla College of Arts, Science and

Commerce (Empowered Autonomous

Status), Kalyan (West), M.S.

Dr. Pravin N. Muli

Department of Chemistry,

Smt. Sindhutai Jadhao Arts and Science

Mahavidyalaya, Mehkar, Buldhana, M.S.

Bhumi Publishing

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PREFACE

The book *Recent Progress in Chemical, Biological and Pharmaceutical Sciences* presents a comprehensive overview of emerging trends, innovative methodologies, and significant advancements across the interconnected domains of chemical, biological, and pharmaceutical sciences. In the present era of rapid scientific evolution, interdisciplinary research has become the cornerstone for addressing complex global challenges related to health, environment, and sustainable development.

This volume brings together contributions from researchers, academicians, and industry experts who have shared their valuable insights and findings in diverse yet complementary areas. The chapters included in this book reflect current progress in chemical synthesis, analytical techniques, drug discovery, biotechnology, molecular biology, and novel therapeutic strategies. Special emphasis has been placed on recent developments in pharmaceutical sciences, including drug delivery systems, pharmacological evaluations, and advancements in medicinal chemistry.

The editors have made sincere efforts to ensure that the content is both informative and accessible to a wide audience, including undergraduate and postgraduate students, research scholars, and professionals. Each chapter is designed to provide a balanced combination of theoretical background and practical applications, thereby facilitating a deeper understanding of the subject matter.

We firmly believe that this book will serve as a valuable academic resource and a reference guide for researchers seeking to explore new dimensions in these scientific fields. It is our hope that the knowledge compiled in this volume will inspire further research, innovation, and collaboration among scientists worldwide.

The editors express their heartfelt gratitude to all contributors for their scholarly efforts and timely submissions. We also acknowledge the support of reviewers and publishers in bringing this work to fruition. Any suggestions for improvement in future editions will be greatly appreciated.

- Editors

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DEVELOPMENT AND ASSESSMENT OF ESSENTIAL OIL CONTAINING HERBAL ANTI-PRURITIC LOTION

Laxmi Tiwari, Manorama Ratre and Sharang Bali
School of Pharmacy,
Chouksey Engineering College, Bilaspur, Chhattisgarh-495001 India
Corresponding author E-mail: laxmitiwari@gmail.com, mannoratre94@gmail.com,
sharangb@cecbilaspur.ac.in

Abstract

Pruritus is a common dermatological condition characterized by persistent itching, irritation, and inflammation of the skin. Conventional treatments such as corticosteroids and antihistamines may produce adverse effects when used for long durations. Therefore, herbal topical formulations have gained increasing attention due to their safety, effectiveness, and minimal side effects. The present study aimed to formulate and evaluate a herbal anti-pruritic lotion and investigate its physicochemical properties, antimicrobial activity, anti-inflammatory potential, and stability.

Three formulations (F1, F2, and F3) were prepared using different concentrations of herbal ingredients and emulsifying agents. The prepared formulations were evaluated for physicochemical parameters including appearance, pH, viscosity, spreadability, homogeneity, and skin irritancy. Antimicrobial activity was assessed against Staphylococcus aureus and Escherichia coli using the broth dilution method to determine the Minimum Inhibitory Concentration (MIC). Anti-inflammatory activity was evaluated by egg albumin denaturation assay. Stability studies were conducted under accelerated and real-time conditions for three months.

Among the formulations, F2 exhibited the most desirable physicochemical properties with acceptable pH (6.2), moderate viscosity (2500 cP), and ideal spreadability (5.2 cm). The patch test confirmed that F2 produced no skin irritation in volunteers. Antimicrobial evaluation showed that F2 possessed the lowest MIC values (0.5% against Staphylococcus aureus and 1.0% against Escherichia coli), indicating superior antibacterial activity. The anti-inflammatory study demonstrated concentration-dependent inhibition of protein denaturation with maximum inhibition of 58.19% at 2% concentration, comparable to diclofenac sodium. Stability studies revealed minimal variations in pH and viscosity, particularly for F2.

Overall, the findings suggest that formulation F2 possesses promising anti-pruritic, antimicrobial, and anti-inflammatory properties with good stability and skin compatibility, indicating its potential for the management of pruritic skin conditions.

Keywords: Herbal Lotion, Pruritus, Antimicrobial Activity, Anti-Inflammatory Activity, Stability Study.

1. Introduction

Pruritus, commonly referred to as itching, is one of the most frequent symptoms associated with dermatological disorders such as eczema, dermatitis, allergic reactions, and microbial infections. Persistent itching may lead to scratching, skin damage, and secondary infections, significantly affecting the quality of life of patients (Weisshaar & Dalgard, 2009).

Conventional treatments for pruritus include antihistamines, corticosteroids, and topical anti-inflammatory agents. Although these therapies are effective, long-term use may cause several adverse effects such as skin thinning, irritation, and hormonal disturbances (Yosipovitch & Bernhard, 2013).

In recent years, herbal medicines have gained considerable attention due to their therapeutic efficacy and lower risk of adverse effects. Medicinal plants contain various bioactive compounds such as flavonoids, phenolic compounds, and terpenoids, which possess antimicrobial, anti-inflammatory, and antioxidant activities (Pandey & Rizvi, 2009). These properties make herbal formulations promising alternatives for the management of skin disorders.

Topical dosage forms such as lotions are widely used in dermatological therapy because they are non-greasy, easily spreadable, and suitable for application on large areas of the skin (Barel *et al.,* 2014). An ideal anti-pruritic lotion should possess suitable physicochemical properties including appropriate pH, viscosity, spreadability, and stability to ensure therapeutic effectiveness and patient acceptability.

Therefore, the present study aimed to develop and evaluate a herbal anti-pruritic lotion using different concentrations of herbal ingredients and to investigate its physicochemical properties, antimicrobial activity, anti-inflammatory potential, and stability.

2. Materials and Methods

2.1 Materials

All herbal ingredients, essential oils, emulsifiers, stabilizers, and preservatives used in the preparation of the herbal anti-pruritic lotion were of pharmaceutical grade. The microbial strains Staphylococcus aureus and Escherichia coli used for antimicrobial testing were obtained from a microbiology laboratory. Diclofenac sodium was used as the standard drug for the anti-inflammatory study. All reagents used were of analytical grade (Barel *et al.,* 2014).

2.2 Preparation of Herbal Anti-Pruritic Lotion

Three formulations (F1, F2, and F3) were prepared using different concentrations of herbal extracts, essential oils, and emulsifying agents. The oil phase and aqueous phase were prepared separately and heated to approximately 70°C. The oil phase was gradually added to the aqueous phase with continuous stirring to form a uniform emulsion. The mixture was allowed to cool while stirring continuously until a smooth lotion was obtained. The prepared formulations were stored in airtight containers for further evaluation (Aulton & Taylor, 2018).

2.3 Physicochemical Evaluation

- **Appearance:** The formulations were visually examined for colour, clarity, consistency, and overall appearance. Uniform colour, absence of lumps, and lack of phase separation were considered acceptable characteristics for topical formulations (Barel *et al.,* 2014).
- **pH Measurement:** The pH of each formulation was measured using a calibrated digital pH meter. Maintaining pH close to the natural skin pH helps to avoid irritation and maintain skin barrier function (Lachman *et al.,* 2013).
- **Viscosity:** Viscosity was measured using a Brookfield viscometer to determine the flow behavior and application properties of the lotion formulations.
- **Spreadability:** Spreadability was determined by measuring the spreading diameter of the formulation between two glass plates under a specified weight.
- **Homogeneity:** The formulations were examined for uniform distribution of ingredients and absence of phase separation.
- **Skin Irritancy Test:** A patch test was conducted on ten healthy volunteers to evaluate the presence of skin irritation such as erythema or edema (Draelos, 2015).

2.4 Antimicrobial Activity

Antimicrobial activity of the formulations was evaluated against Staphylococcus aureus and Escherichia coli using the broth dilution method to determine the Minimum Inhibitory Concentration (MIC). The lowest concentration showing no visible bacterial growth after incubation at 37°C for 24 hours was recorded as the MIC value (CLSI, 2018).

2.5 In-Vitro Anti-Inflammatory Activity

Anti-inflammatory activity was evaluated using the egg albumin denaturation assay. The reaction mixture containing egg albumin, phosphate buffer saline, and the test sample was heated to induce protein denaturation. Absorbance was measured at 660 nm using a UV-visible spectrophotometer. Diclofenac sodium served as the positive control (Mizushima & Kobayashi, 1968).

2.6 Stability Studies

Stability studies were conducted under accelerated and real-time storage conditions for three months. The formulations were evaluated periodically for changes in pH, viscosity, colour, odour, and homogeneity according to ICH stability guidelines (ICH, 2003).

3. Result and Discussion

The prepared formulations (F1, F2, and F3) were evaluated for physicochemical properties, antimicrobial activity, anti-inflammatory potential, and stability.

3.1 Appearance

All three formulations (F1, F2, and F3) have observed visually for colour, consistency, and clarity.

Table 1: Appearance

Formulations	Appearance	Acceptable Standard (Anti-Pruritic Lotion)
F1:	Pale yellow, smooth texture, slightly thick consistency.	Uniform colour, smooth texture, no lumps, no phase separation
F2:	Pale cream, smooth texture, moderate thickness.	Uniform colour, smooth texture, elegant appearance
F3:	Light green, uniform, thin consistency.	Uniform appearance, no grittiness

Standard Requirements for Anti-Pruritic Lotion (Appearance): An ideal anti-pruritic lotion should have: Uniform colour without discoloration, Smooth and homogeneous texture, No grittiness or lump formation, No phase separation, Moderate consistency (neither too thick nor too runny) & Elegant and patient-acceptable appearance

(a) (b) (c)

Figure 1: Appearance (a = F1, b = F2 & c = F3)

The variation in appearance among the formulations have attributed to differences in the concentration of essential oils and emulsifiers used in the formulations. F2, with the optimal emulsifier concentration, exhibited a visually appealing and uniform texture, indicating better formulation stability.

3.2 pH Measurement

The pH values of the samples have measured to ensure skin compatibility.

Table 2: pH Measurement

Formulations	pH Value	Anti-Pruritic Standard
F1	5.8	Slightly above ideal but acceptable
F2	6.2	Higher side (may reduce barrier support)
F3	5.5	Ideal

Desirable pH Range (Anti-Pruritic Lotion): 4.5 – 5.5 (Ideal) & 4.5 – 6.0 (Acceptable upper limit)

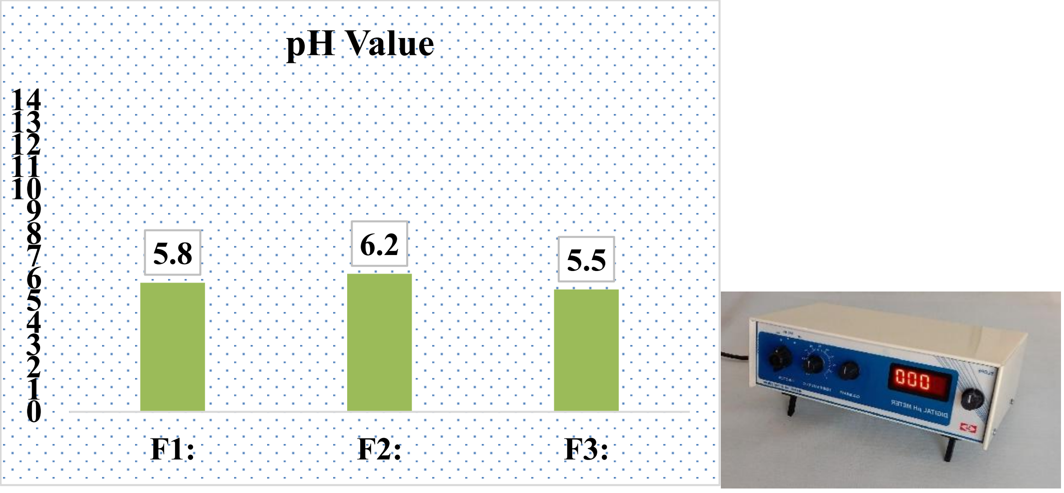


Figure 2: pH Measurement

The pH values of all formulations have within the acceptable range of 5.5–6.5 for topical applications, ensuring skin compatibility. F2, with a pH of 6.2, closely matches the natural pH of the skin, making it the most suitable for prolonged use.

3.3 Viscosity Measurement

The viscosity of the formulations has measured to evaluate spreadability and application properties.

Table 3: Viscosity Measurement

Formulations	Viscosity (cPs)	Anti-Pruritic Standard
F1	3000 cP	Ideal
F2	2500 cP	Ideal
F3	2000 cP	Lower ideal limit

Ideal Viscosity Range: 2,000 – 5,000 cP (Dermatological lotion standard)

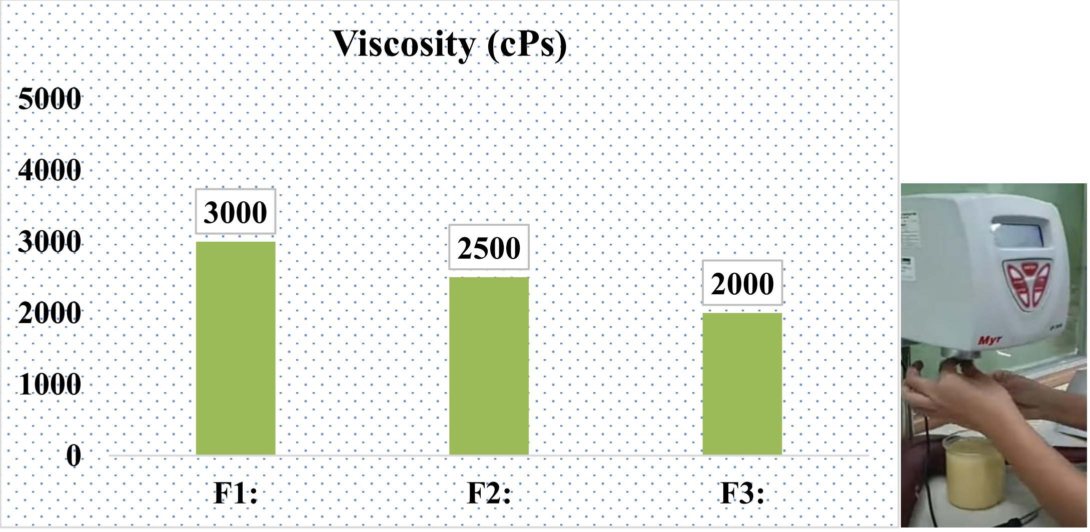


Figure 3: Viscosity Measurement

F1 exhibited the highest viscosity, contributing to better application control and uniform spreading. The lower viscosity of F3 may lead to runny application, whereas F2 exhibited moderate viscosity suitable for ease of use.

3.4 Spreadability

Spreadability has evaluated by measuring the spreading diameter of the samples under a specified weight.

Table 4: Spreadability of Samples

Formulations	Spreadability (cm)	Standard
F1	4.8	Slightly low
F2	5.2	Ideal
F3	6.0	Ideal

Desirable Spreadability: 5 – 7 cm

F2 has showed the least spreadability due to higher viscosity, ensuring controlled application without excessive spreading. F3, with higher spreadability, may indicate ease of application but may reduce product efficacy due to lower adhesion to the skin. F2 has showed moderate spreadability due to moderate viscosity, may indicate ease of application.

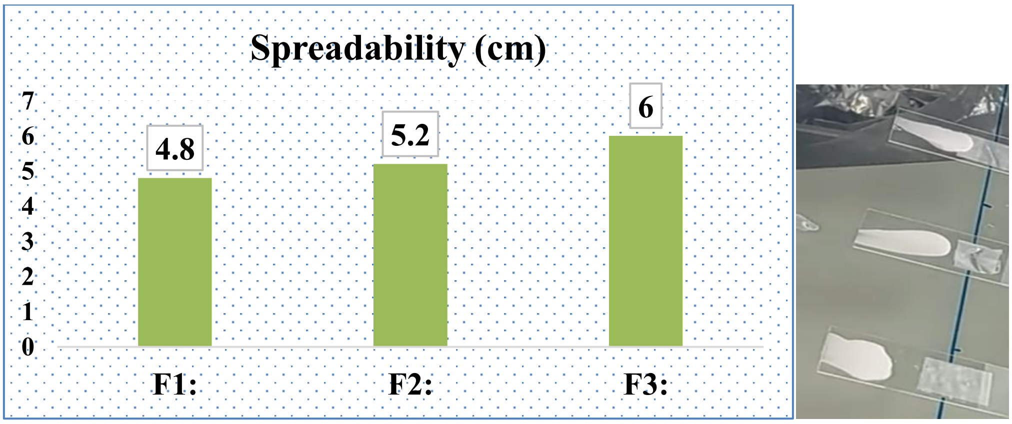


Figure 4: Spreadability of Samples

3.5 Homogeneity and Uniformity

All formulations have evaluated for uniform distribution of ingredients and absence of phase separation.

Table 5: Homogeneity and Uniformity

Formulations	Homogeneity and Uniformity	Standard
F1:	Homogeneous and stable.	Acceptable
F2:	Homogeneous and stable.	Acceptable
F3:	Slight phase separation observed after 24 hours.	Not ideal

Standard: No phase separation, No creaming & Stable after centrifugation

Figure 5: Homogeneity and Uniformity

F1 and F2 demonstrated excellent homogeneity and uniformity, ensuring even distribution of active ingredients. The slight phase separation in F3 could be due to lower stabilizer concentration.

3.6 Skin Irritancy Test: Patch Test

The patch test has conducted on 10 volunteers to assess skin irritancy.

Table 6: Skin Irritancy Test: Patch Test

Formulations	No Irritation (%)	Mild Irritation (%)	Severe Irritation (%)
F1	90	10	00
F2	100	00	00
F3	80	20	00

Ideal Standard: 100% non-irritant & No erythema / edema

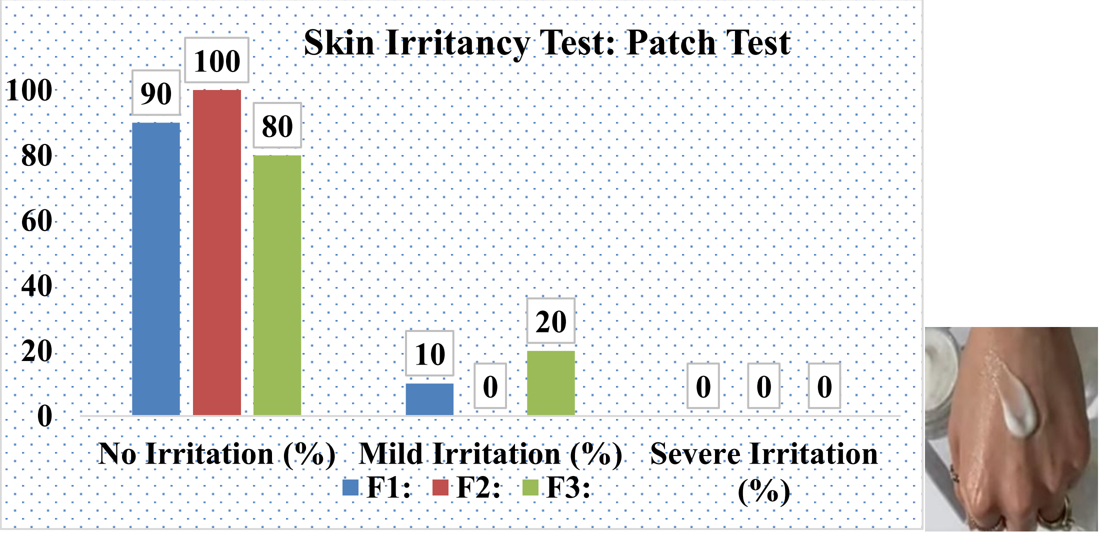


Figure 6: Skin Irritancy Test: Patch Test

F2 has found to be the most skin-friendly formulation, showing no signs of irritation in any volunteer. The mild redness observed in F1 and irritation in F3 could be due to variations in essential oil concentrations.

3.7 In-Vitro Assessment

3.7.1 Anti-microbial Testing: Determination of Minimum Inhibitory Concentration (MIC)

The antimicrobial activity of the developed herbal anti-pruritic lotion formulations (F1, F2, and F3) was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the broth dilution method to determine their Minimum Inhibitory Concentration (MIC). The results demonstrated a clear concentration-dependent inhibitory effect for all three formulations.

Table 7: MIC of Formulations (0.125–2% v/v)

Micro-organism	Conc. (% v/v)	F1	F2	F3	MIC Values (%)
Against Staphylococcus aureus	0.125 %	Growth	Growth	Growth	F1 = 1.0 F2 = 0.5 F3 = 2.0
	0.25 %	Growth	Growth	Growth	
	0.5 %	Growth	**No Growth**	Growth	
	1.0 %	**No Growth**	No Growth	Growth	
	2.0 %	No Growth	No Growth	**No Growth**	
Against *Escherichia coli*	0.125 %	Growth	Growth	Growth	F1 = 2.0 F2 = 1.0 F3 = 2.0
	0.25 %	Growth	Growth	Growth	
	0.5 %	Growth	Growth	Growth	
	1.0 %	Growth	**No Growth**	Growth	
	2.0 %	**No Growth**	No Growth	**No Growth**	

Against Staphylococcus aureus, all formulations showed visible microbial growth at lower concentrations (0.125% and 0.25%). Formulation F2 inhibited bacterial growth at 0.5%, indicating superior antibacterial efficacy, while F1 showed inhibition at 1.0%. In contrast, F3 required a higher concentration of 2.0% to completely inhibit growth. The MIC values were found to be 1.0% for F1, 0.5% for F2, and 2.0% for F3. These findings indicate that F2 possessed the strongest activity against the Gram-positive organism.

In the case of *Escherichia coli*, all formulations exhibited growth up to 0.5% concentration, suggesting comparatively lower sensitivity of the Gram-negative organism. F2 demonstrated inhibition at 1.0%, whereas both F1 and F3 required 2.0% concentration to suppress bacterial growth. The MIC values were determined as 2.0% for F1, 1.0% for F2, and 2.0% for F3.

The comparatively higher resistance shown by *E. coli* may be attributed to the presence of an outer lipopolysaccharide membrane in Gram-negative bacteria, which acts as a permeability barrier and restricts the entry of hydrophobic phytoconstituents present in essential oils. On the other hand, Gram-positive bacteria such as *S. aureus* lack this outer membrane, making them more susceptible to membrane-disrupting phytochemicals.

Overall, the results confirm that all formulations possess antimicrobial potential; however, F2 exhibited the lowest MIC values against both test organisms, indicating its superior efficacy. The enhanced activity of F2 may be attributed to the optimal concentration and better diffusion of bioactive constituents such as terpenoids and phenolic compounds in the formulation. These findings support the potential application of the developed herbal lotion in managing microbial-associated pruritic skin conditions.

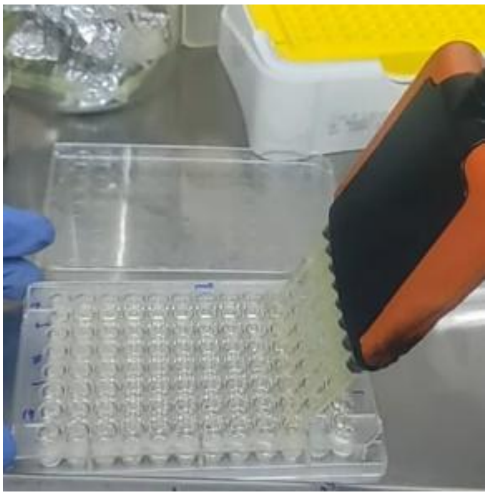


Figure 7: Minimum Inhibitory Concentration (MIC) of Samples

3.7.2 Anti-inflammatory Testing: Egg Protein Denaturation Assay

The anti-inflammatory activity of the formulated herbal anti-pruritic lotion was evaluated using the egg albumin denaturation assay, a widely accepted in vitro method for assessing protein stabilization. Protein denaturation is one of the key mechanisms involved in inflammation, leading to the formation of auto-antigens and tissue damage. Therefore, inhibition of protein denaturation is considered an important indicator of anti-inflammatory potential.

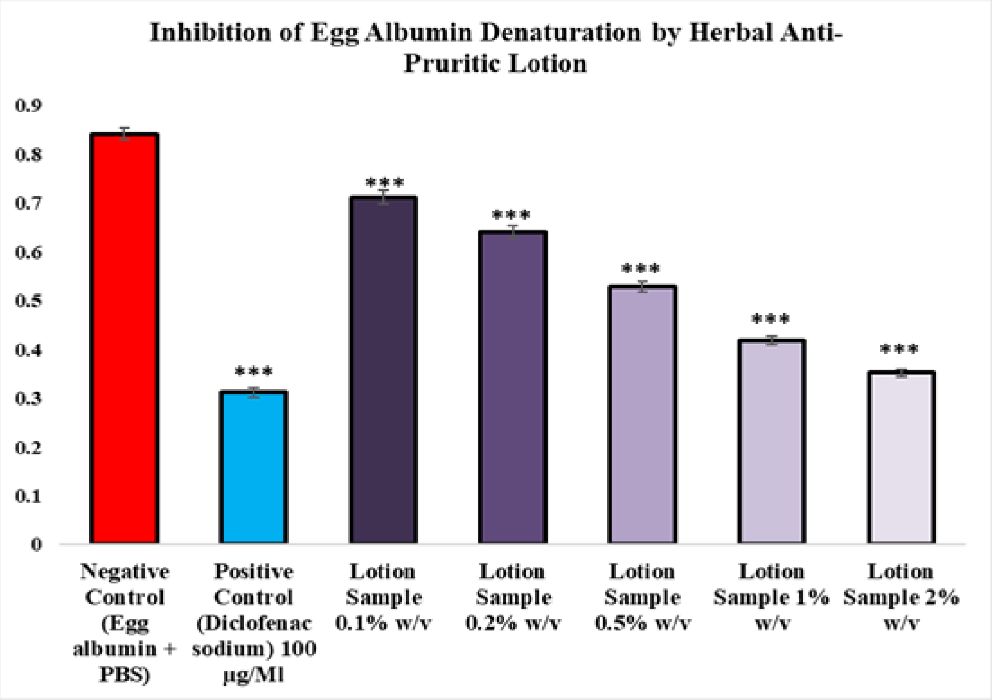
Table 8: Inhibition of Egg Albumin Denaturation by Herbal Anti-Pruritic Lotion

S. No.	Sample	Conc.	Absorbance at 660 nm (Mean ± SD, n=3)	% Inhibition of Protein Denaturation
1.	Negative Control (Egg albumin + PBS)	—	0.842 ± 0.012	—
2.	Positive Control (Diclofenac sodium)	100 µg/mL	0.312 ± 0.010 ***	62.94%
3.	Lotion Sample	0.1% w/v	0.712 ± 0.015 ***	15.44%
4.	Lotion Sample	0.2% w/v	0.641 ± 0.014 ***	23.86%
5.	Lotion Sample	0.5% w/v	0.528 ± 0.011 ***	37.29%
6.	Lotion Sample	1% w/v	0.418 ± 0.009 ***	50.36%
7.	Lotion Sample	2% w/v	0.352 ± 0.008 ***	58.19%

Data were expressed as Mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test. All treated groups showed ***$p < 0.001$ when compared to Negative Control

In the present study, the negative control (egg albumin + PBS) showed a high absorbance value of 0.842 ± 0.012, indicating maximum protein denaturation. The positive control, diclofenac sodium (100 µg/mL), demonstrated a significant reduction in absorbance (0.312 ± 0.010) with 62.94% inhibition of protein denaturation (***p < 0.001), confirming the reliability of the experimental model.

The herbal lotion exhibited a concentration-dependent inhibitory effect. At 0.1% w/v, the formulation showed 15.44% inhibition (0.712 ± 0.015), which increased to 23.86% at 0.2% w/v (0.641 ± 0.014). Further enhancement in activity was observed at higher concentrations, with 37.29% inhibition at 0.5% w/v (0.528 ± 0.011), 50.36% at 1% w/v (0.418 ± 0.009), and a maximum of 58.19% at 2% w/v (0.352 ± 0.008). Statistical analysis using one-way ANOVA followed by Dunnett's post hoc test indicated that all treated groups showed highly significant differences (***p < 0.001) when compared to the negative control.



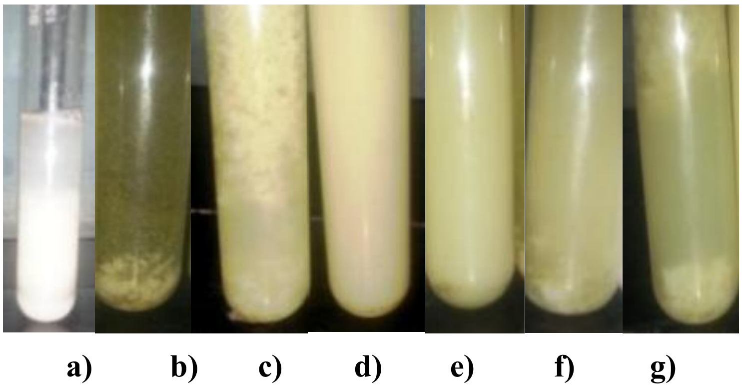

a) b) c) d) e) f) g)

Figure 8: Percentage Inhibition of Protein Denaturation a) Negative Control, b) Positive Control, c) 0.1% w/v, d) 0.2% w/v, e) 0.5% w/v, f) 1.0% w/v & g) 2.0% w/v

The progressive decrease in absorbance values with increasing concentration suggests effective stabilization of protein structure by the herbal formulation. Notably, the 2% w/v concentration exhibited inhibition comparable to diclofenac sodium, indicating strong anti-inflammatory efficacy at higher doses.

The observed activity may be attributed to the presence of phytoconstituents such as flavonoids, phenolic compounds, and terpenoids in the formulation. These bioactive compounds are known to possess antioxidant and membrane-stabilizing properties, which help in preventing heat-induced protein denaturation and reducing inflammatory mediator activity.

Overall, the results demonstrate that the herbal anti-pruritic lotion possesses significant in vitro anti-inflammatory activity in a dose-dependent manner. The comparable efficacy at higher concentration supports its potential therapeutic application in managing inflammatory and pruritic skin conditions. Further in vivo studies are recommended to confirm these findings and establish clinical relevance.

3.8 Stability Testing

3.8.1 Accelerated Stability Testing

The lotion's physical properties (pH, viscosity, colour, odour, and homogeneity), chemical stability (active constituents' content), and microbiological stability have monitored over a 3-month period. F1, F2, and F3 exhibited minor changes in pH and viscosity, with F2 showing the highest stability.

Table 9: (a) pH Changes Over 3 Months (Accelerated Testing)

pH	Initial Value	Month 1	Month 2	Month 3	Limits
F1	5.8	5.7	5.7	5.6	5.5–7.0
F2	6.2	6.1	6.1	6.1	5.5–7.0
F3	5.5	5.5	5.4	5.3	5.5–7.0

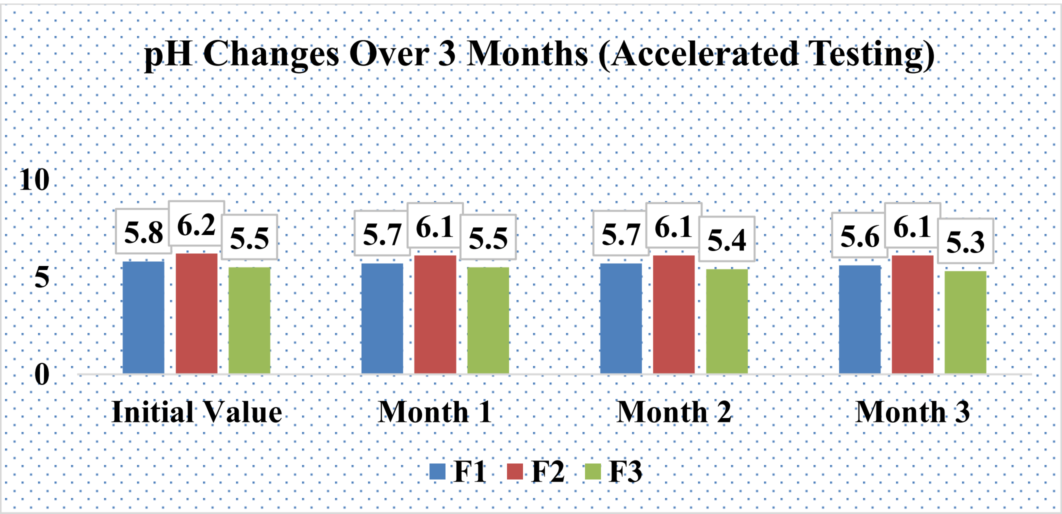


Figure 9: (a) pH Changes Over 3 Months (Accelerated Testing)

Table 10: (b) Viscosity (cP) Changes Over 3 Months (Accelerated Testing)

Viscosity (cP)	Initial Value	Month 1	Month 2	Month 3	Limits
F1	3000	2950	2900	2850	±10% variation
F2	2500	2480	2460	2450	±10% variation
F3	2000	1800	1600	1550	±10% variation

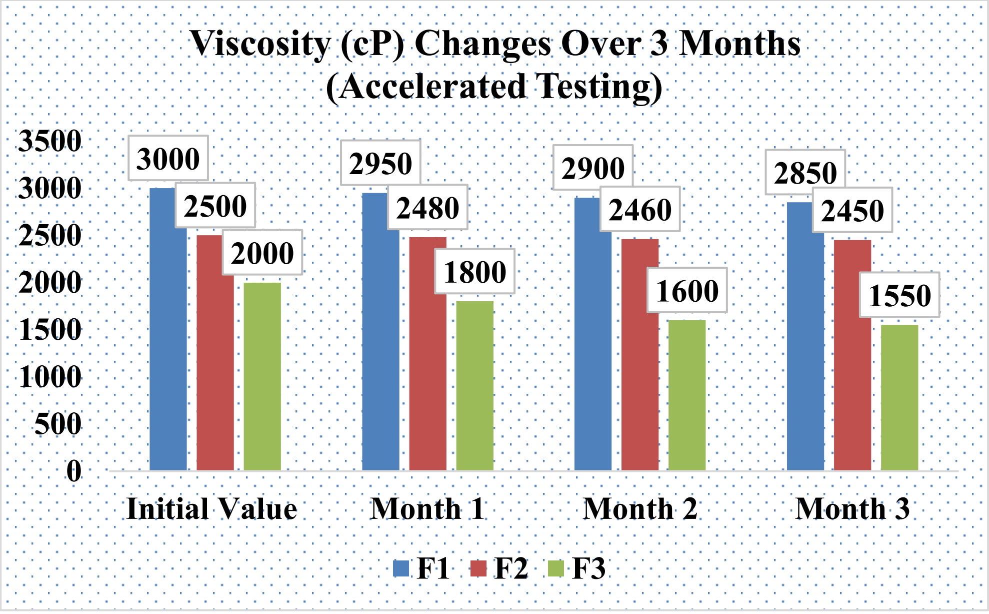


Figure 10: (b) Viscosity (cP) Changes Over 3 Months (Accelerated Testing)

F3 showed the most significant degradation in pH and viscosity, suggesting it is less stable under accelerated conditions. F2 maintained better consistency, indicating it has a more robust formulation suitable for extended storage. All formulations remained within acceptable limits for colour, odour, and microbial growth.

3.8.2 Real-Time Stability Testing

Minimal changes have observed across all formulations. F2 exhibited superior stability compared to F1 and F3.

Table 11: (a) pH Changes Over 3 Months (Real-Time Testing)

pH	Initial Value	Month 1	Month 2	Month 3	Limits
F1	5.8	5.7	5.6	5.5	5.5–7.0
F2	6.2	6.1	6.0	6.0	5.5–7.0
F3	5.5	5.4	5.3	5.1	5.5–7.0

F2 demonstrated the least variation in pH and viscosity under real-time conditions, making it the most stable formulation. No significant changes in colour, odour, or microbial growth have observed across all formulations. The real-time stability results support the predictions from accelerated stability testing, highlighting F2 as the best candidate for commercialization.

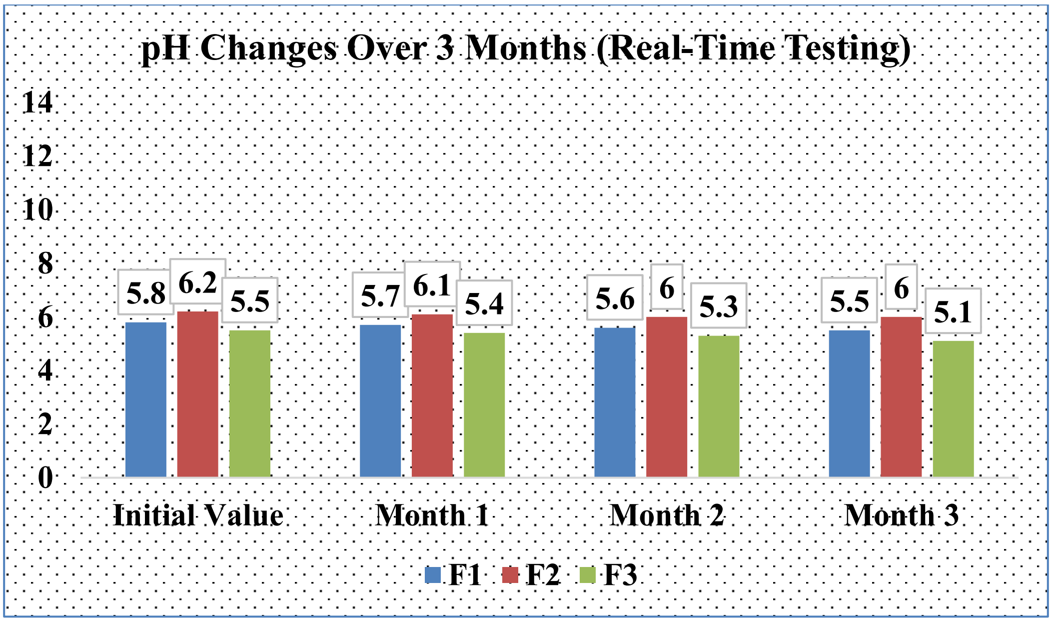


Figure 11: (a) pH Changes Over 3 Months (Real-Time Testing)

Table 12: (b) Viscosity (cP) Changes Over 3 Months (Real-Time Testing)

Viscosity (cP)	Initial Value	Month 1	Month 2	Month 3	Limits
F1	3000	2900	2850	2750	±10% variation
F2	2500	2490	2480	2460	±10% variation
F3	2000	1900	1700	1650	±10% variation

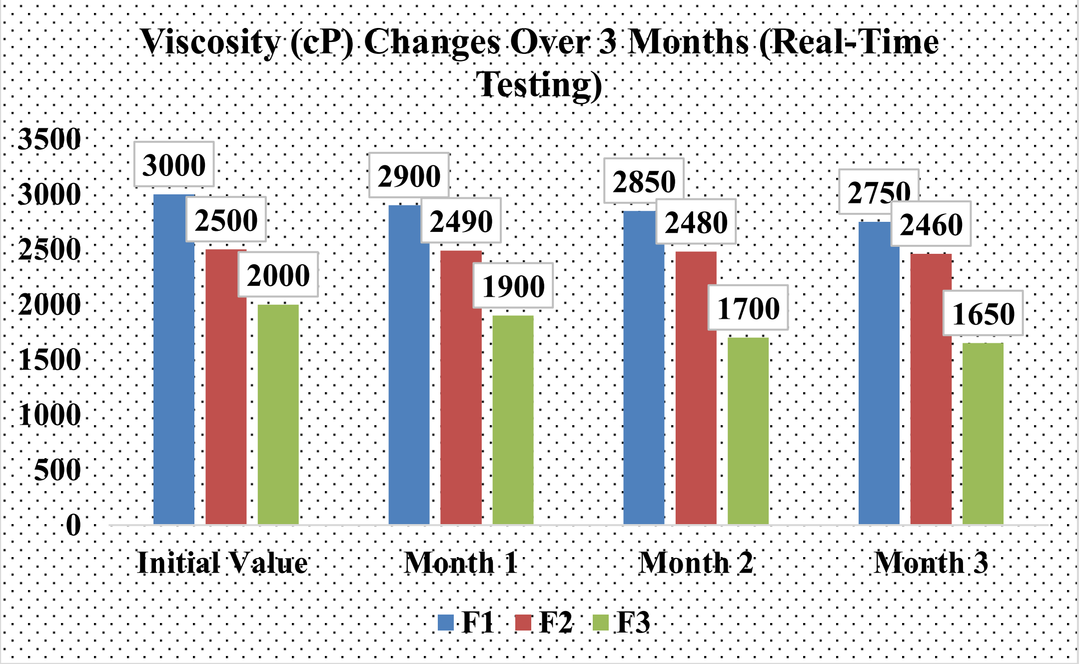


Figure 12: (b) Viscosity (cP) Changes Over 3 Months (Real-Time Testing)

Conclusion

The present study successfully formulated and evaluated a herbal anti-pruritic lotion using different concentrations of herbal ingredients. The prepared formulations were assessed for physicochemical properties, antimicrobial activity, anti-inflammatory potential, and stability.

Among the three formulations, F2 demonstrated the most favorable characteristics including acceptable pH, moderate viscosity, ideal spreadability, excellent homogeneity, and absence of skin irritation. Additionally, F2 exhibited superior antimicrobial activity and significant anti-inflammatory effects compared to the other formulations. Stability studies further confirmed that F2 remained stable under both accelerated and real-time storage conditions.

Therefore, formulation F2 can be considered the most promising candidate for further development and potential commercialization as a safe and effective herbal anti-pruritic topical preparation.

Acknowledgment

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RECENT ADVANCES IN UNDERSTANDING THE CELIAC DISEASE

Yuvasri S.K and Lavanya Krishnadhas
Department of Biochemistry,
PSG College of Arts and Science, Coimbatore – 641004
Corresponding author E-mail: yuvasrisk2005@gmail.com, lavanya@psgcas.ac.in

Abstract

Celiac disease is a chronic autoimmune disorder which is caused by the gluten intake, which is a storage protein present in the wheat, rye and barley. The raise in the global consumption of the wheat-based food causes the gluten related disorders including celiac disease, non-celiac gluten sensitivity and IgE mediated wheat allergy. Celiac disease primarily occurs in individuals carrying the human leukocyte antigen HLA-DQ2 and HLA-DQ8 haplotypes. The disease involves both the innate and adaptive immune response, leading to inflammation, villous atrophy, crypt hyperplasia and increased intra epithelial lymphocytes in the small intestine, these changes impair nutrient absorption. The disease presents with both gastrointestinal symptoms such as diarrhoea, abdominal pain, bloating as well as extraintestinal manifestations including anaemia, neurological disorders and autoimmune conditions. Diagnosis is commonly performed through serological tests for specific antibodies, followed by confirmation using endoscopy and duodenal biopsy. Currently, the only effective treatment for celiac disease is strict gluten free diet, although emerging therapeutic strategies such as enzymatic degradation of gluten and gluten sequestering agents are being explored. This review summarizes the epidemiology, mechanisms, pathophysiology, biochemical manifestations, diagnosis and potential approach for the celiac disease.

Keywords: Celiac Disease, Gluten, HLA-DQ2 Gene, HLA-DQ8 Gene, Autoimmune Disorder.

Introduction

Wheat is one of the most vital staple foods across the world and its need among the people is also increasing day by day. Since 1955, its usage among the people has been triplicated. The yield of the wheat is largely raised due to the advancement in agricultural practices. There is a change in the protein quantity content and composition due to this reason. Because of the higher intake of wheat, there is a increase in rise of disorders that is caused against wheat constituents in the body (Stordal and Kurppa, 2025). Wheat is the mixture of starch, proteins, micronutrients, fibers which attributes to its culinary and viscoelastic properties and it also confers the wheat allergy, celiac disease, non-celiac gluten/ wheat sensitivity. A type of storage protein which is present in wheat, rye and barley is called gluten. It consists of mixture of prolamins especially proline and glutamine (Manza *et al.,* 2024).

The disorders and symptoms associated with this wheat consumption is of two types, namely immunological and non-immunological reactions. Immunological reactions are caused by eliciting hypersensitivity to wheat proteins and that constitute about 13%. Gluten is the major storage of wheat protein which constitutes glutenin and gliadin constituting about 75% - 85% of the protein quantity (Stordal and Kurppa, 2025).

Among the population, 10% is being affected by gluten related problems which constitutes of three types namely, celiac disease, non-celiac gluten sensitivity and IgE mediated wheat allergy. The inflammation in the intestine is by the innate and adaptive immune response that is being caused by the triggering activity of the gluten. Gluten along with other food compartments contribute to the development of inflammatory bowel disease (IBD) and functional gastrointestinal disorders (FGIDs) (Cenni *et al.,* 2023).

According to the previous research done by Martin *et al.,* 2024, zonulin and other molecules is regulated by gluten in which it is being involved in the intestinal permeability. Furthermore, if there is improper hydrolysis of the gluten, its respective peptide is being absorbed and triggers the immune inflammatory response by raising the levels of interferon γ, intraepithelial CD3^{+} T cell lymphocytes and toll like receptors (TLR).

Celiac Disease

Celiac disease is a genetic disorder and immune mediated response to gluten that is characterised by inflammatory damage to the small intestine due to the inappropriate T cell mediated immune response (Raiteri *et al.,* 2022). It affects the population around 0.5 – 1 %. It is being widely spreading especially among the women and the children worldwide (Besser and Khosla, 2023).

Celiac disease was found to be the food related disorders, but now it has many specific characteristics with tissue specific autoimmune disease like type 1 diabetes and rheumatoid arthritis. The autoimmune characteristics of celiac disease include human leukocytes antigen association, production of the specialized autoantibodies and immune mediated destruction of the enterocytes. However, celiac disease is distinct from other autoimmune conditions, as it only shows symptoms while being exposed to certain external stimulus (Iverson and Sollid, 2023).

The prevalence of the autoimmune disorders like celiac disease and wheat related allergy is due to the consumption of the gluten foods in their diet. The protein that acts as a antigen in celiac disease is the gliadin, where the human body intestinal enzymes and peptidases cannot degrade them. The proteolysis of the proline peptides is inhibited by the pancreatic, gastric and intestinal membrane enzymes, which causes the accumulation in the small intestine (Wu *et al.,* 2021). The People with celiac disease are characterised by changes in the gut structure that triggers and induce the production of autoantibodies. The main characteristics of injury in the small intestine is the villous atrophy with crypt hyperplasia and higher production of intraepithelial lymphocytes (Fontana *et al.,* 2025).

The celiac disease is a chronic illness with no specific medical treatment for the cure of the disease. Apart from changing their diet, people cannot recover from the disease as they age or experience the remission. So, the only possible way to recover is having gluten free diet throughout their lifetime. Though if there is a lack in intaking the gluten free diet, the progression of the disease will continue and injury to the small intestine and inflammation resides for a long period (Posterick and Ayars, 2023).

The gut microbiota plays a very important role in the body. The composition of gut bacteria is distinct in genetically predisposed children when compared to the lower risk children. The patient with celiac disease has a higher level of gut bacteria like *bacteroids, E.coli, proteobacteria* and *staphylococcus* and the lower level of *lactobacillus* and *bifidobacteria* (Mentesidou *et al.,* 2025)

Epidemiology

The incidence of the celiac disease may vary between the regions of the world. The difference is due to the variations in the gluten consumption, use of antibiotics and genetics. It affects the adult as well as children but most commonly it affects women when compared to men (Garcia *et al.,* 2025). The prevalence of celiac disease across the world is about 1.4%. according to the community-based studies, the prevalence of celiac disease is about 1.04% (Meena *et al.,* 2024).

The celiac disease is found to be increased in the late 20th and early 21st centuries, though the recent reports say that they are levelling up at higher rates in some countries (Lexner *et al.,* 2024). It is believed that the modern wheat varieties are the major reason for the development of disorders related to gluten intolerance. But, the modern lifestyles, changes in the food pattern and preparations, disruption of the intestinal barrier in the case of viral diseases and those which causes the dysbiosis of the intestine etc., these all contributes to the increased prevalence of celiac disease globally (Stastna *et al.,* 2023).

Mechanism of Action

The pathogenesis of the celiac disease, which is an autoimmune disorder is caused by the combination of the genetic, environmental factors and genetic variables that triggers an immune response in the body. In case of genetics, celiac disease occurs in genetically exposed people with particularly major histocompatibility complex haplotypes DQ2 or DQ8 in the HLA – human leukocyte antigen are more likely to develop the celiac disease. In case of environmental factors, the gluten intake causes the celiac disease in adults as well as in infancy. According to some of the recent research, rotavirus related viral infection is also found to be the major factor for the development of this celiac disease recently.

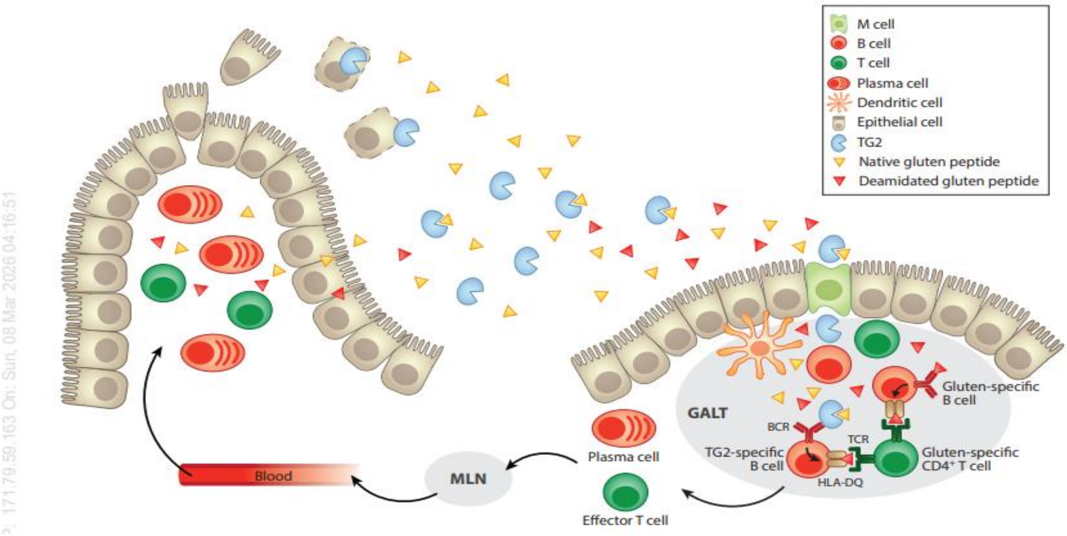
Innate immunity and adaptive immunity are the two basic immunities involved in the gut immune response by gluten. In terms of innate immunity, gluten's non immunogen dominant peptide (P31-P43) which may directly stimulate the growth and development of epithelial lymphocytes, $CD8^+$ T cells, natural killer cell proliferation and interferon γ production. Moreover, it increases the intestinal epithelial cell expression of the NKG2D receptor ligands and the natural killer cells ability to kill the intestinal epithelial cells, which in turn increases the intestinal permeability. Regarding the adaptive immunity, some bigger peptides in the gluten may pass through intestinal mucosal barrier and reach intestinal lamina propria due to the increased intestinal permeability which is brought up by the infection and causes the damage to the intestines. Finally, after the IECs death, villi would become blunt because if the interaction between the gliadin and CD4$^+$ T cells in the intestinal tract's lamina propria and as well as crypt proliferation by generating the pro inflammatory cytokines that cause the immune cell chemotaxis. (Sharma *et al.,* 2020)

The most important gene for the development of the celiac disease are the leukocyte histocompatibility antigen genes HLA-DQ2 encoded by (HLA-DQA1*05-DQB1*02) and HLA-DQ8 encoded by (HLA-DQA1*03-DQB1*0302) on the chromosome 6q21. 90% of the people with celiac disease have the haplotype HLA-DQ2; and the 5% of the people with celiac have the haplotype HLA-DQ8 (Aboulaghras *et al.,* 2023). These genes encode HLA-DQ2 and HLA-DQ8 on (APCs) Antigen Presenting Cells, which in turn primarily binds to gluten and is broken down in the small intestine to produce the HLA polypeptide complexes that are presented to CD4$^+$ T cells. The T Cell activation induces the APCs to secrete the interleukin 15 (IL – 15), causing the proliferation of the intraepithelial lymphocytes (IELs). The IELs in turn expresses the natural killer cells receptor-NKG2D on the surface of the cell and causes the epithelial cells to express the NKG2D ligand major histocompatibility complex (MHC) related gene A (MICA). The natural killer cells identify the MIC via NKG2D receptor and kill the intestinal epithelial cells, which increases the intestinal permeability. So, T Cells secrete interleukin 21 (IL- 21) and IFN- γ which causes the inflammation and epithelial damage in the small intestine. On the other hand, T Cell activation causes activation of B Cells and plasma cells which induces the antibody production like anti–gluten antibodies and anti-transglutaminase- 2 antibodies (Ge and Chen, 2024).

Both the anti-gluten antibodies and the anti-transglutaminase 2 antibodies are produced during the celiac disease. Though these antibodies are useful for diagnosing diseases, their pathogenic role is an unanswered question. The B Cell receptor is involved in the effective transport of the gluten antigens to HLA-DQ2 and HLA-DQ8 for presenting the CD4$^+$ cells via endocytosis of gluten peptides and TG2 gluten complexes. Thus, the people with celiac disease whoever is consuming gluten diet have a significant concentration of the TG2 specific plasma cells in their intestine (Besser and Khosla, 2023).

Mechanism of Celiac Disease

It is interesting to know that many people with HLA-DQ2 and HLA-DQ8 do not have celiac disease. Infection that modifies the immune response, alterations in the gut flora after the antibiotic use and changes during the pregnancy are the factors which causes the gluten intolerance in the individuals (Kahrs *et al.,* 2019).



(Iverson and Sollid, 2023)

Pathophysiology

The patients with celiac disease showed crypt hyperplasia, villous atrophy, focal atrophy, partial atrophy or full atrophy, inflammation ranging from mild to severe cases, changes in the shape of the epithelium, epithelial damage, mucosal edema and goblet cell depletion (Manzoor *et al.,* 2022).

Villous atrophy and crypt hyperplasia reduces the villous to crypt ratio in patients with celiac disease. The ratio is less than 2:1 in untreated persons and in extreme conditions it may range as low as 1:1. The normal ratio in healthy individual is 3:1 which promotes the absorption very well (Mandile *et al.,* 2022). In addition to it, when intraepithelial lymphocytes especially CD8$^+$ T cells enter the epithelial layer represents the inflammatory reaction to gliadin peptides. Increased immune cell infiltration of T cells, B cells and plasma cells is visible in the lamina propria beneath the epithelium (Elli *et al.,* 2024).

Clinical Manifestations

The patients with celiac disease have the manifestations like positive anti transglutaminase antibody, dermatitis herpetiformis, gluten ataxia, abnormal liver enzymes, type 1 diabetes mellitus, autoimmune thyroid issues, psoriasis, chronic urticaria (Machado, 2023).

Gluten neuropathy, gluten encephalopathy, temporal lobe epilepsy, multiple sclerosis, cognitive symptoms called “foggy brain” which improves when the gluten is restricted but reappears when the gluten is intake. It also causes the psychiatric disorders like bipolar disorder, depression, apathy, autism, eating disorder and sleeping disorders (AI-Toma *et al.,* 2025).

Gastrointestinal symptoms like abdominal pain, diarrhoea, vomiting, bloating, indigestion, to non-gastrointestinal symptoms like fatigue, anaemia, headaches and cognitive dysfunction. Untreated celiac disease patients have a lifelong health complication (Shuler *et al.,* 2023).

Diagnosis

Currently, the diagnosis of celiac disease is done by the combination of serological testing, endoscopy and duodenal biopsy. A patient with the intestinal and extraintestinal symptoms must undergo the serological analysis for the diagnosis of celiac disease. IgA-class TGA is the specific antibody for celiac disease, and they should undergo serological analysis to identify antibody. The enzyme linked immunosorbent assay is used to conduct the IgA-class TGA test. Some of the patients may be IgA deficient, so they should assess IgG levels. The gold standard for diagnosing celiac disease in adult is by endoscopy and duodenal biopsy samples. The endoscopy findings have a higher specificity for celiac disease. Though endoscopy is taken, the patients must also undergo biopsy since 33% of the people show normal appearance in endoscopy (Losurdo *et al.,* 2021).

Endomysial (EMA)- IgA Testing is mostly used as a secondary confirming assay for patients with equivocal tTg-IgA results. When the patients have low level of tTg-IgA antibodies, it is necessary to take endomysial testing. Even a positive endomysial (EMA)-IgA test, biopsy is required to confirm the celiac disease. A cytosolic protein that is being widely produced in the adult enterocytes is intestinal fatty acid binding protein (I-FABP) which serves as a biomarker for the intestinal epithelial damage. The villous enterocytes of the distal jejunum, which is the main location of the early mucosal injury in celiac disease shows a high expression of the I-FABP. It shows the changes in the intestinal permeability (Wang and Wu, 2025).

The histological assessment of the small intestine mucosa is the gold standard for the diagnosis of celiac disease before the serum analysis of TGA. The enterocyte thickness, luminal surface, ratio to villus to crypt, quantity of the intraepithelial lymphocytes should be considered while evaluating the biopsies. Pathologist primarily use Marsh Oberhuber classification to assess the mucosal lesions. Marsh types 2 -4 strongly indicates the celiac disease (Wieser *et al.,* 2024).

Prevention and Treatment

The people with celiac disease must not intake gluten related food items and they are advised to intake the gluten free diet to reduce the severe progression of the disease. If they continue to intake gluten, it may cause clinical symptoms like intestinal damage and raising the risk of causing small intestinal adenocarcinoma, oesophageal cancer and melanoma in the future. People who is intaking the gluten free diet are likely advised to intake fruits, vegetables, fish, meat and gluten free foods for their betterment (Aljada *et al.,* 2021).

Addition of naturally occurring amino acid to gluten, which area linked to reduction in T cell stimulatory qualities. The patients with celiac disease challenged with E82 bread had lower gluten induced IFN γ production of the T cells and gluten immunogenic peptides in stools. (Discepolo *et al.,* 2024). During the gluten free diet, there is a successful reduction in the gastrointestinal symptoms which includes the reduction of the stomach pain, bloating. But the diarrhoea and reflux may take a longer day to fade away even there is a continuous gluten free diet (Simon *et al.,* 2023).

To lower the effects of gluten exposure with people of celiac disease, many studies focused on the new therapies like enzyme degradation and gluten sequestration. An oral enzyme *Aspergillus niger* derived from prolyl Endo protease can break down the antigenic proteins and reducing the immunogenicity and intestinal damage. It is resistant to pepsin and is of the ranges from 2 to 8. BL-7010 is a high molecular weight, non-absorbable polymer which has a strong affinity to gliadin. It prevents the absorption of the toxic and non-immunogenic gluten peptides, which alters the inflammatory and immune response that is being linked to celiac disease (Santonicola *et al.,* 2025).

Conclusion

Celiac disease is a long term immune mediated disorder that develops due to the consumption of gluten related food items. The interaction between the gluten peptides and specific genetic factors induces an abnormal immune response which damages the lining of the small intestine and interferes with the nutrient absorption. The prevalence and awareness of the gluten related disorders have increased due to the world's increasing dependence of wheat-based diets. Accurate diagnosis using the serological markers and intestinal examination, followed by the struct adherence to the gluten free diet continue to be the cornerstone of celiac disease management. The continued research and the increased public awareness are important to improve the early diagnosis, enhance the treatment strategies and reduce the complications associated with this disease.

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A REVIEW ON EPIGENETIC REGULATION OF MICRORNAs IN CANCER

Suwetha B and Lavanya Krishnadhas
Department of Biochemistry, PSG College of Arts & Science, Coimbatore – 14
Corresponding author E-mail: suwethabaskar14@gmail.com, lavanya@psgcas.ac.in

Abstract

MicroRNAs (miRNAs) are small, naturally occurring, single-stranded non-coding RNA molecules that play a crucial role in regulating gene expression. They are involved in many important biological processes, including immune response, neural development, DNA repair, apoptosis, oxidative stress and cancer progression. Although miRNAs are mainly located within cells, a considerable number are also found in body fluids as circulating miRNAs, where they function as extracellular signalling molecules that influence cell growth and differentiation. Proper control of miRNA activity is essential for maintaining normal cellular functions and disturbances in this regulation are commonly linked to various diseases, including cancer, cardiovascular, metabolic, neurological and immune-related disorders. Circulating miRNAs have garnered significant interest due to their abnormal expression patterns, which are closely associated with disease development, particularly in cancer. Many circulating miRNAs have been identified as potential biomarkers for both solid and blood-related tumours, contributing to improved diagnosis, prognosis and prediction of treatment outcomes. Furthermore, analysing circulating miRNAs may help detect individuals at risk of cancer and provide new opportunities for targeted therapies. In this review, we summarise recent progress on the mechanisms regulating miRNA biogenesis and the interplay between DNA Methylation and miRNA.

Keywords: Circulating miRNAs, Gene Expression, Biomarkers, Cancer, Diagnosis, Apoptosis, Prognosis and Therapeutic Targets.

Introduction

MicroRNA (miRNA) biogenesis is a complex process that involves multiple steps, from transcription in the nucleus to the production of mature miRNA in the cytoplasm. This process is intensively studied due to the exciting regulatory roles of miRNAs, with applications in both basic research and therapeutics. The six primary processes that define miRNA levels include transcription of the primary miRNA (pri-miRNA) transcript, processing of the primary miRNA transcript into precursor miRNAs (pre-miRNA), export of the precursor miRNA to the cytoplasm, processing of the precursor miRNA into the mature miRNA, recognition and inhibition of target transcripts and mechanisms to regulate stability and decay of mature miRNAs (Bartel *et al.,* 2019).

RNA polymerase II first transcribes a primary microRNA transcript from the DNA. The pre-miRNA is contained in the primary sequence and forms a hairpin structure that is recognised and cleaved by the Microprocessor complex consisting of the RNase III enzyme DROSHA (double-stranded RNA-specific endoribonuclease) and its cofactor DGCR8 (DiGeorge Syndrome Critical Region 8), resulting in the processed hairpin-shaped pre-miRNA. The Exportin 5 (XPO5) protein then exports the pre-miRNA to the cytoplasm, where the pre-miRNA is further processed by the RNase III enzyme Dicer, and its cofactor Trans-Activation Response RNA- Binding protein (TRBP), to produce a double-stranded RNA duplex consisting of the mature miRNA and its complementary strand, known as the passenger strand. The duplex is unwound and the mature miRNA is loaded into the RNA-induced silencing complex (RISC). The RISC/miRNA complex then binds to target mRNAs and represses their translation (McGeary *et al.,* 2019).

miRNA biogenesis and regulation

The genesis of miRNAs originates in the nucleus of the cell. RNA polymerase II transcribes miRNA genes, yielding primary miRNA transcripts (pri-miRNAs). These pri-miRNAs are extensive, capped and polyadenylated RNA molecules that contain one or more miRNA hairpin structures. The microprocessor complex, which includes the RNase III enzyme Drosha and its cofactor DGCR8, identifies and cleaves the pri-miRNAs to produce precursor pre-miRNAs. Drosha specifically recognises these precursor pri-miRNAs and processes them into pre-miRNAs. Recent research has revealed additional factors that influence this step, such as the RNA helicase Brr2a, which interacts with HYL1 to modulate pri-miRNA structures and enhance miRNA production. The pre-miRNAs, approximately 70 nucleotides in length and featuring a distinctive stem-loop structure, are then transported from the nucleus to the cytoplasm via the exportin-5/Ran-GTP complex. The RNase III enzyme Dicer further processes the pre-miRNAs in the cytoplasm. Dicer identifies the stem-loop structure of the pre-miRNAs and cleaves them to generate mature miRNAs. Recent studies have shed light on the structural determinants of Dicer's substrate recognition, particularly the importance of 5′ ends for efficient processing. For example, (Nguyen *et al.* 2023). demonstrated that internal loops in the lower stem of primary miRNA transcripts facilitate single cleavage by the human microprocessor. Furthermore, research has revealed the structural basis of Dicer's interaction with pre-miRNAs, providing insight into its catalytic mechanism. Additionally, growing evidence suggests that Dicer-independent pathways exist for miRNA biogenesis. Notably, the catalytic activity of Argonaut 2 has been shown to process certain miRNAs, such as miR-451, bypassing the requirement for Dicer (Lee *et al.,* 2023).

Mature miRNAs, typically spanning 21 to 23 nucleotides and existing as single-stranded molecules, are incorporated into the RNA-induced silencing complex (RISC) upon maturation. The RISC is composed of Argonaut proteins and other cofactors. The miRNA embedded within the RISC directs the complex to the complementary sequences located in the 3’Untranslated Regions (3′UTRs) of the target mRNAs. The interaction between the RISC and the target mRNA leads to either the suppression of translation or the degradation of the mRNA, thereby modulating gene expression (Treiber *et al.,* 2019).

The stability and activity of miRNAs can be modulated by various mechanisms. For instance, the role of epigenetic modifications in miRNA biogenesis is gaining increasing recognition. Modifications such as methylation and acetylation can directly impact miRNA stability and binding affinity to target mRNAs. miRNAs can also be regulated by competing endogenous RNAs (ceRNAs), which are non-coding RNA molecules that bind to miRNAs and prevent them from engaging with their target mRNAs (Yang *et al.,* 2021).

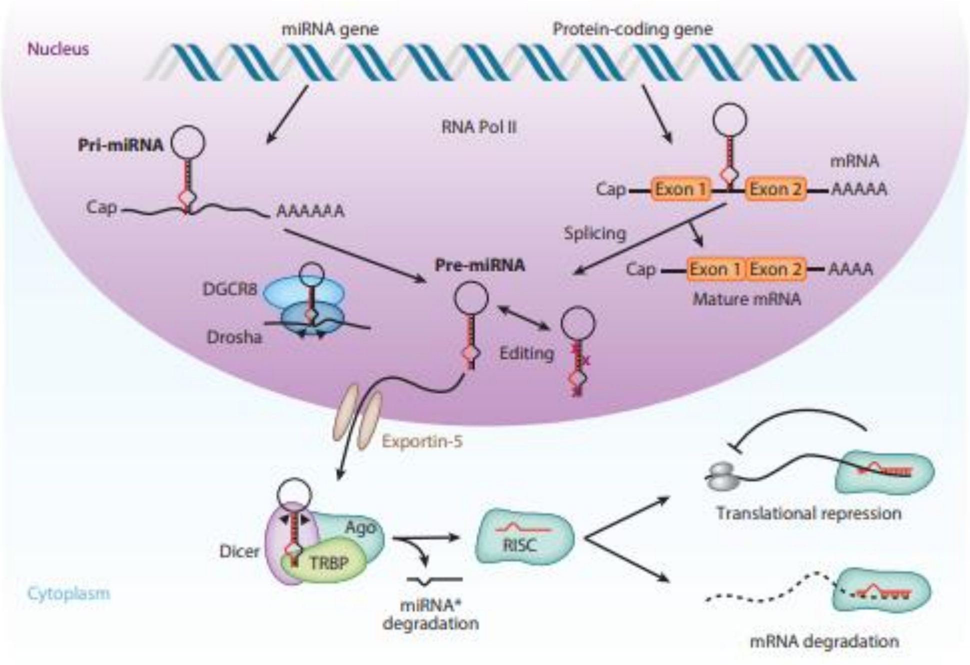

Figure 1: Biogenesis and Mechanism of Action of microRNA

Figure 1 describes the biogenesis of microRNA (miRNA) from transcription in the nucleus to its maturation in the cytoplasm. It shows the processing of pri-miRNA by Drosha-DGCR8, export by Exportin-5. And further cleavage by Dicer to form mature miRNA.

Role of DNA Methylation and miRNAs as Biomarkers

The aberrant expression of miRNAs has been the subject of intense investigation for years in cancer research. MiRNAs can be easily detected in tissues, but also in circulation by non-invasive liquid biopsy. Their stability and ease of detection render miRNA a suitable biomarker for human cancer diagnosis, prognosis and therapeutics. Furthermore, many studies are ongoing to propose miRNA-based cancer therapies. Liquid biopsy is increasingly being used for helping cancer diagnosis due to several advantages compared with the conventional biopsy, such as minimal invasiveness, pain and risk of complications. Circulating tumour-related miRNAs can be found in several bodily fluids and can be used for cancer screening, diagnostics and prognostics. These miRNAs are intensively studied in several cancer types and are easily detectable in serum and plasma (Koh *et al.,* 2022).

Other biological fluids can also be informative. In urine samples, it is possible to detect miRNAs that serve as diagnostic and prognostic biomarkers for oesophageal, cervical, bladder, colorectal and prostate cancers. The aberrant expression of miRNAs has been found in cerebrospinal fluid, pancreatic juice, sputum, and pleural effusion and could be used as potential biomarkers for brain, pancreatic, and lung cancers, respectively (Okuda *et al.,* 2021).

DNA methylation profiles of miRNAs can be used as a signature to define tumour type, clinical prognosis and treatment response. Indeed, dysregulation of DNA methylation is likewise ubiquitous across various cancer types and is considered a hallmark of cancer. Disease and exposure-related methylation changes are detectable in blood, potentially allowing them to serve as biomarkers for cancer and immune response.

Interplay Between miRNA and DNA Methylation

Additional studies further support the role of miRNA loss in promoting DNA hypermethylation and gene silencing. DNA methyltransferases (DNMTs) are enzymes responsible for adding methyl groups to CpG sites in DNA, thereby regulating gene expression through epigenetic modification. Reintroduction of certain miRNAs has been shown to decrease DNMTs. levels and restore the expression of tumour suppressor genes. For example, miR-148a and miR-152 inhibit DNMT1 in cholangiocarcinoma and liver cells, resulting in reduced global methylation and reactivation of genes such as Ras Association Domain Family member 1, isoform A (RASSF1A) and p16. Similarly, miR-342, which is downregulated in colorectal cancer, can suppress DNMT1 and induce demethylation of multiple tumour suppressor gene promoters, including ADAM23, Histidine Triad Nucleotide-binding protein 1 (HINT1), RASSF1A and Reversion-inducing Cysteine-rich protein with Kazal motifs (RECK). In glioma, miR-185 also targets DNMT1 and modulates DNA methylation at various gene loci. Overall, these findings indicate that MiRNAs are small non-coding RNAs with a length of 18 to 25 nucleotides (Chen *et al.,* 2024). MiRNA biogenesis involves three main steps: firstly, the fragment located in an intragenic region or gene desert is transcribed by RNA polymerase II (*pol II*), which produces an mRNA with a length of about 3000 to 5000 bases, called primary miRNA (pri-miRNA). In the second step, pri-miRNA is shortened to produce a 70-base precursor miRNA (pre-miRNA), which is shortened by DROSHA and DGCR8. Finally, the export receptor export 5 (Exp5) directly interacts with the pre-miRNA to transport it into the cytoplasm and then the Dicer cleaves the pre-miRNA into short fragments with a length of 22 bases. Then, its double strand is dissociated into a single strand. AGO-2 relates to one of the mature strands to form an RNA-induced silencing complex (RISC). According to the miRNAs' progression, they can be used as oncogenes (Bofill *et al.,* 2024*).* One example is miR-125b, which is downregulated in cancer-promoting cell proliferation and cell-cycle progression (Chakaraborty *et al.,* 2019).

Conclusion

MicroRNAs are essential regulators of gene expression that maintain cellular homeostasis by controlling processes such as proliferation, differentiation, apoptosis and stress responses. In cancer, the normal balance of miRNA expression is frequently disrupted and one of the major mechanisms underlying this dysregulation is DNA methylation. Hypermethylation of CpG in the promoter regions of tumour suppressor miRNAs leads to their silencing, thereby allowing the overexpression of oncogenic targets and promoting tumour progression. Conversely, several miRNAs directly regulate DNA methyltransferases and their downregulation results in abnormal methylation patterns and widespread epigenetic alterations. This reciprocal interaction between miRNAs and DNA methylation forms a complex regulatory network that significantly contributes to tumour initiation, growth and metastasis. Understanding the mechanisms of epigenetic control of miRNAs highlights their potential as diagnostic biomarkers, prognostic indicators and therapeutic targets. Therefore, further research focusing on the clinical validation and therapeutic modulation of epigenetically regulated miRNAs may provide promising strategies for improved cancer diagnosis and treatment.

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GREEN SOLVENT-PROCESSED IRIDIUM(III) COMPLEXES FOR HIGH-EFFICIENCY PHOSPHORESCENT OLEDs

A. Maheshwaran, P. Santharaman*,
S. Dorothy, M. Dinesh Kumar and V. Kanchana
Department of Chemistry,
AMET University, Kanathur, Chennai – 603 112, Tamil Nadu, India
*Corresponding author E-mail: santharamanpaulraj86@gmail.com

Abstract

This chapter discusses the creation of high-efficiency phosphorescent organic light-emitting diodes (OLEDS) using iridium (III) complexes fabricated using eco-friendly solvents. Replacing conventional vacuum thermal evaporation with solution-based fabrication processes including spin-coating has great benefits in terms of low-cost, large-area, and roll to roll production. However, this method normally utilizes the use of dangerous aromatic or chlorinated solvents. Here, we discuss about the molecular architecture of novel iridium (III) complexes with a high level of solubility in the green solvent cyclohexanone. The aggregation behavior of the complexes can be controlled by modifying the ancillary ligands in an intelligent fashion, either by the addition of alkyl chains or other polar functionalities and makes the complexes compatible with solvents that are environmentally friendly. These solutions processed emitters are highly performing when included within an appropriate host matrix. External quantum efficiencies of devices with these realistically architecture complexes have reached greater than 24% and current efficiencies of greater than 60 cd/m^2, some of the highest ever recorded in solution-processed green OLEDs. Finally, this chapter shows that the development of iridium complexes to process green solvents is a potential and feasible direction to achieve a compromise between high device functionality and eco-friendly and sustainable production of OLEDs.

Keywords: Green Solvent Processing, Iridium(III) Complexes, Phosphorescent OLEDs Solution-Processed OLEDs, Eco-Friendly Solvents.

Introduction

Phosphorescence organic light emitting diodes (PHOLEDs) have received significant interest in academic and industrial communities because of their potential uses in display and solid-state lighting systems [1,2]. The singlet and triplet excitons can be harvested by PHOLEDs, which allows them to attain near-theoretical internal quantum efficiencies (IQEs) (Figure 1**)** [3,4]. Generally, the processing of PHOLEDs can be done by dry or wet methods. Although it is possible to achieve OLED devices with optimized high external quantum efficiencies (EQE) by thermal deposition, a number of drawbacks, including high energy usage, high production cost, and low yield are associated with it. Although the use of vacuum deposition allows easy movement of charges and excitons to recombine leading to the achievement of better device performance, solution-processed PHOLEDs possess considerable benefits of material reduction and low manufacturing costs. [5,6].

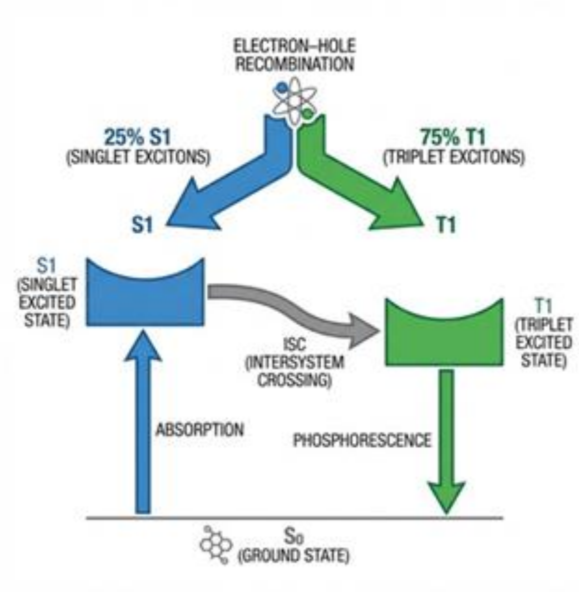


Figure 1. Electrical excitation is commonly observed in organic light-emitting devices (OLEDs)

In recent years, heteroleptic green-emitting iridium(III) complexes derived from 5H-benzo(c)(1,5)naphthyridin-6-one have demonstrated excellent performance in solution-processed phosphorescent organic light-emitting diode (PHOLED) devices [7-10]. Generally, homoleptic Ir(III) complexes exhibit higher chemical and thermal stability than their heteroleptic counterparts. Thompson *et al.* reported widely adopted strategies for the selective synthesis of homoleptic meridional (*mer)* and facial (*fac*) isomers [11-12]. The formation of these *mer* and *fac* isomers is primarily governed by reaction parameters such as temperature, solvent, and substrate. Although the photophysical and electroluminescent (EL) characteristics of *fac*-isomers based on phenylpyridine (ppy) have been extensively studied in PHOLEDs (Figure 2), the properties of mer-isomers remain comparatively underexplored. Most research efforts have concentrated on ppy-based *fac*-isomers because of their symmetrical structures and high PLQYs in solution [13-20]. However, challenging the traditional perception of the relatively reduced efficacy of mer-based Ir(C^N)$_3$ complexes, PHOLED devices employing *mer*-tris(2-phenyl-4-methylpyridinato-N,C^2) [*mer*-Ir(m-ppy)$_3$]. These devices exhibited remarkable EL performance comparable to that of PHOLEDs based on *fac*-Ir(ppy)$_3$ and *fac*-Ir(*m*-ppy)$_3$ [21-22]. Motivated by these findings, we aimed and produced two novel homoleptic green-emitting *mer*-isomers: *mer*-tris[5-hexyl-8-trifluoromethyl-5H-benzo(c)(1,5)naphthyridin-6-one]iridium(III) (*mer*-G1) and mer-tris[5-ethylhexyl-8-trifluoromethyl-5H-benzo(c)(1,5)naphthyridin-6-one]iridium(III) (*mer*-G2). When high kinetic barricade associated with *mer-fac* isomerization enables the isolation of pure *mer*-isomers in excellent yields. This large barrier is likely attributed to the rigid bridged amide framework and the significant C-N rotational barriers [12, 23]. Furthermore, the incorporation of bulky -CF_3 substituents in the main ligands helps reduce self-quenching effects. Moreover, the introduction of longer alkyl chains enhanced the dissolution of the Ir(III) complexes and enhances their photoluminescence quantum yields. In addition, good surface morphological stability at room temperature was observed, which may arise from improved miscibility between host and dopant molecules due to the presence of longer alkyl chains. Consequently, these structural features make the complexes highly suitable for solution-processed PHOLED applications [24,25].

Significantly, both Ir(III) complexes exhibited high PLQYs in solution, which motivated additional investigation of their photophysical and EL properties. Accordingly, the complexes, mer-G1 and mer-G2, were employed as dopants in solution-processed phosphorescent organic light-emitting diodes (PHOLEDs) using tris(4-carbazoyl-9-ylphenyl)amine (TCTA) and 2,2',2''-(1,3,5-benzinetriyl)-tris(1-phenyl-1H-benzimidazole) (TPBi) as host materials. Notably, the device incorporating *mer*-G2 as the dopant exhibited outstanding performance of EL, which was comparable to that of earlier reported fac-Ir(C^N)$_3$-based emitters in solution treated PHOLEDs. An extraordinarily high maximum current efficiency (CE_{max}) of 67.81 cd A^{-1} and an external quantum efficiency (EQEmax) of 20.03% were achieved for the mer-G2-based device. To the best of our knowledge, this represents the first report of Ir(C^N)$_3$-based mer-isomers exhibiting both high PLQYs and excellent EL performance in solution-processed PHOLEDs with high luminous efficiency in the green region (see Figure 1) [26]. Encouraged by these results, we further modified the molecular structure by replacing one of the main ligands in mer-G2 with a multifunctional ancillary ligand, 4-(2-ethoxyethoxy)picolinate, to obtain a new heteroleptic Ir(III) complex (G3). Typically, solution-processable heteroleptic Ir(III) complexes are synthesized through multistep procedures that involve complicated synthetic routes and additional purification processes. To overcome these limitations, the solubilizing 2-ethoxyethanol (-EO_2) group in G3 was introduced over a tandem response strategy, thereby eliminating the need for intermediate isolation in addition to complex purification stages. In general, the fabrication of OLED devices often relies on toxic chlorinated solvents viz. chloroform, chlorobenzene (CB), or o-dichlorobenzene (o-DCB). Although these solvents can reduce production costs and enhance device performance, they pose significant risks to human health and the environment [27-29]. Therefore, the development of eco-friendly solvents and efficient solution-processing techniques is essential for sustainable large-scale OLED manufacturing. In addition, achieving efficient carrier injection and maintaining a well-balanced emissive layer (EML) are vital for realizing high-performance OLED devices. Among various hole-transporting materials, PEDOT:PSS has been widely used as a universal hole injection layer (HIL) and has been extensively studied for more than three decades. Several strategies have been explored to further enhance PHOLED performance, including the use of purified PEDOT:PSS or modified PEDOT:PSS (*m*-PEDOT:PSS) [30,31].Based on these considerations, high-performance green PHOLEDs were fabricated using bis5-ethylhexyl-8-trifluoromethyl-5H-benzo[c][1,5]naphthyridin-6-oneiridium(III) (G3) as the emitter, m-PEDOT:PSS as the hole injection layer, and the non-halogenated solvent cyclohexanone for device fabrication. The resulting devices exhibited an impressive maximum EQE of 23.60% and a CEmax of 85.22 cd A^{-1}. To the best of our knowledge, these values represent among the highest reported efficiencies for green solution-processed PHOLEDs. When G3 was employed as the emitter and m-PEDOT:PSS served as the hole injection layer, the optimized PHOLED devices exhibited enhanced performance, achieving a maximum EQE of 24.56% and a current efficiency of 87.96 cd A^{-1}**.** These results demonstrate the excellent potential of the designed Ir(III) complexes for high-efficiency green PHOLED applications.

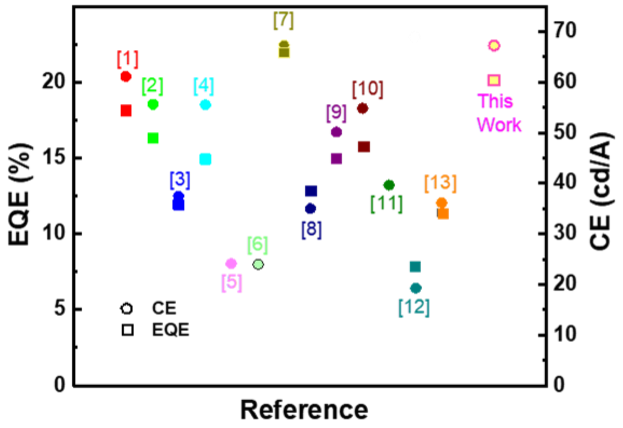
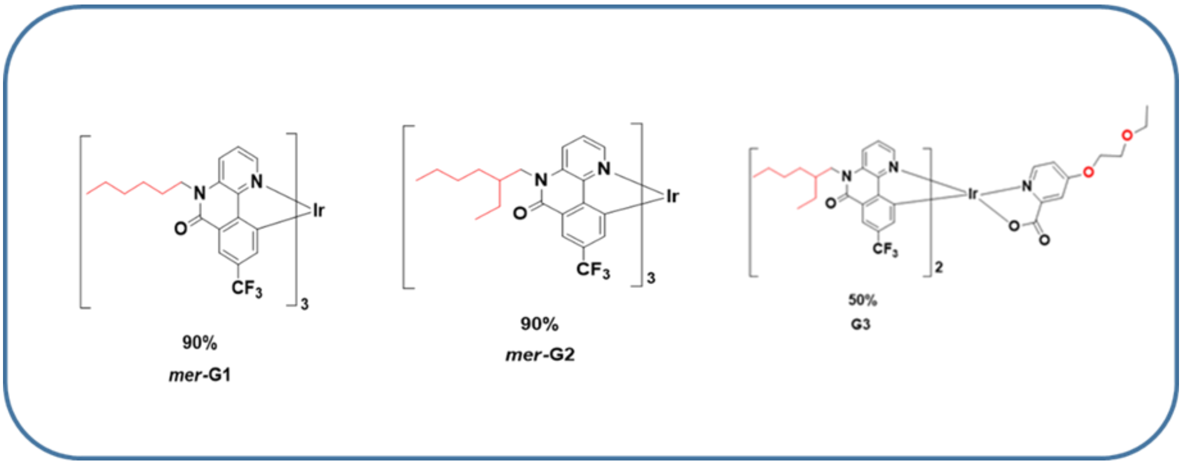


Figure 2: Literature comparison of *fac* isomers in green solution-processed PHOLEDs with *mer*-G2

Recent Development of green Solvent Processed Ir(III) complexes

Recently, we introduced a set of stable and highly efficient heteroleptic green-emitting Ir(III) complexes suitable for solution-processed phosphorescent OLED (PHOLED) technologies. In this chapter, to further develop the device performance of homoleptic green Ir(III) complexes in solution-processed PHOLEDs, designed and synthesized two new homoleptic Ir(III) complexes, namely) *mer*-G1 and *mer*-G2, both exhibiting high PLQYs. Interestingly, the synthetic approach the *mer*-isomers were obtained at elevated temperatures, while no formation of *fac*-isomers was detected during the reaction. The target mer-isomers were successfully obtained in high purity. Furthermore, these mer-isomers did not undergo isomerization under either thermal or photochemical conditions, indicating the presence of a pronounced kinetic barrier sandwiched between the mer and *fac*- structural variants.

High-vacuum train sublimation was employed to purify all synthesized Ir(III) complexes, affording highly pure products. Generally, mer-Ir(C^N)$_3$ complexes possess C_1 symmetry, which results in non-first-order ^{1}H NMR spectra with the total number of resonances corresponding to the number of aromatic protons present in the Ir(III) complexes [15].To further develop highly efficient heteroleptic green solution-processable PHOLEDs and to demonstrate the performance-enhancing effect of a multifunctional ancillary ligand, a new Ir(III) complex, bis5-ethylhexyl-8-trifluoromethyl-5H-benzo[c][1,5]naphthyridin-6-oneiridium(III) (G3), was designed and successfully synthesized following the synthetic route illustrated in Scheme 1.



Scheme 1: Synthesised Ir(III) complexes

The modified hole injection material m-PEDOT:PSS was prepared according to previously reported procedures [31,32]. The G3 Ir(III) complex was purified using column chromatography and recrystallization via suitable solvents. UV-Vis absorption and PL spectra of *mer*-G1 and *mer*-G2 were recorded in degassed 2-methyltetrahydrofuran (2-MeTHF) solution (1×10^{-5}M), as illustrated in Figure 3(a), (b), and c, the resultant data are summarized in Table 1. The intense absorption band around 330 nm is attributed to the spin-allowed π–π* transitions of the cyclometalated ligands, while the broad tail absorption band extending from 380 - 450 nm can be allotted to charge-transfer transitions, including singlet metal-to-ligand charge transfer (^{1}MLCT) and spin-forbidden triplet ^{3}MLCT. When UV irradiation, both Ir(III) complexes exhibited bright green emission at room temperature. The photophysical properties of mer-isomers are well known to differ from those of *fac*-isomers, typically showing noticeable red shifts and broader emission bands in the PL and EL spectra. In the present case, both mer-G1 and mer-G2 displayed broad and structureless emission maxima centered at 528 nm. This observation confirms that the ensuing complexes exist as the mer configuration, since *fac*-isomers usually exhibit narrower and more structured PL emission spectra. Moreover, the low temperature PL spectra of both complexes displayed structured emission bands accompanied by a rigidochromic blue shift relative to the room-temperature spectra. This behaviour indicates that the emissive excited state predominantly originates from a ligand-centered (^{3}π-π*) excited state rather than from ^{3}LLCT or ^{3}MLCT states, which typically produce structureless emission spectra. In the neat film state, both Ir(III) complexes showed a slight red shift in their PL spectra compared to the solution spectra, suggesting the presence of aggregation and intermolecular interactions among the Ir(III) molecules in the solid state.

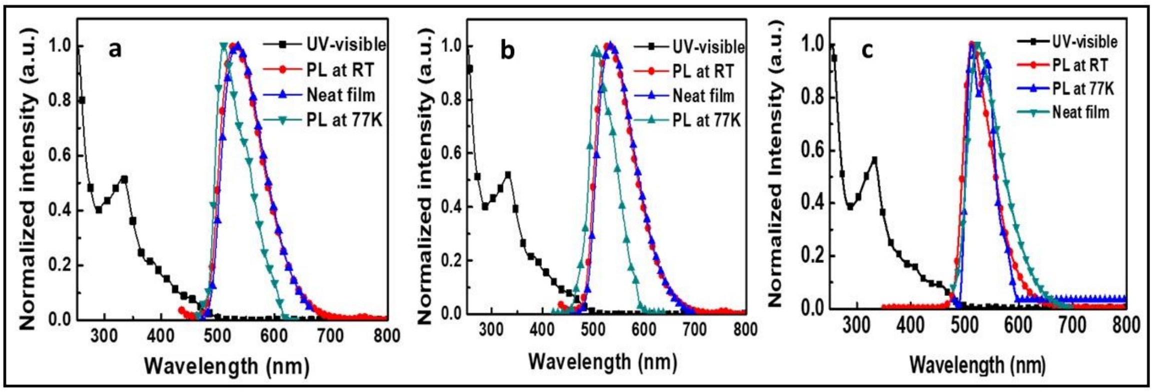


Figure 3: UV-Vis and PL spectra of (a) *mer*-G1, (b) *mer*-G2, and (c) G3 in solution and solid state.

The introduction of different alkyl chains did not significantly influence the photophysical or electrochemical properties of the synthesized Ir(III) complexes. The triplet lifetimes (τ) of mer-G1 and mer-G2 were measured in degassed dichloromethane (DCM) solution (1×10^{-5} M). The photoluminescence decay lifetimes (τ) of *mer*-G1 and *mer*-G2 were measured in degassed dichloromethane (DCM) solution (1×10^{-5} M). The measured τ values were 0.30 and 0.23 μs for *mer*-G1 and *mer*-G2, correspondingly. Particularly, *mer*-G2 exhibited a relatively shorter excited-state lifetime compared with *mer*-G1, which could effectively suppress self-quenching effects in PHOLED devices. To gain further insight into the electronic structures of the synthesized Ir(III) complexes, density functional theory (DFT) calculations were performed, and the corresponding frontier molecular orbital distributions are illustrated in Figure 4. The highest occupied molecular orbitals (HOMOs) of the Ir(III) complexes are primarily distributed over the π orbitals of the coordinated phenyl rings and the d orbitals of the Ir(III) center. The HOMO-1 orbital is mainly localized on the metal center and partially on the coordinated pyridine moiety. In contrast, the lowest unoccupied molecular orbitals (LUMO and LUMO+1) are predominantly located on one of the benzo(c)(1,5)naphthyridine ligands and partially distributed over the other two ligands. For the heteroleptic complex G3, the HOMO is mainly distributed over the Ir(III) d orbitals and the π orbitals of the -CF_3-substituted phenylpyridine ligand, whereas the LUMO is primarily localized on the ancillary ligand with partial contribution from the Ir center. The PLQYs of *mer*-G1 and *mer*-G2 were measured in degassed 2-methyltetrahydrofuran (2-MeTHF) solution at 298 K using the well-known green emitter Ir(tppy)$_3$ (Φ = 0.50) as the reference [12]. Both complexes showed relatively high PLQYs of 48% and 51% for mer-G1 and mer-G2, correspondingly, which are comparable to the standard green phosphorescent emitter Ir(tppy)$_3$. Consequently, mer-G2 demonstrated superior performance, exhibiting higher external quantum efficiency (EQE) and current efficiency (CE) than its parent Ir(III) complex. This enhancement can be directly attributed to its higher PLQY and shorter excited-state lifetime. Notably, these values represent some of the highest PLQYs reported so far for mer-isomer-based Ir(III) complexes emitting in the green region. The optical band gaps of *mer*-G1 and *mer*-G2 were estimated from the absorption edges of their UV-Vis spectra, yielding similar values of approximately 2.5 eV. The photophysical properties of the heteroleptic complex G3 were also investigated. Figure 6(c) presents the UV-Vis absorption and photoluminescence spectra of G3 in anhydrous DCM solution (1×10^{-5} M) as well as in neat films at room temperature, while low-temperature phosphorescence spectra were recorded in 2-MeTHF at 77 K. The key photophysical, thermal, and electrochemical parameters of the complexes are summarized in **Table 1**. Complex G3 displayed an intense absorption band at 335 nm, which is attributed to spin-allowed ligand-centered (^{1}LC) π–π* transitions within the ligands. Additional lower-energy absorption bands observed between 408 and 460 nm were assigned to mixed ^{1}MLCT and ^{3}MLCT transitions facilitated by strong spin–orbit coupling induced by the iridium center. The optical band gap (Eg_{opt}) of G3 was also determined from the UV-Vis absorption edge.

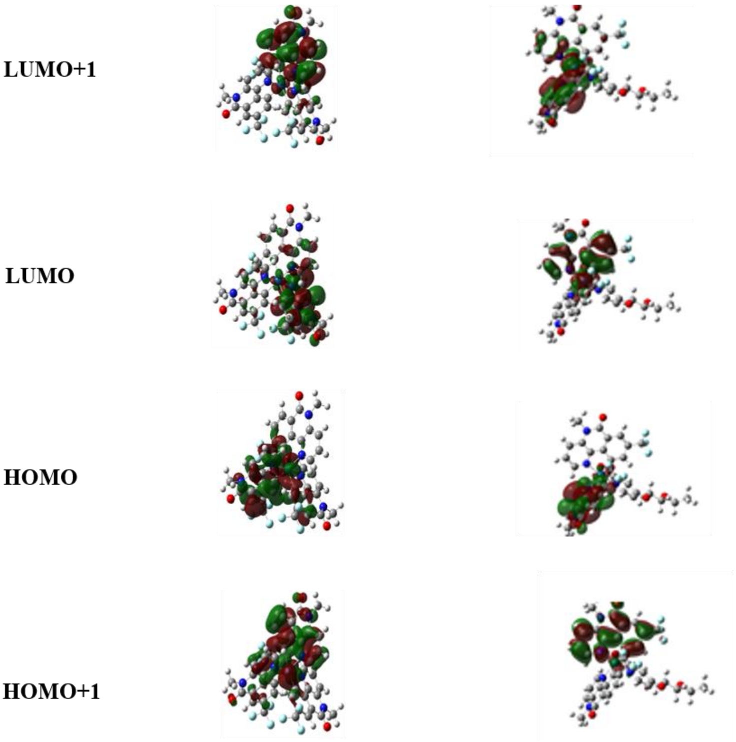


Figure 4: DFT analysis of *mer*-G1, *mer*-G2 (right), and G3 (left).

When photoexcitation, G3 displayed intense green phosphorescence in degassed DCM solution with an emission maximum at 513 nm, suggesting that the emissive excited state is predominantly derived from triplet charge-transfer states such as ^{3}MLCT or ^{3}LLCT rather than ligand-centered ^{3}LC (^{3}π–π*) states. The low-temperature phosphorescence spectrum of G3 exhibited structured emission bands, indicating partial ligand-centered character of the emissive triplet state. The photoluminescence decay lifetime (τ) of G3 measured in solution at 298 K was 0.78 μs (Figure 7(b)). The PLQY of G3 was determined using $Ir(ppy)_3$ (Φp = 40% in DCM) as the reference [32]. Remarkably, G3 showed a very high PLQY of 88%, which is significantly higher than that of the commercial green phosphorescent emitter $Ir(ppy)_3$. The enhanced PLQY and solubility of G3 can be attributed to the incorporation of the $-EO_2$ solubilizing group, which improves molecular dispersion and reduces intermolecular quenching effects. Consequently, G3 demonstrated superior electroluminescent performance compared with the other Ir(III) complexes, as evidenced by its higher PLQY and favorable excited-state dynamics.

Table 1: Photophysical and electrochemical properties of Ir(III) complexes.

Complex	λ_{PL} a) [nm]		τ b) [μs]	PLQYs c) [%]		T_1 d) [eV]	HOMO e) [eV]	LUMO e) [eV]	E_g^{opt} e) [eV]
	Solution	**Film**	**Solution**	**Solution**	**Film**	**Solution**			
mer-G1	528	534	0.30	42	83	2.43	-5.2	-2.8	-2.5
mer-G2	528	530	0.23	51	90	2.45	-5.2	-2.8	-2.5
G3	513	528	0.27	88	95	2.41	-5.9	-3.4	-2.5

a) Measured in 2-MeTHF solution at room temperature (1×10^{-5} M).

b) Measured in degassed DCM.

c) PLQYs of the thin films composed of TCTA:TPBi:15% Ir(III) complexes measured on the quartz substrate.

d) E_T measured from low-temperature emission phosphorescence spectra.

e) HOMO calculated from onset oxidation potential from CV, LUMO = E_g (optical) + HOMO.

Solution-processed PHOLED performances of green Ir(III) complexes

The electroluminescent performance of the complexes and to understand the influence of the two different alkyl chains, solution-processed green-emitting PHOLEDs were fabricated. The devices were constructed with the architecture ITO/PEDOT:PSS (30 nm)/EML (25 nm)/TPBi (40 nm)/LiF (1 nm)/Al (120 nm), where the emissive layer consisted of TCTA and TPBi as mixed hosts in a 1:1 ratio with a dopant concentration of 15 wt%. The corresponding energy level diagram is illustrated in Figure 5. The use of two host materials with different charge-transport properties helps to achieve sensible carrier transport within the EML, thereby improving device lifetime and preventing charge accumulation at the interfaces. Devices incorporating *mer*-G1 and *mer*-G2 as dopants exhibited outstanding electroluminescent (EL) performance, and the key parameters of the device are given in Table 2. Among them, the device using *mer*-G2 demonstrated superior performance compared with the mer-G1-based device. Specifically, the mer-G2 device achieved a maximum external quantum efficiency (EQEmax) of 20.03% and a maximum current efficiency (CEmax) of 67.81 cd A^{-1} with Commission Internationale de l'Éclairage (CIE) coordinates of (0.36, 0.58). In contrast, the mer-G1 device showed an EQE_{max} of 18.47% and a CEmax of 62.24 cd A^{-1} with nearly identical CIE coordinates. The electroluminescent characteristics of the devices using *mer*-G1 and *mer*-G2 are shown **in** Figure 6. These EQE and CE values are between the highest reported for solution-processed green PHOLEDs employing *mer*-isomer-based emitters. In addition, the devices exhibited remarkably high brightness values of 43,492 cd m^{-2} and 38,877 cd m^{-2} for *mer*-G1 and *mer*-G2, respectively. Both devices displayed similar EL emission peaks centered at 528 nm. The radiative (kr) and non-radiative (knr) decay rate constants were estimated using the relationships kr=ΦPL/τkr=

$\Phi/\tau kr=\Phi PL/\tau$ and $\Phi PL=kr/(kr+knr)\Phi = kr/(kr + k\{nr\})\Phi PL=kr/(kr+knr)$. The calculated kr and knr values were 1.40×10^6 and 1.93×10^6 s^{-1} for *mer*-G1, and 2.22×10^6 and 2.13×10^6 s^{-1} for mer-G2, respectively. Consistent with the AFM results, the device incorporating mer-G2 exhibited better EL performance, achieving the highest EQE among PHOLEDs based on homoleptic Ir(III) complexes. The superior performance of *mer*-G2 can be attributed to its higher PLQY, shorter excited-state lifetime with higher radiative decay rate. To further determine the impact of the new ancillary ligand on the electroluminescent characteristics of G3, solution-processed PHOLED devices (A, B and C) were prepared using the configuration of ITO/PEDOT:PSS or m-PEDOT:PSS (40 nm)/TCTA:TPBi:15 wt% dopant (20nm)/TPBi (30nm)/LiF (1nm)/Al (150nm). Chlorobenzene and cyclohexanone were the processing solvents that were utilized in the fabrication of the devices. Balanced carrier transport in the emissive layer was done using the mixed host system that comprises TCTA and TPBi. Figure 5 illustrates its energy level diagram and Table 2 summarizes the EL performance parameters of the fabricated devices. The current density (J) vs voltage (V) and luminance (J-V-L), current efficiency-current density-power efficiency (CE-J-PE) and EQE vs lumuminance performance of the G3-based devices prepared with various hole injection layers (HILs) and solvents are shown in Figure 7. In order to explore the effects of the altered hole injection layer, device B was made by using m-PEDOT:PSS that incorporated chlorobenzene as the solvent. The device B had a maximum luminance (L_{max}) of 36,762 cd m 2, EQE_{max} of 22.06, and CE_{max} of 79.06 cdA^2. The highest luminance exhibited by device B was 28,844 cd m^{-2}, and the values of the efficiency significantly increased, with an EQEmax of 24.56% and a CEmax of 87.96 cd A^{-1}. The use of m-PEDOT:PSS resulted in significant improvement of both CE and EQE relative to the instrument using traditional PEDOT:PSS **(Table 3)**. The values are one of the highest reported efficiencies of green solution-processed PHOLEDs. Moreover, device C was prepared with cyclohexanone as the processing solvent and m-PEDOT:PSS as the HIL to test how the performance of the device would be affected by using non-halogenated solvents rather than using halogenated one. Usually, device C had a high luminance of 36762 cd m^{-2}, with an EQE_{max} of 23.60 percent and a CE_{max} of 85.22 cd A^{-1}. These findings show that the non-halogenated solvent cyclohexanone has little effect on the morphology of the emissive layer and the working of the device and was able to achieve the same efficiencies as those achieved with the chlorinated solvent chlorobenzene. In general, these findings indicate that the addition of the multifunctional 4-(2-ethoxyethoxy)picolinate ligand into the Ir(III) complex remarkably improves the photophysical and electroluminescent characteristics. The G3 complex, in comparison to mer-G2, had higher PLQY, CE, EQE and brightness of the device. The multifunctional nature of the 4-(2-ethoxyethoxy)picolinate group that not only increases the solubility of the Ir(III) complex but also increases the rate of charge transport and emission in the PHOLED devices can be attributed to the superior performance of G3.

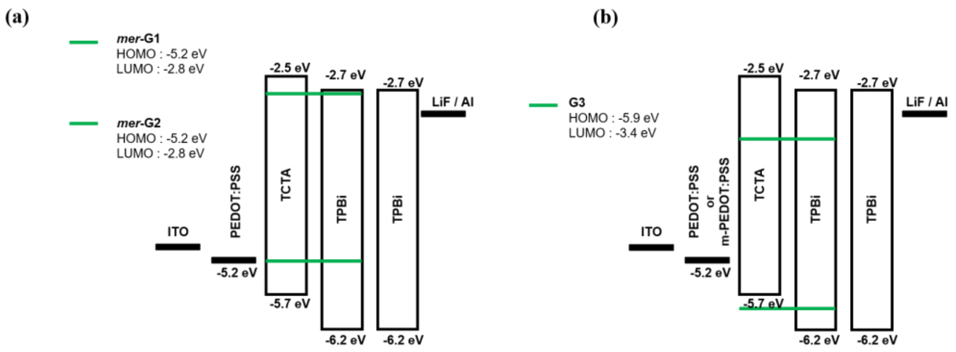


Figure 5: Device energy level diagram (a) *mer*-G1, *mer*-G2, and (b) G3.

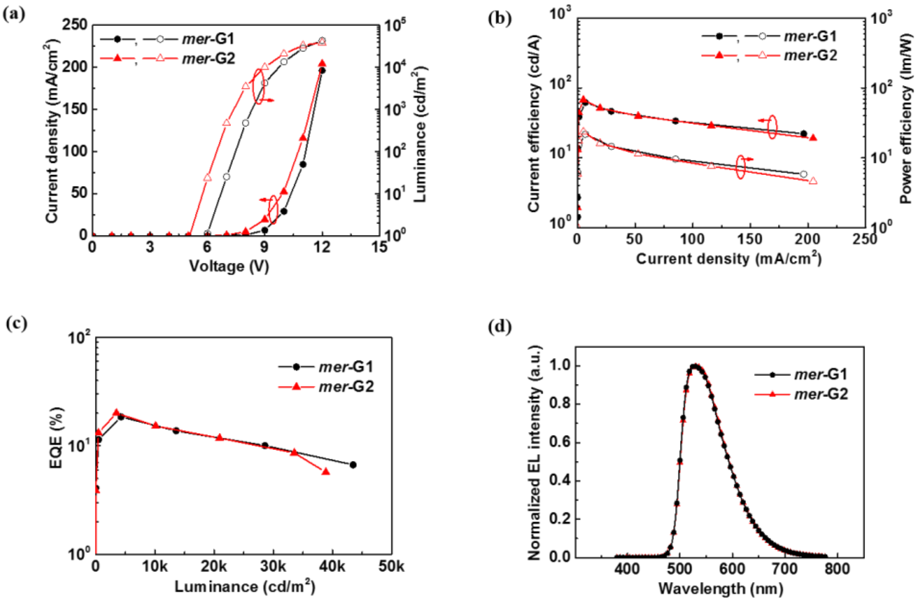


Figure 6: (a) J-V-L plots, (b) CE-J-PE plots, (c) EQE-luminance plots, and (d) EL spectra of devices *mer*-G1 and *mer*-G2.

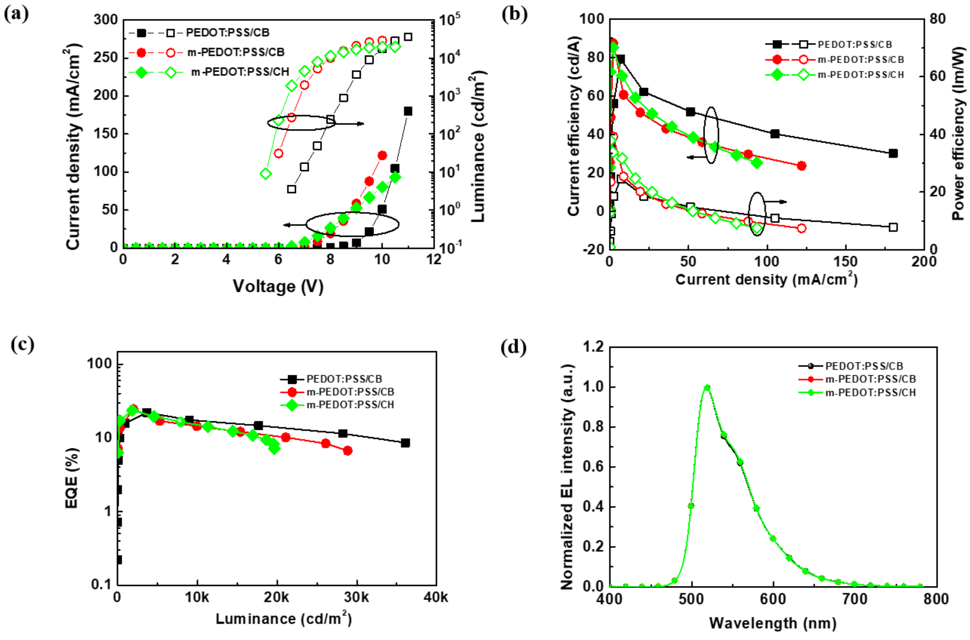


Figure 7: (a) J-V-L plots, (b) CE-J-PE plots, (c) EQE-luminance plots, and (d) EL spectra of G3.

Table 2: Key performances of device with complexes *mer*-G1 and *mer*-G2.

Complexes	**V$_{on}$ [V]**	**L$_{max}$ [cd m^{-2}]**	**EL$_{max}$$^{c)}$ [nm]**	**EQE [%]**	**CE [cd/A]**	**PE [lm/W]**	**CIE$^{c)}$ [x,y]**
				Max, @10000 cd m^{-2}	**Max, @30 mA cm^{-2}**		
***mer*-G1**	5.95$^{a)}$, 8.32$^{b)}$	43492	526	18.47, 15.57	62.24, 47.49	21.73,14.50	(0.36, 0.58)
***mer*-G2**	5.06$^{a)}$, 7.38$^{b)}$	38877	528	20.03, 15.52	67.81, 47.55	23.67, 14.68	(0.36, 0.58)

a) Measured at 1 cd m^{-2}; $^{b)}$ Measured at 1000 cd m^{-2}; $^{c)}$ Measured at 30 mA cm^{-2}.

Table 3: Device performance of green solution-processed PHOLEDs using G3 as guest

Device	**HIL**	**Dopant**	**OV [V]**	**EQE [%]**	**CE [cd A^{-1}]**	**PE [lm W^{-1}]**	**CIE [x, y]**	**EL$_{max}$ [nm]**	**Maximum Luminance**
				Max, @1000 cd m^{-2}	**Max, @10 mA cm^{-2}**		**@10 mA cm^{-2}**		**(cd m^{-2})**
A	PEDOT:PSS (chlorobenzene)	**G3**	6.0$^{a)}$, 8.6$^{b)}$	22.06, 17.02	79.06, 75.35	26.41, 23.02	0.320, 0.620	518	36762
B	*m*-PEDOT:PSS (chlorobenzene)	**G3**	5.6$^{a)}$, 6.8$^{b)}$	24.56, 19.26	87.96, 59.16	39.25, 25.35	0.320, 0.620	518	28844
C	*m*-PEDOT:PSS (cyclohexanone)	**G3**	5.3$^{a)}$, 6.5$^{b)}$	23.60, 21.06	85.22, 68.19	37.92, 28.94	0.300, 0.634	518	19569

$^{a)}$Measured at 1cd m^{-2}; $^{b)}$Measured at 1000 cd m^{-2}

Conclusion

PHOLEDs have become a popular subject of research in high-efficiency display and solid-state lighting projects. Three new Ir(III) complexes emitting green were designed and synthesized in solution to use in PHOLEDs, two homoleptic meridional isomers that are *mer*-G1 and *mer*-G2, and a heteroleptic complex, G3. All the complexes had very high PLQYs and high electroluminescent capabilities. The *mer*-G2 device gave a maximum current efficiency (CE_{max}) of 67.81 cd A $^{-1}$ and a maximum external quantum efficiency (EQE_{max}) of 20.03, which is one of the highest recorded performances of mer-isomer emitters. Moreover, heteroleptic complex G3 had better device performance with EQE_{max} of 24.56 and CEmax of 87.46 cd A $^{-1}$. Notably, the solvent cyclohexanone, which is an environmentally friendly solvent was also used to obtain high device efficiencies, indicating the possibility of using ecofriendly solution-processing methods to process PHOLEDs in large scale.

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PHENYLKETONURIA: AN OVERVIEW OF GENETICS, PATHOPHYSIOLOGY, TREATMENT AND MANAGEMENT

Mari Kavitha L and Lavanya Krishnadhas
Department of Biochemistry, PSG College of Arts and Science, Coimbatore -14
Corresponding author E-mail: kavithalmk15@gmail.com, lavanya@psgcas.ac.in

Abstract

Mutations in the phenylalanine hydroxylase (PAH) gene are the primary cause of phenylketonuria (PKU), a frequent inborn defect of phenylalanine metabolism. Hyperphenylalaninemia (HPA) or high levels of phenylalanine in the blood, is caused by a lack of the PAH enzyme, which stops phenylalanine from being converted to tyrosine. PKU can cause serious neurological issues like intellectual impairment, convulsions and developmental delays if left untreated. Preventing these effects requires early discovery through neonatal screening. The main treatment consists of supplementing with phenylalanine-free amino acid formulations and restricting phenylalanine in the diet for the remainder of one's life. For some people, pharmacological treatments such sapropterin dihydrochloride and enzyme substitution therapy (pegvaliase) are potentially an option. This review focuses on pathophysiology, diagnosis, treatment and management of phenylketonuria.

Keywords: Phenylketonuria, Phenylalanine Hydroxylase Gene, Hyperphenylalaninemia, Phenylalanine Metabolism, Newborn Screening, Sapropterin Dihydrochloride, Pegvaliase.

Introduction

Phenylketonuria is the most common inborn error of the phenylalanine-tyrosine amino acid metabolism. The phenylalanine hydroxylase gene is located on chromosome 12q22-24.1, which has more than 1,180 bi-allelic variants. This is an autosomal recessive disorder which lead to deficiency of enzyme phenylalanine hydroxylase which catalyze the conversion of phenylalanine to tyrosine with the help of cofactor like BH4 (tetrahydrobiopterin), molecular oxygen and non-heme iron (Hillert *et.al*,2020). Delayed diagnosis or treatment, the disorder exhibits itself with mild to severe intellectual disability (Orphanet, 2020) Subsequently, Phenylalanine (Phe) accumulates in the plasma, causing unfavourable effects on various systems, affecting nervous system leading to symptoms like myoclonus, mental retardation and seizures, causing hypopigmentation in skin and urinary compounds (Vinueza, 2023).

The Phenylalanine Hydroxylase enzyme is generally expressed in hepatocytes and hydroxylase Phenylalanine to Tyrosine with the aid of the BH4 cofactor as well as a non-heme iron and molecular oxygen (Carducci *et.al.,*2020). Notably, the BH4 and DNAJC12 can also serves as mentor for the Phenylalanine Hydroxylase enzyme monomer to fold correctly. Therefore, the pathogenic variation of DNAJC12 and abnormalities of BH4 metabolism can also account for tiny percentage of HPA. The most significant abnormalities of PKU is HPA, where the phenylalaninemia content are higher above the typical 35-120 μmol/L (Spronsen *et.al.,*2021)

Discovery

In 1934, Asbjorn Folling identified phenylpyruvic acid in the urine of two severely mentally retarded siblings and later in eight other similar patients, leading to the discovery phenylketonuria defining a new inborn error of metabolism that chiefly affects the brain. It then designates the way by which he isolate and characterize the compound using classical organic chemistry to, flourished that the condition follows an autosomal recessive inheritance pattern, associated with the blockage of phenylalanine metabolism with evidently elevated blood phenylalanine and established early biochemical and loading tests to identify both affected individuals and asymptomatic carriers, determining PKU as a model disease in medical genetics and providing a glimpse into Folling's scientific approach and personality (Folling,1994).

Epidemiology

One of the most prominent inborn disorders of metabolism, PKU affects 1 in 100,00 live births in Europe and 1 in 40,000 in Turkey. According to a study examined that 14 out of 485,634 live births between January 1965 and December 2014, 64% of the patients were adults, 35% had cognitive disabilities and 78% were not blood-related relationships (Jomarron *et.al.,*2021). In the United States is about 1 in 15,000 had shown positive to PKU. African, Hispanic and Asian groups possessed lower incidence rates than Caucasian and Native American populations. PKU incidence fluctuates significantly over the world (Stone and Los, 2023). The IVS10 mutation has been found to be the cause of PKU (PAH gene) in Chile, with a 25% recurrence rate similar to that of Spain (Vinueza, 2023).

White or East Asian people tend to have the ethnic prevalence (1 in 10,000-15,000 live births). From a geographical perspective, it is usually higher in European and Middle Eastern nations and lowest in Asian nations, with the notable exception of China (Elhawary *et.al.,*2022).

Etiology

The main contributory factor of HPA pathogenetic variants in the PAH gene located on chromosome 12, which are passed on through an autosomal recessive manner and results in either the total absence of PAH protein or the production of PAH monomer with minimal or no activity (Spronsen *et.al.,*2021).

PKU can be triggered by more than a thousand mutation, the most prevalent of which substitutes Tryptophan with Arginine at position 408(i.e., Arg408Trp). PKU-causing mutation in PAH often result in diminished Pah expression or activity, which raises Phenylalanine concentrations and lowers Tyrosine concentrations in the blood. The majority of PAH variation leading to PKU developed by PAH misfolding and/or instability (Stone and Los, 2023). Mild hyperphenylalaninemia (120-600µmol/L), mild PKU (mPKU 600-1200µmol/L), moderate PKU (900-1200µmol/L) and classic PKU (cPKU >1200µmol/L) are categorised according to the concentration of phenylalanine in plasma. Phenylalanine will build up in both the brain and bloodstream if PKU fails to be detected within the first week of life or is not treated. This can result in severe intellectual incapacity, behavioural and psychiatric issues, epilepsy, dermatitis, pale skin and hair pigmentation (Dababneh *et.al.,*2022)

Mechanism of Action

PAH is an iron-containing monooxygenase enzyme, made up of four subunits, that facilitates the conversion of Phenylalanine to Tyrosine through hydroxylation (Figure 1).

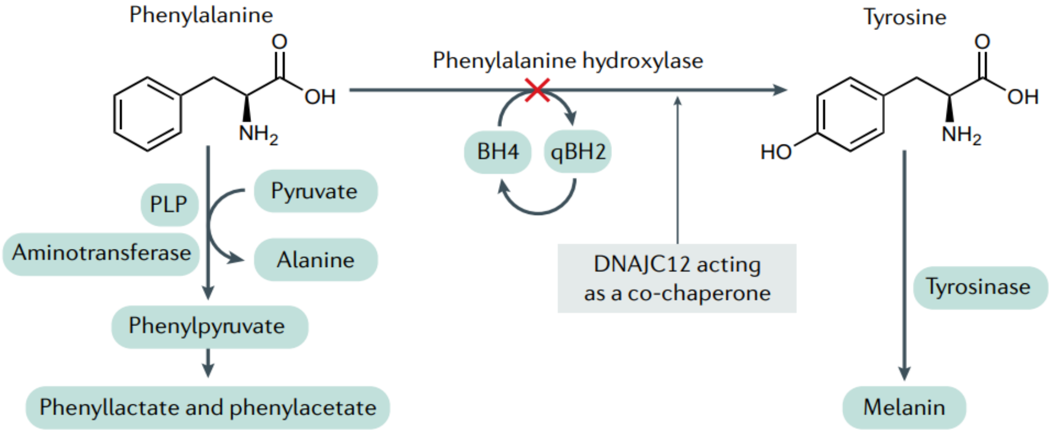

Figure 1: Mechanism of Phenylalanine metabolism (Spronsen *et al.,* 2021)

This process necessitates the presence of molecular oxygen as a cofactor and the reduced pterin BH4 as a co-substrate. The hydroxylation performed by PAH represents the rate-limiting step in the intermediary metabolism of L-Phenylalanine.

Despite the availability of advanced research methodologies, the precise amount of Phenylalanine used in net protein metabolism remains unclear. By comparing the Phenylalanine needs of healthy children and adults with those of individuals with PKU, we estimate that approximately 10–20% of usual dietary Phenylalanine intake is used during routine protein turnover, while the rest is converted to Tyr through the action of PAH.

Tyrosine has various metabolic pathways, including the synthesis of the neurotransmitters dopamine, adrenaline and norepinephrine, its conversion to thyroxine in the thyroid gland and to melanin in melanocytes, as well as its complete breakdown into acetoacetate (a ketone) and fumarate, which are the intermediate of Kreb's cycle for energy use. An inherited or functional deficiency in PAH activity results in HPA, a mild deficiency of Tyrand, in severe cases, leads to the urinary excretion of phenylpyruvate (which is formed from the spontaneous deamination of Phe) and other phenylketone bodies (Spronsen *et al.,* 2021).

Pathophysiology

Problems with PAH lead to the failure to convert phenylalanine into tyrosine, causing a buildup of phenylalanine and a shortage of tyrosine. Tyrosine is essential for the creation of neurotransmitters, the transformation into thyroxine and melanin and the breakdown into acetoacetate and fumarate, which can be used for energy (Spronsen *et al.,* 2021).

It is suggested that high levels of phenylalanine in the bloodstream compete with other amino acids for attachment to the large amino acid transporter (LAT1; also known as SLC7A5) at the blood-brain barrier. This results in increased levels of phenylalanine in the brain and decreased levels of neurotransmitter precursors. There is also increasing evidence that a deficiency in mitochondrial energy and oxidative stress are significant factors in the pathophysiology of PKU (Dobrowolski *et al.,* 2022).

Phenylketonuria has been extensively researched regarding its potential connection to being overweight and obese. Some retrospective studies indicated that PKU patients have a higher BMI and show a greater frequency of overweight, especially among females (Burrage *et.al.,*2012). Nonetheless, subsequent research revealed that the average BMI of individuals with PKU is similar to that of healthy individuals, although specific groups, such as those with classical PKU, may exhibit elevated BMI. Consequently, ongoing follow-up with tailored dietary supervision is advisable to mitigate the risk of obesity and associated health issues (Rodrigues *et.al.,*2021)

Diagnosis

The majority of patients are identified through newborn screening (NBS) instead of through symptoms. Nevertheless, there are still nations that have either incomplete or no NBS in place. Traditionally, individuals with PKU exhibited significant delays in both motor skills and intellectual development; however, it is now recognized that a small number of patients suffer little to no neurological difficulties despite elevated blood Phenylalanine levels (>1200 µmol/L) (Wegberg *et.al.,*2025).

Phenylketonuria is detected via NBS, which should be accessible in every country across Europe. For effective NBS, a solid infrastructure is necessary, wherein blood samples are collected from all newborns within a few days post-birth and these samples are analyzed by a well-equipped laboratory capable of efficiently handling and evaluating dried blood spots (DBS). Although less invasive methods for diagnosing PKU in urine exist, they are not commonly utilized since many disorders can be detected in DBS. Notably, NBS was first developed to identify classic PKU. In the 1960s, Dr. Robert Guthrie, a medical microbiologist, was the pioneering individual to gather heel blood samples from newborns and to create a screening test aimed at detecting PKU. In 1962, following the screening of just 800 newborns, the first PKU case was identified (Guthrie and Susi, 1963). Familial screening has become a routine standard in numerous clinics and is particularly essential for women from countries where the NBS program was not fully operational at the time of their births (Wegberg *et.al.,*2025).

Individuals migrating or seeking refuge from these regions risk having undetected PKU as most host nations lack a PKU screening program for newcomers (Schiergens *et al*., 2018).

Genotyping The gene responsible for PAH is situated on chromosome 12 (region q22–24.1) comprising 13 exons and 12 introns, encompassing a total of 100 kb of genetic material (Wegberg *et.al.,*2025).

Treatment

In 1953, Bickel *et.al*.,. effectively treated a 4-year-old girl diagnosed with PKU. The child was placed on a diet devoid of Phenylalanine and Tyr (Bickel *et.al.,*1954).

Since a Phe-restricted diet is low in proteins, which results in a deficiency of amino acids, it is important to provide Phe-free medical mixtures containing L-amino acids. The inclusion of these medical mixtures is vital for promoting normal growth and well-being, as they provide proteins, calories and additional nutrients (Stroup *et al.,*2017).

The primary element of PKU treatment is a Phe-restricted diet. This means consuming foods with low protein content that have ≤ 50 mg Phenylalanine per g of protein and providing a medical mixture to guarantee adequate intake of other amino acids, vitamins, minerals and fatty acids (Pena *et.al.,*2015).

For newborn infants, breastfeeding typically does not lead to issues as it contains only 46 mg Phenylalanine per 100 ml of milk (Wegberg *et al.,* 2025). Sapropterin dihydrochloride, the synthetic version of BH4, can be administered as treatment to 25–50% of PKU patients who respond to BH4. Lowering Phenylalanine levels is achieved through enhanced folding and greater stability of the mutated PAH enzyme after taking sapropterin dihydrochloride (Qu *et al.,* 2019).

Since 2018, the PAL enzyme from Anabaena variabilis (AvPAL) has received approval for use in patients aged 16 and older who suffer from PKU and have high blood Phenylalanine levels over 600 µmol/l, despite following a Phenylalanine limited diet (Thomas *et al.,* 2018). The pegylated form of the recombinant AvPAL enzyme, referred to as pegvaliase, lowers blood Phenylalanine levels via a pathway that does not depend on PAH. Ongoing treatment is necessary for life. As dietary constraints are eased at the age of 12, allowing blood Phenylalanine levels to rise to 600 µmol/l, it is possible that adherence to treatment may diminish. While treatment options have improved since PKU therapies were available in 1953, the existing strategies still face challenges, especially regarding compliance with a Phenylalanine restricted diet (Nulmans *et al.,* 2025).

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RECENT PROGRESS IN CHEMICAL, BIOLOGICAL AND PHARMACEUTICAL SCIENCES

Jaymala Kumawat*[1] and Vrushali Neve[2]
[1]Vishwakarma University, School of Pharmacy, Pune, Maharashtra, India
[2]Dr.D.Y. Patil institute of Pharmaceutical Sciences and Research, Pune, Maharashtra, India
*Corresponding author E-mail: jaymala.kumawat@vupune.ac.in

Abstract

The rapid evolution of chemical, biological, and pharmaceutical sciences has significantly transformed modern healthcare and industrial applications. Emerging technologies such as artificial intelligence (AI), nanotechnology, genomics, and biotechnology have accelerated drug discovery, improved disease diagnosis, and enhanced therapeutic outcomes. Green chemistry promotes sustainability, while biologics and gene therapies provide targeted treatment options. Interdisciplinary approaches integrating computational tools and life sciences are shaping the future of personalized medicine. This chapter discusses recent trends, technological advancements, applications, challenges, and future perspectives in these dynamic scientific fields.

1. Introduction

Chemical, biological, and pharmaceutical sciences are fundamental pillars of modern science, contributing significantly to healthcare, agriculture, and environmental sustainability. Over the past few decades, rapid technological advancements have transformed these disciplines. Emerging tools such as artificial intelligence, nanotechnology, and genomics have enhanced the understanding of complex biological systems and accelerated the development of innovative therapeutic strategies. These advancements play a crucial role in addressing global challenges, including infectious diseases, chronic disorders, and environmental pollution, thereby supporting sustainable development and improving overall quality of life for present and future generations.

2. Emerging Trends in Chemical Sciences

2.1 Green Chemistry and Sustainable Development

Green chemistry focuses on designing chemical processes that reduce or eliminate hazardous substances.

Key Principles:

- Use of renewable raw materials
- Reduction of toxic by-products
- Energy-efficient reactions
- Eco-friendly solvents

Green Chemistry Model

Raw Materials → Chemical Process → Product + Waste ❌

↓

Green Chemistry → Safe Process → Product + Minimal Waste ✅

2.2 Artificial Intelligence in Chemistry

AI and machine learning are widely used for:

- Predicting chemical reactions
- Designing new drug molecules
- Optimizing synthesis pathways

AI in Drug Design

Data Collection → Algorithm Training → Molecular Prediction → Screening → Lead Optimization

2.3 Nanotechnology and Advanced Materials

Nanotechnology deals with materials at the nanoscale (1–100 nm).

Applications:

- Drug delivery systems
- Biosensors
- Imaging techniques

Nanoparticle Drug Delivery

Drug → Encapsulation in Nanoparticle → Target Site → Controlled Release

3. Emerging Trends in Biological Sciences

3.1 Genomics and Gene Editing

Technologies like CRISPR-Cas9 enable precise gene modification.

Applications:

- Treatment of genetic disorders
- Cancer therapy
- Agricultural improvements

Gene Editing Process: Target DNA → Guide RNA Binding → Cas9 Cleavage → Gene Correction

3.2 Microbiome and Human Health: The human microbiome plays a key role in:

- Digestion
- Immunity
- Mental health
- Applications:
- Probiotics
- Disease prevention

3.3 Advances in Molecular Biology

Modern tools:

- PCR
- DNA sequencing
- Cell imaging
- Impact:
- Better understanding of diseases
- Drug mechanism analysis

4. Emerging Trends in Pharmaceutical Sciences

4.1 Targeted Drug Delivery

Drugs are designed to act on specific targets.

Advantages:

- Increased efficacy
- Reduced side effects

4.2 Biologics and Monoclonal Antibodies

Used in:

- Cancer therapy
- Autoimmune diseases

4.3 Nanotechnology in Drug Delivery

Nanodrug Delivery

Drug Loading → Nanocarrier → Blood Circulation → Target Cell → Drug Release

4.4 Personalized Medicine and Pharmacogenomics

Treatment based on individual genetic profile.

Benefits:

- Customized therapy
- Reduced adverse drug reactions

4.5 mRNA Vaccines

A breakthrough in vaccine technology.

Features:

- Rapid development
- High efficacy

4.6 Gene and RNA-based Therapy

Includes:

- RNA interference
- Gene therapy

5. Interdisciplinary Approaches

Fields Integrated:

- Chemistry
- Biology
- Computer science

Flowchart: Interdisciplinary Research

Chemistry + Biology + IT
↓
Data Analysis
↓
Drug Discovery
↓
Clinical Application

6. Discussion

The integration of advanced technologies has significantly enhanced research efficiency and healthcare outcomes. AI has reduced the time required for drug discovery, while nanotechnology has improved drug delivery systems. Gene editing tools have revolutionized treatment strategies for genetic disorders.

However, challenges such as ethical concerns, high costs, and regulatory barriers remain. The balance between innovation and safety is crucial for sustainable development in these fields.

7. Challenges and Future Perspectives

7.1 Challenges

- High cost of research and development
- Regulatory issues
- Drug resistance
- Ethical concerns in gene editing

7.2 Future Directions

Flowchart of Future Trends

AI + Nanotechnology + Genomics
↓
Smart Healthcare System
↓
Personalized Medicine
AI-driven drug discovery
Regenerative medicine
Sustainable pharmaceutical processes

Conclusion

Emerging trends and technological advances in chemical, biological, and pharmaceutical sciences are transforming modern healthcare and industry. Innovations such as AI, nanotechnology, and genomics have improved drug discovery, diagnosis, and treatment.

Interdisciplinary approaches will continue to play a crucial role in addressing global challenges. Future advancements will focus on precision medicine, sustainability, and improved healthcare accessibility.

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THERAPEUTIC VERSATILITY OF SULPHA DRUGS: FROM INFECTION CONTROL TO ADVANCED BIOMEDICAL INNOVATIONS

Kuldeep Singh and Raman Singh*
Department of Applied Chemistry,
Amity University Madhya Pradesh, Gwalior, Madhya Pradesh, India.
*Corresponding author E-mail: bhadauria.raman@gmail.com

Abstract

Sulpha drugs, also known as sulfonamides, are among the earliest synthetic antibacterial agents developed in medicine era. Their pharmacological importance was first recognized through the work of Scientist Gerhard Domagk in the early 20th century. These drugs act by inhibiting the enzyme dihydropteroate synthase in bacteria, thereby blocking the synthesis of folic acid(an essential component required for bacterial growth and DNA formation). As compared to human cells, they obtain folic acid from the diet and do not synthesize it, sulpha drugs selectively target bacterial cells without significantly harming human tissues.

Initially, sulfonamides were widely used for the treatment of infections such as urinary tract infections (UTI), respiratory tract infections (RTI), and wound infections (WI). Later, combination therapy—especially sulfamethoxazole with trimethoprim—was introduced to enhance antibacterial efficacy by blocking two subsequent steps in folate metabolism. Structural amendments of the sulfonamide nucleus have also led to the development of several non-antibacterial drugs. These include sulfonylureas for diabetes management, thiazide diuretics for hypertension, carbonic anhydrase inhibitors for glaucoma, and certain anti-inflammatory and anticancer agents.

In recent years, sulfonamide containing compounds have accomplished interest in biomedical research and development due to their role as versatile pharmacophores in enzyme inhibition, targeted drug delivery systems, and novel antimicrobial materials. However, the emergence of bacterial resistance remains a significant clinical concern, highlighting the urgence for rational drug design and combination therapy. Overall, sulpha drugs show outstanding therapeutic versatility, spreading from infection control to diverse biomedical and pharmaceutical era.

Keywords: Sulpha Drugs, Sulfonamides, Antibacterial Mechanism, Folic Acid Pathway, Resistance, Biomedical Innovation.

Introduction

Sulfa drugs, or sulfonamides, encompass both antibiotic and non-antibiotic medications employed in the treatment of infections, diabetes, inflammation, and various other applications in microbiology, pharmacology, medicinal chemistry, and therapeutics(R. Singh & K. Halve, 2025). They cover up both antibiotic mechanisms (e.g., inhibition of folate synthesis in bacteria) and non-antibiotic roles (e.g., antidiabetic agents such as sulfonylureas, anti-inflammatory drugs, and carbonic anhydrase inhibitors).

Sulfonamide antibiotics drugs primarily inhibit bacterial folic acid synthesis, making them bacteriostatic. Since sulpha drugs only stop folic acid production (they don't directly kill bacterial cells), the immune system must eliminate the inhibited bacteria. Hence, they are classified as bacteriostatic antibiotics. They are basically organosulfur group with

- The basic structure $R{-}S(=O)_2{-}NR_2$ or $R{-}SO_2NR'R''$ or $R{-}S(=O)_2{-}NR'R''$, where each R is some organic group,
- And contains sulfonyl group (O=S=O)
- And attached to an amine group ($-NH_2$).

In medicine, the term "sulfonamide" is sometimes used as a synonym for sulphonamides, sulfa drugs or sulpha drugs, a derivative or variation of sulfanilamide. The original antibacterial sulfonamides are synthetic antimicrobial agents that contain the sulfonamide group. Some sulfonamides are also devoid of antibacterial activity, e.g., the anticonvulsant sultiame. The sulfonylureas and thiazide diuretics are newer drug groups based upon the antibacterial sulfonamides.

Allergies to sulfonamides and adverse drug reactions to sulfa antibiotics is approximately 3%, close to penicillin; hence medications containing sulfonamides are prescribed carefully.

Table 1: Basic Structure of Sulphonamides

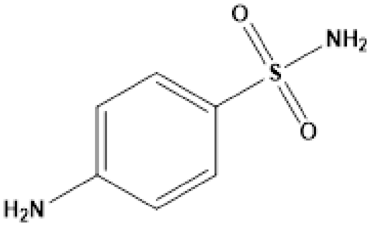
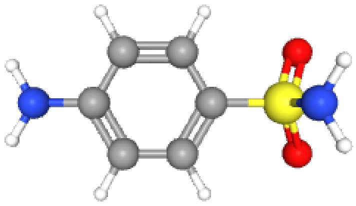
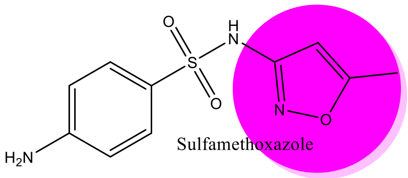
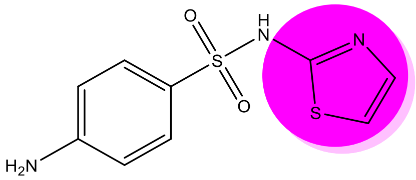
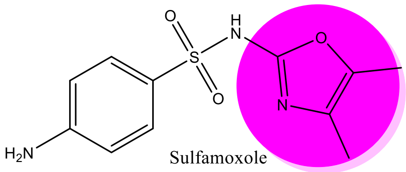
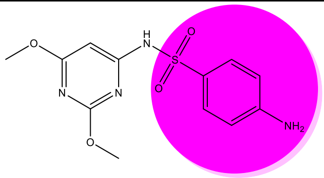
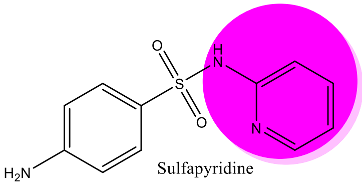
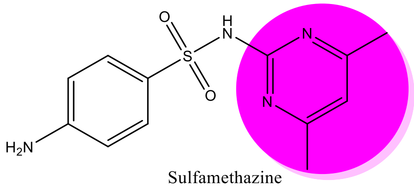
	
Chemical Structure	**Ball and Stick Model**

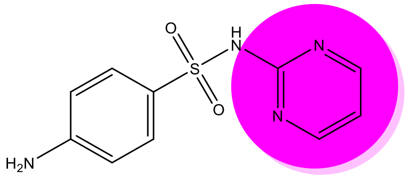
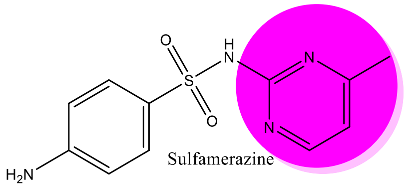
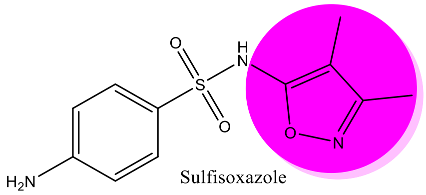
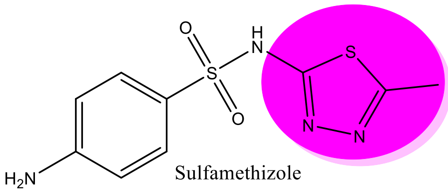
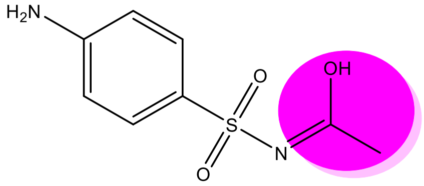
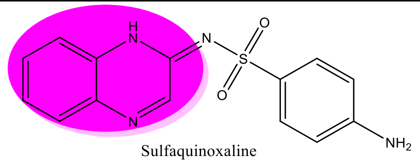
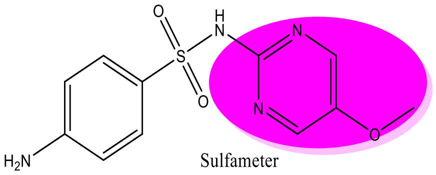
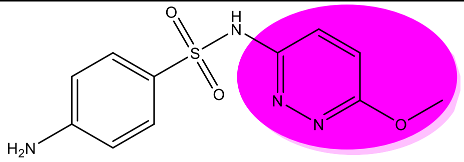
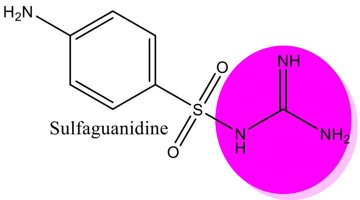
Table 2: Example of Sulpha drug containing compounds

S.No.	Name of Compound	S.No.	Name of Compound
1	Sulfamethoxazole	11	Sulfacetamide
2	Sulfathiazole	12	Sulfaquinoxaline
3	Sulfamoxole	13	Sulfameter
4	Sulfadimethoxine	14	Sulfamethoxypyridazine
5	Sulfapyridine	15	Sulfaguanidine
6	Sulfamethazine	16	Sulfaphenazole
7	Sulfadiazine	17	Sulfachlorpyridazine
8	Sulfamerazine	18	Sulfisomidine
9	Sulfisoxazole	19	Sulfalene
10	Sulfamethizole	20	Sulfamonomethoxine

Importance of Sulpha Drug Containing Compounds

Several sulfonamide drugs are widely used in clinical practice for different therapeutic purposes. Sulfamethoxazole, commonly combined with trimethoprim as co-trimoxazole, is frequently prescribed for urinary tract and respiratory tract infections. Sulfadiazine is administered along with pyrimethamine in the treatment of toxoplasmosis. Sulfisoxazole is mainly used for ear infections and may be given in combination with erythromycin. Sulfasalazine is effective in managing ulcerative colitis and other inflammatory bowel diseases. Sulfacetamide is applied topically for acne and as eye drops for conjunctivitis. Silver sulfadiazine is widely used in burn wound infections to prevent microbial growth. Sulfapyridine was historically important in the treatment of pneumonia, while Sulfathiazole has been used for gastrointestinal infections. Together, these examples highlight the broad therapeutic applications of sulfonamide drugs. Structural representation of all sulpha drugs represented in table 2.

S.No.	Name of the Compound	Structure
1	Sulfamethoxazole	 Sulfamethoxazole
2	Sulfathiazole	 Sulfathiazole
3	Sulfamoxole	 Sulfamoxole
4	Sulfadimethoxine	 Sulfadimethoxine
5	Sulfapyridine	 Sulfapyridine
6	Sulfamethazine	 Sulfamethazine

7	Sulfadiazine	 Sulfadiazine
8	Sulfamerazine	 Sulfamerazine
9	Sulfisoxazole	 Sulfisoxazole
10	Sulfamethizole	 Sulfamethizole
11	Sulfacetamide	 Sulfacetamide
12	Sulfaquinoxaline	 Sulfaquinoxaline
13	Sulfameter	 Sulfameter
14	Sulfamethoxypyridazine	 Sulfamethoxypyridazine
15	Sulfaguanidine	 Sulfaguanidine

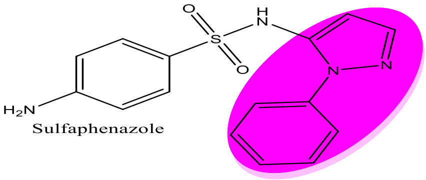
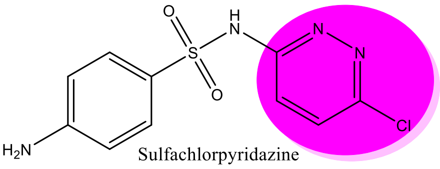
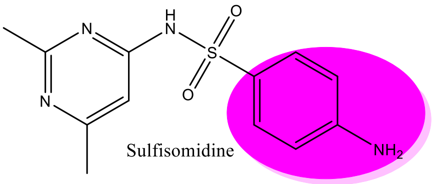
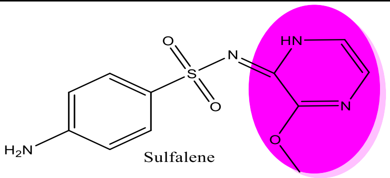
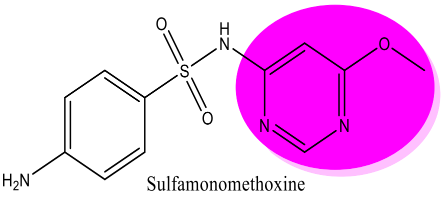
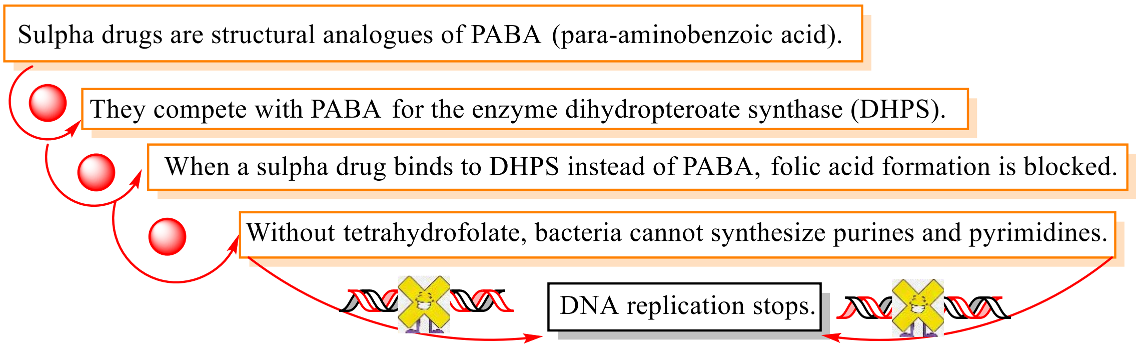
16	Sulfaphenazole	
17	Sulfachlorpyridazine	
18	Sulfisomidine	
19	Sulfalene	
20	Sulfamonomethoxine	

Figure 1: Structural representation of substituted sulpha drugs

Mechanism of Action

Inhibition by Sulpha Drugs (Sulfonamides)-Sulpha drugs act by inhibiting bacterial folic acid synthesis, which is essential for DNA and cell replication.



Sulpha drugs are structural analogues of PABA (para-aminobenzoic acid).

They compete with PABA for the enzyme dihydropteroate synthase (DHPS).

When a sulpha drug binds to DHPS instead of PABA, folic acid formation is blocked.

Without tetrahydrofolate, bacteria cannot synthesize purines and pyrimidines.

DNA replication stops.

Figure 2: Inhibition by Sulpha Drugs (Sulfonamides)

Sulfadiazine inhibits DHPS by mimicking PABA, preventing folate production and stopping bacterial growth. Important point is that they are Bacteriostatic and the reason behind is that Sulpha drugs do not directly kill bacteria. They only stop bacterial growth and multiplication, allowing the immune system to eliminate the infection. Further from literature review it is expressed that Target enzyme of this bioactive compound is Dihydropteroate synthase and type of inhibition is competitive inhibition. Nature of action is bacteriostatic and pathway

The general chemical structure of Sulpha Drugs/sulfonamides, are derivatives of p-aminobenzenesulfonamide.

Competitive Inhibition- Sulpha drugs are structural analogues of para-aminobenzoic acid (PABA), a natural substrate required by bacteria for folic acid synthesis. Because of this structural similarity, sulpha drugs competitively inhibit the bacterial enzyme dihydropteroate synthase (DHPS) by binding to its active site in place of PABA. When a sulpha drug engages DHPS, the actual formation of dihydropteroic acid is blocked, thereby stopping the synthesis of folic acid. Consequently, tetrahydrofolate, the active form of folic acid (necessary for one-carbon transfer reactions), is not produced. In the absence of tetrahydrofolate, bacteria are unable to produce essential purines and pyrimidines necessary for nucleic acid formation. Consequently, DNA replication and cell division are stopped, leading to suppression of bacterial growth (bacteriostatic effect).

Chemical Knowledge: Sulpha drugs are chemically classified as para-amino benzene sulfonamide derivatives, indicating they contain a benzene ring with a para ($–NH_2$) group and a sulfonamide ($–SO_2NH–$) functional group. These structural moieties are responsible for their pharmaceutical activity. Chemically, sulphonamides behave as weak acids. Weak acid as the hydrogen attached to the sulfonamide nitrogen can dissociate and their pKa values typically range from 5-10, totally depending on the type of substituent attached to the N^1 position of the sulfonamide group. Substitution with heterocyclic rings generally reduces the pKa, increases acidity, and upgrades water solubility. At physiological pH 7.4, many sulpha drugs exist chiefly in their ionized form, which facilitates renal excretion and increases their distribution in body fluids. The degree of ionization also influences their antibacterial activity and pharmacokinetic behavior. In terms of chemical stability, sulphonamides are stable in solid (dry) form under normal storage conditions, but must be protected from moisture (Excessive) and heat to maintain effective potency. All physicochemical properties mentioned above play an important role in determining their absorption, distribution, and overall therapeutic effectiveness.

Essential Structural Requirements of Sulfonamides

The antibacterial activity of sulfonamides is significantly dependent on para-aminobenzenesulfonamide nucleus(A. K. Halve *et al.,* 2007; A. K. A. K. Halve *et al.,* 2008; K. Singh *et al.,* 2025; R. Singh *et al.,* 2024). For structural mimicry of para-aminobenzoic acid (PABA) free para-amino ($–NH_2$) group is needed, results in significant antimicrobial activity. Further facilitates competitive inhibition of the bacterial enzyme dihydropteroate synthase. Permanent modification of this amino group generally results in marked reduction or decrease of antibacterial activity, except in cases where reversible prodrug strategies are employed. The $–SO_2NH–$ constitutes the core pharmacophore moiety responsible for antibacterial action. This moiety is essential for interaction with the active site of dihydropteroate synthase and is therefore fundamental providing base for the inhibition of bacterial folic acid biosynthesis.

Substitution at the N^1 position, (particularly with heterocyclic aromatic rings), significantly influences physicochemical and pharmacokinetic properties. Electron-withdrawing heterocycles increases the acidity of the sulfonamide proton, thereby increasing ionization at physiological pH. This modification improves:

- Aqueous solubility,
- Optimizes systemic absorption,
- And enhances antimicrobial potency.

Further incorporation of bulky substituents at appropriate positions enhances plasma protein binding, which extends the duration of action. Such structural changes are characteristic of long-acting sulfonamides. Furthermore, combination of –N=N– linkage produces compounds with limited systemic absorption. These derivatives function primarily as prodrugs, undergoing bacterial cleavage in the colon to release the active sulfonamide moiety, thereby exerting localized intestinal therapeutic effects.

Collectively, these structural determinants define the structure–activity relationship (SAR) profile of sulfonamides and govern their pharmacodynamic and pharmacokinetic behavior. So it is concluded that Para-amino group and Sulfonamide moiety is essential parameters, heterocyclic ring increases activity, electron withdrawing groups increase potency and Blocking NH_2 permanently results in inactive results. Other Key Structure–Activity Relationship (SAR) determinants of Sulfonamides are depicted in figure.

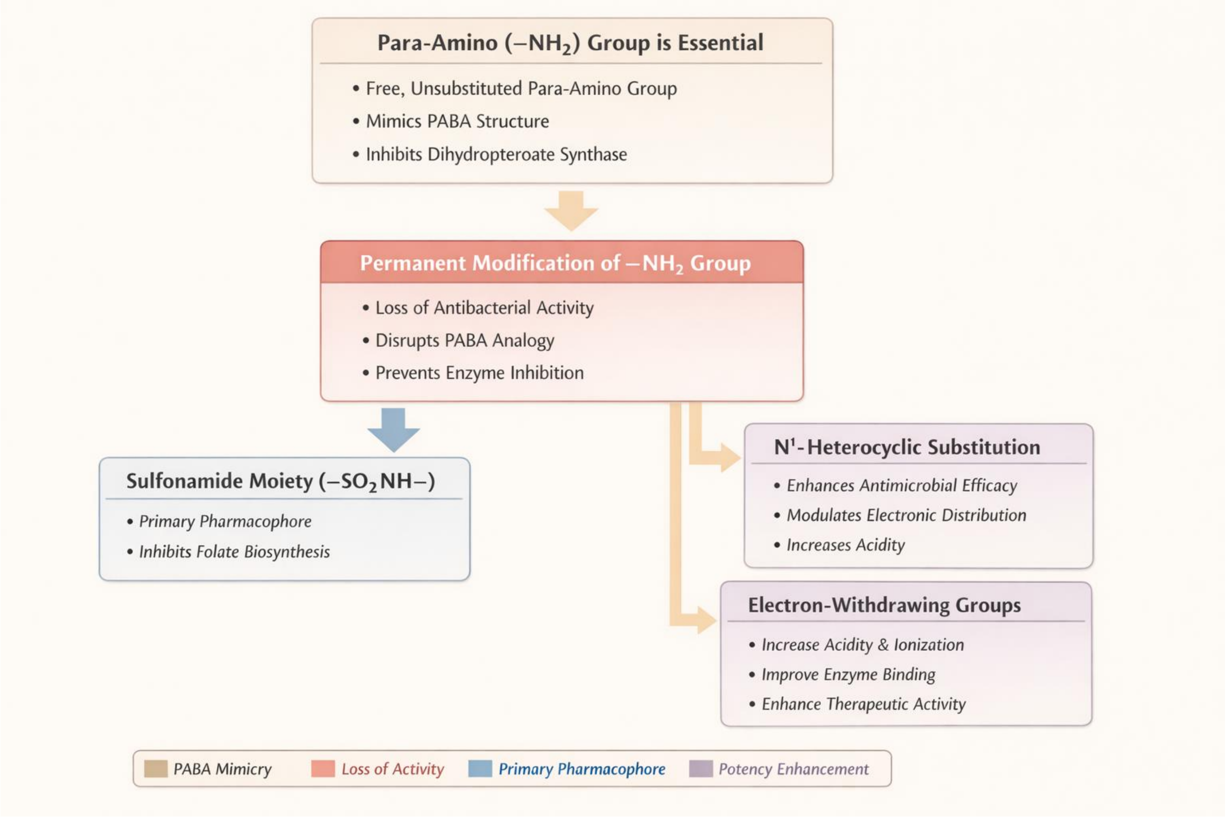


Figure 3: Collectively represented Structure Activity Relationship

The above-mentioned SAR collectively defines the molecular requirements necessary for optimal antibacterial performance of sulfonamide derivatives.

Table 3: Summary list of sulpha drugs/sulphonamides with their name, properties, biological activity

S.No.	Name of the Compound	Moiety or structural feature responsible for Structure activity relationship	SAR/Biological activity	Reference
1	Sulfamethoxazole	p-NH_2; $-SO_2NH-$; N^1 isoxazole	Isoxazole lowers pKa, optimal systemic absorption; synergistic effect with trimethoprim/ Broad-spectrum antibacterial	(Balraj *et al.,* 1991), (Hoofnagle, 2012b),(C. Wang *et al.,* 2026)
2	Sulfathiazole	p-NH_2 substitution and thiazole moiety	Aromatic heterocycle enhances DHPS binding/ Broad-spectrum antibacterial	(Inoue *et al.,* 2026)(Sherif *et al.,* 2026)(A. P. Singh *et al.,* 2021)
3	Sulfamoxole	p-NH_2 group and acetamide side chain	Water solubility; suitable for topical use/ Ophthalmic antibacterial	(Kuhne *et al.,* 1976), (Velluti *et al.,* 2014)
4	Sulfadimethoxine	Methoxy pyridazine moiety	Long-acting; electron-withdrawing heterocycle increases potency/ Systemic antibacterial	(Rekg *et al.,* 1988),(Mitrovic *et al.,* 1969),(Korolkovas *et al.,* 1978)
5	Sulfapyridine	Pyrazine moiety	Long half-life; improved absorption/ Systemic antibacterial	(Paniker & Levine, 2001)(Rani *et al.,* 2026),(Kadife *et al.,* 2026)
6	Sulfamethazine	Dimethyl pyrimidine moiety	Moderately long acting, decreases renal excretion/ Veterinary antibacterial	(Tahir *et al.,* 2016)(Abulkhair & El-Adl, 2025)(García-Pastor *et al.,* n.d.)
7	Sulfadiazine	Pyrimidine moiety	Optimal pKa, effective systemic action/ Toxoplasmosis therapy	(Hoofnagle, 2012a)(Alahmadi *et al.,* 2026)(Alqahtani *et al.,* 2026)
8	Sulfamerazine	Monomethyl pyrimidine substitution	Intermediate duration/ Antibacterial	(Aday *et al.,* 2016)(Chen *et al.,* 2021)(Spisz *et al.,* 2021)
9	Sulfisoxazole	Isoxazole moiety	Highly soluble; short acting/ UTI treatment	(Shin *et al.,* 2022)(Grunberg *et al.,* 1970)

10	Sulfamethizole	Thiazole derivative	Rapid urinary excretion/ Urinary antiseptic	(Kambli *et al.,* 2025)(Siddiqui *et al.,* 2018)
11	Sulfacetamide	Acetamide substitution	Solubility, topical/ Ophthalmic antibacterial	(Agarwal *et al.,* 2016)(Ahmad *et al.,* 2023)
12	Sulfaquinoxaline	Quinoline heterocyclic ring	Extended half-life, veterinary use/ Coccidiosis	(Nikoofard *et al.,* 2017)(Ghorab *et al.,* 2011)
13	Sulfameter	Dimethoxypyrimidine substitution	Long acting; sustained plasma level/ Systemic antibacterial	(Ohara *et al.,* 1995)(Nakahata *et al.,* 2018)
14	Sulfamethoxypyridazine	Methoxypyridazine ring	Long duration/ Antibacterial	(LAPEYSSONNIE *et al.,* 1961)(Sanfilippo & Morvillo, 1968)
15	Sulfaguanidine	Guanidine moiety	Poor absorption, intestinal action/ Intestinal infections	(Abulkhair, 2025)(Cleaver, 2003)
16	Sulfaphenazole	Thiazole moiety	Potent heterocyclic sulfonamide/ Broad spectrum	(Fleming *et al.,* 2001)(Myung *et al.,* 2012)
17	Sulfachlorpyridazine	Chloropyridazine	Electron-withdrawing Cl increases potency/ Veterinary antibacterial	(Trafton & Lind, 1963)(Nanda & Batterman, 1957)
18	Sulfisomidine	Pyrimidine containing compound	Intermediate duration/ Antibacterial	(Kassem *et al.,* 1981)(Rakshith *et al.,* 2025)
19	Sulfalene	Dichlorophenyl and pyrimidine	Very long-acting, high protein binding/ Malaria (with pyrimethamine	(Lil'in *et al.,* 1979)(Bobrov *et al.,* 1984)
20	Sulfamonomethoxine	Isoxazole moiety	Optimal pKa, combination therapy/ Broad-spectrum antibacterial	(Kung *et al.,* 2019; Z.-L. Wang *et al.,* 2025)

Conclusions

In Conclusion, Sulpha drugs (sulfonamides) are among the most significant discoveries in modern chemotherapy, first recognized for their antibacterial activity by Gerhard Domagk in the early 20th century, and they laid the foundation of the antibiotic era. They act by competitively inhibiting dihydropteroate synthase (DHPS) in the bacterial folic acid pathway, showing selective toxicity because humans obtain folic acid from diet rather than synthesizing it; since they inhibit growth instead of killing bacteria directly, they are classified as bacteriostatic agents. The development of new strategy for synthesing sulpha drug containg pharamacophore is crucial for advancing the field of medicinal chemistry and addressing the AMR (antibiotic resistance). Today, sulfonamide-containing compounds are seeking attention in biomedical research for enzyme inhibition, targeted drug delivery, nanomedicine, anticancer studies, and treating a wide range of bacterial infections. Overall, sulpha drugs clearly illustrate that its chemical structure governs therapeutic diversity and have received considerable attention from scientific community.

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ADVANCES IN TRIPHALA RESEARCH

K. Lalitha

Department of Pharmaceutics, Faculty of Pharmacy,
Gokaraju Rangaraju College of Pharmacy, Nizampet, Bachupally,
Hyderabad – 500090, Telangana, India
Corresponding author E-mail: kalakotalalitha8094@grcp.ac.in

Introduction and Traditional Background

Ayurveda represents one of the oldest holistic healthcare systems originating in India, emphasizing individualized treatment, disease prevention, and overall well-being. The term is derived from Sanskrit, where "Ayu" denotes life and "Veda" signifies knowledge, collectively meaning "science of life." This system focuses on maintaining harmony between body, mind, and spirit by considering an individual's constitution (Prakriti) [Bairwa VK *et al.,* 2025].

Historically, Ayurveda has been preserved through classical texts authored by scholars such as Charaka, Sushruta, and Vagbhata [Thakar VJ*et al.,*2010]. Its philosophical roots are deeply embedded in the Vedas, where knowledge was believed to be divinely revealed to sages [Jaiswal YS *et al.,2017*].

Within this system, Triphala is a widely recognized polyherbal formulation composed of three fruits. The name itself originates from Sanskrit—"Tri" meaning three and "Phala" meaning fruits [Goya-Jorge E *et al.,2025*]. It consists of *Emblica officinalis*, *Terminalia bellerica*, and *Terminalia chebula* [Peterson CT *et al., 2017*].

Ancient Ayurvedic literature highlights its long-term use for promoting longevity and maintaining health. Classical texts recommend its consumption with honey and ghee for sustaining vitality and delaying aging [Bairwa VK *et al.,2025*].

Triphala has traditionally been used for multiple therapeutic purposes, including digestive regulation, detoxification, and rejuvenation. It is also associated with antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory effects [Parmar HS *et al.,* 2011].

Chemical and Phytochemical Profiling

Composition of Triphala

Triphala is formulated by combining equal proportions (1:1:1) of:

- *Emblica officinalis* (Amalaki)
- *Terminalia bellerica* (Bibhitaki)
- *Terminalia chebula* (Haritaki)

This combination creates a synergistic formulation widely used in Ayurvedic medicine [Arnold JT *et al.,* 2022].

Phytochemical Constituents

Triphala is rich in biologically active compounds, primarily polyphenols, tannins, and flavonoids. Analytical studies indicate that it contains approximately 38±3% polyphenols and 35±3% tannins**,** with gallic acid commonly used as a marker compound.

Major constituents identified include:

- Gallic acid, tannic acid, syringic acid, epicatechin
- Ascorbic acid
- Emblicanin A & B, ellagic acid, corilagin

Each individual component contributes specific phytochemicals:

- *E. officinalis*: ascorbic acid, gallic acid
- *T. bellerica*: tannins, gallic acid
- *T. chebula*: chebulinic acid, ellagic acid

These compounds collectively contribute to Triphala's therapeutic properties [Prakash D *et al.,* 2000; El-Mekkawy S *et al.,* 1995; Iyer KM *et al.,* 1958; Bhattacharya SK *et al.,* 2000; Jamwal KS *et al.,* 1959; Reddy BM *et al.,* 1994; Grampurohit ND *et al.,* 1986; Lin TC *et al.,* 1990].

Individual Components and Their Properties

Amalaki (*Emblica officinalis*)

Amalaki is rich in vitamin C and polyphenols such as gallic acid and ellagic acid. It exhibits antioxidant, hepatoprotective, immunomodulatory, and antidiabetic activities. It also supports digestion, immunity, and skin health [Goswami B *et al.,* 2022; Gul M *et al.,* 2022].

Bibhitaki (*Terminalia bellerica*)

Bibhitaki contains tannins, oils, and glycosides. It demonstrates laxative, antimicrobial, and anti-inflammatory properties. Traditionally, it is used in respiratory and gastrointestinal disorders [Ashutosh Gupta et al.; Anindita Deb *et al.,* 2016].

Haritaki (*Terminalia chebula*)

Haritaki is known as the "King of Medicines" due to its broad therapeutic potential. It contains tannins, chebulinic acid, and gallic acid. It shows antioxidant, antimicrobial, antidiabetic, and neuroprotective effects [Bulbul MR *et al.,* 2022; Maruthappan V *et al.,* 2010].

Biological and Pharmacological Activities

Antioxidant Activity

Triphala exhibits significant antioxidant and radioprotective properties, effectively safeguarding biological systems from oxidative damage induced by γ-radiation. Studies have demonstrated its ability to protect plasmid pBR322 DNA and microsomal lipids from radiation-induced injury. This protective effect is largely attributed to the high concentration of polyphenols and tannins present in the formulation, which act by neutralizing reactive oxygen species (ROS) and converting them into less reactive, non-toxic molecules. Additionally, Triphala seems to mitigate oxidative stress induced by cold exposure, as evidenced by reductions in lipid peroxidation and modulation of endogenous antioxidant systems, including superoxide dismutase (SOD), catalase (CAT), and non-enzymatic antioxidants such as vitamin C [Rao P. *et al.,* 2017].

Plant-derived antioxidants are widely recognized for their beneficial role in maintaining physiological balance by scavenging reactive oxygen species, thereby preventing cellular damage and associated pathological conditions [Wong CC *et al.,* 2005].

Experimental studies further indicate that oral administration of Triphala (1 g/kg body weight for 48 days) significantly attenuates oxidative stress caused by cold exposure, while also modulating corticosterone levels and lipid peroxidation. Moreover, Triphala and its individual constituents have demonstrated the ability to inhibit radiation-induced DNA strand breaks, highlighting their potential as natural radioprotective agents [Sandhya T *et al.,* 2006].

The formulation exhibits potent free radical scavenging activity against reactive species such as superoxide radicals and DPPH radicals, and effectively inhibits radiation-induced lipid peroxidation. Notably, the synergistic interaction among the three constituent fruits enhances its overall antioxidant efficacy compared to individual components. Mechanistic studies reveal that Triphala not only scavenges superoxide radicals through the xanthine–xanthine oxidase system but also reduces uric acid formation, indicating a dual mode of action. High-performance liquid chromatography (HPLC) analysis has confirmed the presence of gallic acid as a major bioactive compound. Antioxidant activity assessed using Fe^{2+}–ascorbate-induced lipid peroxidation and DPPH assays shows a strong positive correlation with total polyphenol content, underscoring the importance of these compounds in its activity [Naik GH *et al.,* 2006].

In addition to its direct antioxidant effects, Triphala also modulates the gut microbiome, contributing indirectly to its therapeutic potential. Key phytochemicals such as quercetin and gallic acid promote the proliferation of beneficial bacterial species, including *Lactobacillus* and *Bifidobacterium*, while inhibiting pathogenic organisms like *E. coli*. Furthermore, gut microbiota metabolize Triphala-derived polyphenols, such as chebulinic acid, into bioactive metabolites like urolithins, which possess enhanced antioxidant and anti-inflammatory properties. Enzymatic activities such as tannase produced by gut bacteria facilitate this biotransformation. This interaction between Triphala and the gut microbiome is believed to amplify its bioactivity and contribute to the production of a diverse range of anti-inflammatory compounds [Kalaiselvan S *et al.,* 2015].

Anti-inflammatory Activity

Triphala demonstrates pronounced anti-inflammatory potential through modulation of key molecular signaling pathways involved in inflammation. The formulation has been shown to suppress the expression of pro-inflammatory mediators such as TNF-α, IL-1β, IL-6, COX-2, nitric oxide (NO), and prostaglandin E2 (PGE2). These effects are primarily mediated via inhibition of the NF-κB and MAPK pathways, which play central roles in inflammatory responses. Experimental studies indicate that Triphala significantly reduces inflammatory cytokine production and enzymatic activity in disease models such as colitis and arthritis, often exhibiting comparable or superior efficacy to standard anti-inflammatory drugs like indomethacin. The presence of bioactive compounds such as gallic acid and chebulinic acid further enhances its anti-inflammatory activity.

Previous investigations have reported that Triphala effectively reduces inflammation-associated biochemical markers and mitigates joint damage in arthritic models [Rasool M *et al.,* 2007]. Additionally, it has been shown to attenuate cartilage degradation and bone destruction in arthritis-induced conditions [Kalaiselvan S *et al.,* 2015]. The formulation also inhibits NF-κB activation, thereby reducing the production of inflammatory mediators, including IL-17, COX-2, and RANKL. Furthermore, Triphala enhances antioxidant defences while reducing lipid peroxidation in inflamed tissues [Kalaiselvan S *et al.,* 2015].

Antimicrobial Activity

Triphala exhibits broad-spectrum antimicrobial activity against a wide range of pathogenic microorganisms. Its antibacterial efficacy is largely attributed to its high content of tannins and phenolic compounds, which contribute to dose-dependent inhibition of both Gram-positive and Gram-negative bacteria. Studies have demonstrated that Triphala significantly reduces microbial load in infected wounds, including pathogens such as *Salmonella*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Bacillus* species.

Additionally, Triphala has shown inhibitory effects against pathogens associated with urinary tract infections, surgical site infections, and enterococcal infections. Both aqueous and alcoholic extracts exhibit antimicrobial activity, with some studies indicating that aqueous extracts may be more effective against organisms such as *E. coli* and *S. aureus* [Kumar A *et al.,* 2011]. Regular consumption of Triphala has also been associated with reduced susceptibility to intestinal infections [Tambekar DH *et al.,* 2007]. Its effectiveness against antibiotic-resistant bacterial strains further highlights its potential as an alternative or adjunct antimicrobial agent [Mahalakshmi K *et al.,* 2006].

Immunomodulatory Activity

Triphala possesses significant immunomodulatory properties, as evidenced by its ability to influence both humoral and cell-mediated immune responses [Belapurkar P *et al.,* 2014; Srikumar R *et al.,* 2007]. Early studies evaluating its aqueous extract demonstrated enhanced delayed-type hypersensitivity (DTH) responses and increased antibody production.

The immunomodulatory effects of Triphala are largely attributed to its antioxidant constituents, including gallic acid, chebulagic acid, and ellagic acid, which neutralize reactive oxygen species and reduce oxidative stress [Belapurkar P *et al.,* 2014; Srikumar R *et al.,* 2005]. By modulating oxidative pathways, Triphala indirectly regulates immune responses and inflammatory processes. Furthermore, Triphala has been shown to influence cytokine production, including IL-1, TNF-α, and IFN-β, which play critical roles in immune activation and recruitment of immune cells such as neutrophils and macrophages. It also modulates lymphocyte populations, including CD4+ and CD8+ T cells, and regulates cytokines such as IL-2, IL-4, and IFN-γ, thereby contributing to balanced immune function [Srikumar R *et al.,* 2007].

Wound Healing Activity

Wound healing is a complex physiological process involving multiple phases, including inflammation, proliferation, and remodeling. Disruptions in these phases can lead to delayed or chronic wound conditions [Earley MJ *et al.,* 2008].

Triphala has demonstrated significant wound healing potential by accelerating tissue repair and enhancing regeneration. Studies indicate that its application leads to improved wound contraction, increased collagen synthesis, and reduced microbial load at the wound site. Elevated levels of biochemical markers such as hexosamine and uronic acid further support enhanced tissue remodeling.

Incorporation of Triphala into biomaterials, such as collagen sponges, has shown promising results, including improved thermal stability, increased water absorption capacity, and faster wound closure rates. These effects are partly attributed to interactions between polyphenols like epigallocatechin gallate and collagen, which facilitate tissue regeneration and repair [Kumar MS *et al.,* 2010].

Anti-obesity Activity

Obesity is a multifactorial condition influenced by genetic, environmental, and lifestyle factors, and is associated with increased risk of metabolic and cardiovascular disorders [Forhan M *et al.,* 2013].

Experimental studies have demonstrated that Triphala possesses anti-obesity effects, as evidenced by reductions in body weight and adiposity in animal models. These effects are partly attributed to bioactive compounds such as gallic acid, which serves as a key marker compound and contributes to lipid metabolism regulation. [Forhan M *et al.,* 2013].

Ongoing clinical investigations, including randomized controlled trials, are evaluating the efficacy of Triphala in weight management and metabolic health, indicating its potential as a natural therapeutic agent for obesity [Forhan M *et al.,* 2013].

Antidiabetic Activity

Triphala exhibits notable hypoglycemic activity and has been studied for its potential role in diabetes management. Elevated postprandial blood glucose levels, commonly observed in type 2 diabetes, are primarily due to increased carbohydrate digestion and reduced glucose uptake by cells.

Triphala has been shown to inhibit key digestive enzymes such as α-amylase and α-glucosidase, thereby reducing glucose absorption and lowering blood sugar levels. This mechanism is comparable to that of certain antidiabetic drugs [Gurjar S *et al.,* 2012].

Animal studies have demonstrated that oral administration of Triphala extract significantly reduces blood glucose levels in both normal and diabetic models, with sustained effects observed upon repeated dosing. Additionally, Triphala exhibits antioxidant activity by scavenging free radicals and reducing lipid peroxidation, further contributing to its antidiabetic effects [Gurjar S *et al.,* 2012].

Anticancer Activity

Cancer remains a leading cause of mortality worldwide [Siegel RL *et al.,* 2019]. Triphala has shown promising anticancer activity through multiple mechanisms, including inhibition of oxidative stress, induction of apoptosis, and suppression of tumor growth.

In vitro and *in vivo* studies demonstrate that Triphala reduces reactive oxygen species in cancer cells, thereby preventing DNA damage caused by radiation and chemical agents. It has also been shown to inhibit lipid peroxidation and exhibit free radical scavenging activity [Aiswarya Lakshmi AG *et al.,* 2022].

Triphala selectively induces cytotoxicity in cancer cells while sparing normal cells. This effect is associated with increased intracellular ROS levels and activation of apoptotic pathways. Studies have reported its efficacy against various cancers, including breast, prostate, and pancreatic tumors [Sandhya T *et al.,* 2006; Phetkate *et al.,* 2012].

Cardioprotective Activity

Triphala has demonstrated significant cardioprotective properties through its antioxidant and lipid-lowering effects. It reduces oxidative stress markers, improves blood circulation, and regulates cholesterol levels, thereby contributing to cardiovascular health [Babu D *et al.,* 2014].

It also improves hemodynamic parameters and protects against myocardial injury by maintaining antioxidant status and preventing lipid peroxidation [Sarada SK *et al.,* 2007]. Additionally, Triphala has been shown to improve glucose regulation and reduce cardiovascular risk factors such as hyperlipidemia and obesity [Fisher M *et al.,* 2006; Brunzell JD *et al.,* 2008].

Clinical studies further support its role in improving fasting blood sugar levels and overall metabolic health in diabetic patients [Singh N et al,. 2015]. The presence of multiple bioactive compounds targeting cardiovascular pathways highlights its potential in managing cardiovascular and cerebrovascular diseases [Ojha S *et al.,* 2012].

Analgesic, Antipyretic and Anti-ulcer Activity

Triphala has demonstrated analgesic and antipyretic effects comparable to standard non-steroidal anti-inflammatory drugs such as indomethacin, without causing gastric mucosal damage. This indicates its potential as a safer alternative for pain and fever management.

The anti-ulcer activity of Triphala has been studied as part of polyherbal formulations such as NR-ANX-C. These studies reveal that the formulation reduces gastric lesions, acidity, and ulcer formation, while enhancing mucosal protection. The observed effects are attributed to its cytoprotective, antioxidant, and anti-secretory properties [Nair V *et al.,* 2010].

Neuroprotective Activity

Triphala administration has been shown to markedly enhance cognitive function in Alzheimer's disease mouse models, including C57BL/6, 5xFAD, and APP/PS1 strains. This effect is mediated through modulation of the amyloid precursor protein (APP) pathway, resulting in reduced amyloid plaque accumulation and attenuation of neuroinflammatory responses. Additionally, Triphala contributes to the restoration of the gut-brain axis by promoting the proliferation of beneficial gut microbiota such as *Bacteroides*, *Proteobacteria*, and *Actinobacteria*, which play critical roles in maintaining gut homeostasis and supporting neuroprotection. Notably, a dual-mode administration regimen (morning and evening) exhibited superior preventive and therapeutic outcomes, highlighting its potential as a strategy for mitigating Alzheimer's disease through modulation of gut microbiota and control of neuroinflammation [Upadhyay P *et al.,* 2024].

Triphala's effects also extend to the regulation of antioxidant-associated signaling pathways, further reinforcing its neuroprotective mechanisms. Despite promising preclinical evidence, challenges remain in enhancing blood-brain barrier permeability and systemic bioavailability to maximize therapeutic impact. Consequently, rigorously designed clinical studies are warranted to validate its efficacy in managing stress-related cognitive and psychological disorders, underscoring its potential as a natural intervention for promoting cognitive resilience and mental well-being [Wang W *et al.,* 2023].

Recent Research and Clinical Studies

Recent studies emphasize the therapeutic potential of Triphala in managing a wide range of conditions, including metabolic disorders, neurodegenerative diseases, and post-viral complications. Its ability to enhance antioxidant enzyme activity (SOD, GSH-Px) and reduce oxidative stress markers such as MDA and TNF-α has been well documented.

However, clinical evidence suggests that achieving significant therapeutic outcomes may require higher doses or prolonged treatment durations, indicating the need for further well-designed human trials.

Future Prospects

Triphala continues to gain attention as a promising integrative therapeutic agent for modern healthcare challenges. Future research should focus on:

- Clinical validation through large-scale trials
- Mechanistic studies at molecular levels
- Development of advanced drug delivery systems
- Exploration of its role in chronic disease management

With growing scientific validation, Triphala holds substantial potential for integration into evidence-based medicine.

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ECLIPTA PROSTRATA IN TRADITIONAL MEDICINAL SYSTEMS

Monica Sanabam[1], Ningombam Bijaya Devi[1] and Kistu Singh Nongthombam*[2]
[1]Department of Botany,
G.P. Women's College, Dhanamanjuri University, Imphal-795001, Manipur, India
[2]Department of Botany, Kumbi College, Kumbi-795133, Manipur, India
*Corresponding author E-mail: nkistusingh@gmail.com

Abstract

Eclipta prostrata is a medicinal plant with a pivotal role in several major traditional medical systems serving hundreds of millions of people all over the world. *E. prostrata* has special places in Ayurveda, Traditional Chinese Medicine (TCM), Siddha medicine, and Unani medicine, the medical systems that share similar therapeutic prescribing indications but with divergent theoretical bases. This chapter systematically explores the ethnomedicinal history of *E. prostrata* across various traditions, outlines classical formulae and preparatory techniques, and sheds light on the productive collaboration between traditional medicine and contemporary scientific investigation. *E. prostrata* is documented to be a rasayana herb within Ayurvedic medicine, with hepatoprotective and hair growth promotion. The plant is known in Traditional Chinese Medicine as a yin-nourishing herb that treats kidney and liver yin deficiency, especially in cases of premature hair graying and hemorrhagic disorders. Siddha and Unani medicine also focus on hepatic and dermatological applications with the temperamental and humoral systems, respectively. Traditional medicine has been shown in modern ethnopharmacological studies to contain bioactive compounds such as wedelolactone, flavonoids, and thiophenes. Recent clinical trials prove effectiveness in androgenetic alopecia, hepatopathology, and hemorrhagic pathology. This chapter demonstrates that centuries of combined clinical knowledge can be applied in modern pharmaceutical research and incorporated into global healthcare systems.

Keywords: *Eclipta prostrata*, Ayurveda, Chinese Medicine, Ethnopharmacology, Hepatoprotection.

1. Introduction

Eclipta prostrata (L.) L. is a well-known and highly valued medicinal plant in traditional health care systems of the Indian subcontinent and East Asia. Its name is reflected in many languages, with the most well-known attribute, in Sanskrit and Hindi, bhringraj (king of hair); in Chinese, mo-han-lian (ink plant); and in Tamil, karisalankanni (Reddy *et al.,* 2025; Sanyal *et al.,* 2022; Li *et al.,* 2021). Instead of being a folk remedy, *E. prostrata* holds a major place in three large, traditionally acclaimed medical systems, namely Ayurveda, Unani medicine, and Siddha medicine. The modern importance of *E. prostrata* goes well beyond historical curiosity; it is a compelling example of how ancient wisdom, captured in classical writings and preserved through centuries of clinical use, is increasingly confirmed and validated by modern researchers.

This chapter discusses the importance of *E. prostrata* in all the major traditional medicinal systems, the classical formulations and preparations in which it is used, and how traditional knowledge can inform modern pharmaceutical studies.

2. *Eclipta prostrata* In Ayurvedic Medicine

2.1 Historical References and Classical Texts

The Ayurvedic medicine of *E. prostrata* spans back to the classical period of the Sanskrit medical text. In different synonymous names, the plant is referred to in other primary Ayurvedic texts. One of the most authoritative Ayurvedic books, the Charaka Samhita, compiled around 2000 years ago, has praised the extraordinary ability of the bija (seeds) and patra (leaves) of bhringraj to strengthen hair follicles and salacity (Balkrishna *et al.,* 2024). There is another comparable text, Sushruta Samhita, which recommends the use of oil infusions of the leaves of *E. prostrata* to prevent premature graying of hair and alopecia (hair loss) (Choukikar *et al.,* 2023). These classical sources show that *E. prostrata* was already known to have a special affinity for hair health two thousand years ago, and this point is also supported by Ayurvedic practice today. In addition to hair care ability, *E. prostrata* is also described in classical Ayurvedic texts as hepatoprotective plant (Timalsina & Devkota, 2021). The plant is listed in other Ayurvedic compendia as a rasayana - a type of rejuvenator herb said to increase longevity, energy, and stimulate the balance of a system that has been weakened (Molligoda *et al.,* 2023). The rasayana botanical classification indicates a high level of understanding that the plant has a broad-spectrum therapeutic effect that extends beyond organ systems. At the same time, *E. prostrata* is listed in the official Ayurvedic Pharmacopoeia of India, an official list of medicinal plants, as a hepatoprotective agent, confirming classical indications of therapy under modern names.

2.2 Ayurvedic Principles and Doshic Classification

To understand *E. prostrata* in Ayurveda, it is essential to be acquainted with the Ayurvedic principle of tridosha, the three basic principles or bio-forces of functioning of the body, viz., vata (air/ether, movement, dryness), pitta (fire/water, metabolism, transformation), and kapha (water/earth, structure, heaviness) (Jayasundar, 2017). The Ayurvedic theory of disease etiology is that the balance of these doshas is destroyed. The action (sheeta virya) of *E. prostrata* is considered cooling (sheeta virya), and bitter and slightly pungent (rasa), so particular in the pacification of excess pitta dosha (heat element). The cooling action is believed to suppress the inflammatory heat that builds up in the blood, liver, and skin. Also, *E. prostrata* is said to contain vata and kapha doshas, albeit less eminently than its pitta-pacifying effect (Tripathy *et al.,* 2024). The multi-doshic action also helps in the therapeutic flexibility of the plant- it is possible to use it in a wide range of disease manifestations related to the various doshic imbalances.

In Ayurvedic theory, liver is considered as the position of pitta dosha and within which transformative digestive and metabolic fire functions. Accumulation of excess pitta in the liver leads to inflammation, heat, and toxins (Arora & Kumar, 2024). These manifestations directly correspond to the cooling and bitter properties of *E. prostrata* that clear the heat in the liver and promote healthy nutrient conversion. Ayurvedic hair health is, in turn, conceived as one that requires sufficient food and good circulation. It is said that the plant nourishes hair roots, promotes blood flow, reduces heat-blocking effects, and provides essential nutrients. The Ayurveda theoretical paradigms can therefore give a logical explanation why one plant can treat both hepatic disease and loss of hair, both diseases are caused by excess pita and impaired circulatory or nutritive system.

2.3 Traditional Preparations and Administration Methods:

E. prostrata is used by Ayurvedic practitioners with a variety of preparation methods, each tailored to maximize the therapeutic effects for particular disease manifestations. Most common forms are:

- Bhringraj Oil (Tailam): In the traditional practices, the leaves and stems of fresh *E. prostrata* are harvested and combined with either sesame oil or coconut oil. New plant matter is either infused directly into the oil by heating or the juice is extracted and mixed with the oil. This is applied to the scalp's hairy areas to stimulate hair growth, darken early gray hairs, and strengthen hair follicles. The therapeutic value of the base oil is highly dependent on the type of base oil used, i.e. coconut oil is cooling and relaxing, and sesame oil is warming and penetrating. The practising Siddha of South India, especially in Kerala, have perfected this preparation into complex recipes of bhringraj oil mixed with other cooling herbs producing therapeutic products which continue to be in demand in the traditional spas and wellness centres of the region (Raina, 2021).
- Powder (Churna): The dried leaves and stems are ground into powder and used in 250 mg to 2 g in divided doses per day, usually with warm water, honey, or ghee. Internal administration is mostly used in the form of powder, to assist the liver, relieve fever, and improve digestion. The amount is usually calculated based on the constitution of the individual and the degree of severity (Panigrahi *et al.,* 2019).
- Decoction (Kwath): The dried leaves (10-15 g) are boiled in water (240 ml), till the water is brought down to 60-80 ml to produce a concentrated extract of soluble compounds. This is prepared once or twice daily for the treatment of hepatic disorders, respiratory conditions, and general rejuvenation. In the past, the decoction technique was primarily utilized especially in domestic medicine, where *E. prostrata* was grown in home gardens (Pareek & Kumar, 2015).
- Fresh Juice (Rasa): Juice is obtained by pressing fresh leaves and given in 5-10 ml doses and is usually combined with other substances. This form is very common in the treatment of acute hepatic conditions, haemorrhagic conditions and skin conditions and it is believed that fresh preparations are the most vital forceful (Tripathy *et al.,* 2024).

- Polyherbal Formulations: *E. prostrata* is commonly used in Ayurvedic medicine formulations by pairing with other medicinal herbs. This is, for instance, used with *Picrorhiza kurroa* (katuki) and *Phyllanthus niruri* (bhumi amalaki) to formulate superior hepatoprotective compounds. Equally, when used in hair specific applications, it is co-formulated with Brahmi (*Bacopa monnieri*) and Neem (*Azadirachta indica*) to make all-inclusive scalp tonics that both provide nutritional and antimicrobial aspects of hair health (Sharma *et al.,* 2021).

3. *Eclipta prostrata* In Traditional Chinese Medicine

3.1 Historical Documentation and Classical References

E. prostrata is known as either "ecliptae herba" or "mo han lian" in the Traditional Chinese Medicine (TCM) because it makes hair dark (Sapat *et al.,* 2026). The first mention of this plant appears in the Ming Dynasty (1368-1644) in certain large pharmacopeias, such as the Ben Cao Gang Mu, where it is said to possess a yin-nourishing effect. According to TCM theory, medicinal substances are grouped according to their attributes to nourish specific organ systems and correct deficiencies in the most basic fluids and structural materials of the human body, i.e. qi (vital energy), blood, yin, and yang. *E. prostrata* belongs to the classification of yin-nourishing herbs, the substances which restore the depleted yin essence, the basic fluid and structural material in the human body (Yang *et al.,* 2023). This categorization places it in a complex theoretical tradition that is radically different but unexpectedly compatible with Ayurvedic doctrines.

3.2 TCM Organ System Associations and Therapeutic Theory

According to TCM theory, *E. prostrata* is believed to nourish the liver and kidney yin and these parts play major roles in TCM physiology (Meesin *et al.,* 2023). In TCM, the liver stores blood and regulates the circulation of qi and emotions. The kidneys store jing (vital spirit) and control growth, development, and reproductive activity. Notably, the kidney jing is said to control the hair; its sheen, growth, and colour. A lack of kidney yin (heat, dryness, and a lack of nourishing fluids) results in symptoms such as premature graying, hair loss, dizziness, tinnitus (ringing in the ears), and blurred vision. *E. prostrata* treats these problems with the help of restoring yin to the kidneys and alleviating the heat of yin deficiency. The plant is especially useful in TCM for treating haemorrhagic disorders due to yin deficiency with heat, such as epistaxis (blood in the nose), haemoptysis (blood in the cough), and abnormal uterine bleeding (Zhang *et al.,* 2022). Failure of yin to check the yang in the TCM theory results in excess heat, which ruptures the blood vessels, causing them to bleed (Chen & Zhang, 2025). Herbs such as *E. prostrata* are yin-nourishing and do not simply prevent bleeding but address the root cause of bleeding yin insufficiency. Notably, *E. prostrata* is a mild substance in TCM pharmacology, and does not have any of the purgative or tonifying properties of most classical herbs. This gentleness enables it to be used over a long duration without producing the side effects that more aggressive substances do, making it a suitable agent in chronic diseases that have to be treated over an extended period.

3.3 TCM Formulations and Clinical Applications

E. prostrata is used in a number of classical and modern formulas by TCM practitioners. One well-known and commonly used traditional combination is a combination of *E. prostrata* and ligustrum fruit (*Ligustrum lucidum*). This combination comes up with a traditional yin-tonifying formula that focuses on the kidney and liver yin deficiency, with the focus of healing the symptoms of the yin deficiency with heat, especially the graying and loss of hair. Other yin-nourishing herbs, including rehmannia (shu di huang) and Chinese angelica root (dang gui) are usually added into this blend to create polyherbal preparations of high therapeutic complex (Mengru *et al.,* 2025). Practically, TCM practitioners often make use of *E. prostrata*-based formulas as either decocted herbs (jian yao), tablet preparations or patent medicines (cheng yao), which are standardized herbal formulas already containing their appropriate quantities of herbs. The combination of *E. prostrata* and ligustrum has been shown to result in a higher graying percentage of hair than either of the two herbs in isolation, which is confirmed by modern research.

4. *Eclipta prostrata* in Siddha Medicine

4.1 Overview of Siddha Tradition and System Philosophy

Siddha medicine is one of the oldest medical traditions in India, which has its origins in southern India and is passed on mostly in the Tamil language literature. According to foundational mythology, Siddha medicine is divinely originated in the teachings of Lord Shiva to his learned mistress Parvati who transferred them to others as an oral tradition of successful adepts (siddhars) (Jeyavenkatesh *et al.,* 2025). The 18 notable Siddha adepts in the past, such as the legendary Siddhar Agasthyar, who is regarded as the founding father of the system, developed advanced medical theories and practices as recorded in classical Tamil texts. The Siddha system functions on principles similar to those of Ayurveda in part, but with different emphases and terminology. Similar to Ayurveda, Siddha medicine also conceptualizes health as an imbalance of three humors (mukkuttram): vata (airy), pitta (fiery), and kapha (watery) (Baheti *et al.,* 2025). Environmental, dietary, lifestyle, and psychological factors cause this imbalance, which leads to disease. Moreover, Siddha acknowledges five fundamental components of all matter, that is, earth, water, fire, air, and ether, as the basic constituents of food, body, and medicine. The system uses herbal (thavaram), mineral (thadhu), and animal products (jangamam) in therapeutics.

4.2 *E. prostrata* in Siddha Pharmacology

Siddha medicine has various names such as karissalankanni (Tamil), bhangraa, and bhringoraaja, among others. The plant is also widely used as a liver and scalp tonic, indicating its known clinical effectiveness in these areas. The Siddha use *E. prostrata* to treat liver cirrhosis, infectious hepatitis, liver swelling accompanied by swollen spleen (hepatosplenomegaly), and jaundice, all of which are explained in Siddhic terms as a result of pitta excess and heat stagnation in the liver (Narendra & Manasi, 2021). Siddha approach to application is slightly different from Ayurvedic practice: in Kerala and other parts of southern India, Siddha vaithiyars (practitioners) developed a preparation of bhringraj oil by boiling fresh *E. prostrata* leaves in sesame oil, and produced therapeutic products regionally localized. These preparations were made a part of the traditional spa and wellness center (Siddha vaidyashala) in all parts of Kerala and still continue to be used as a treatment for various dermatological and alopecia related issues (Gopi, 2016).

The Siddha system focuses on pathiyam and apathiyam, explicit rules and contraindications of what can be eaten or what cannot be eaten in the course of the medicinal therapy. Once *E. prostrata* is used in the treatment of hepatic disorders, Siddha practitioners advise certain dietary changes: cooling foods (cucumber, coconut, bitter melon) are welcomed, while heating foods (spices, fried foods, alcohol) are discouraged (Kulkarni *et al.,* 2023). This is an integrated treatment methodology by incorporating medicinal plant treatment alongside dietary and lifestyle changes reflects the holistic therapy philosophy of Siddha medicine.

5. *Eclipta prostrata* in Unani Medicine

5.1 Unani System Foundations and Theoretical Framework

Unani medicine, also known as Greco-Arabic medicine or Tibb medicine, is a complex medical tradition with its roots in ancient Greek medicine, which was later developed and perfected in the Islamic world and arrived in South Asia through a series of historical routes. The Indian Unani system is officially recognized as one of the AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy) systems of medicine and is regulated by the Central Council of Indian Medicine. Unani medicine makes four temperaments (akhlat) (phlegm cold-wet, blood hot-wet, yellow bile hot-dry, black bile cold-dry) conceptualizations of health (Ansari, 2021). Quantitative or qualitative imbalances of these temperaments are the cause of disease. Medical substances are categorized by their temperamental effects: heating/cooling and moistening/drying. The therapy aims to correct the patient's temperament imbalance using medication and diet.

5.2 *E. prostrata* in Unani Pharmacology and Clinical Applications

E. prostrata is called bhangra or bhangraa in Unani medicine. In the Unani temperamental system, the plant is moderately cooling and moderately drying, which is considered useful in dealing with conditions that are associated with excessive heat and moisture. *E. prostrata* is specifically applied in the treatment of skin disease, hair loss, fever and several inflammatory diseases. The plant is a component of the traditional Unani preparations and is sometimes added with rose water, which is a cooling Unani compound, to relieve headaches, skin rashes, and ophthalmological inflammation (Ranjan *et al.,* 2025). The combination of E. prostrata and rose water is a classic illustration of the Unani principle of applying the compatible medicine resources and media in order to achieve maximum therapeutic impact. Although marginally covered in contemporary literature relative to its use in Ayurvedic or TCM medicine practice, *E. prostrata* remains in use in the practice of the Unani school of medicine in India and other areas with a long history of Unani medicine, especially for the management of hepatic disease and the treatment of dermatological disorders.

6. Bridge Between Traditional Knowledge and Modern Science

6.1 Ethnopharmacological Research Methodology

Ethnopharmacology has proven to be a fruitful approach for drug discovery and *E. prostrata* is a good example. The traditional applications described in classical literature can be tested as a hypothesis. For example, Ayurvedic signs suggesting hepatoprotection led to the study of liver-protective effects in animal models of chemically induced hepatotoxicity. The research on hemorrhage control is based on TCM indications of hemorrhage control. Phytochemical studies in modern times show that the traditional therapeutic indications are supported by bioactive compounds, including wedelolactone (a coumestan) with hepatoprotective and anti-inflammatory action, flavonoids such as luteolin with antimicrobial and antioxidative action, and thiophene derivatives with antimicrobial activity against multidrug-resistant pathogens (Jahan *et al.,* 2014).

6.2 Validation of Traditional Claims through Clinical Research

More and more traditional applications of E. prostrata are adapted to modern clinical trial. A herbal shampoo containing E. prostrata extract was compared to placebo in a study of men with androgenetic alopecia (male pattern baldness), and objective evidence that this traditional claim that this plant is an herbal promoter of hair in male subjects was significantly more evident in participants using the herbal extract compared to placebo at 16 weeks- objective (Lee *et al.,* 2017). In an Indian clinical trial, an open-label study in 12 weeks, 65% of the participants who used the bhringraj oil reported that their hair was growing and their hair shedding slowed down (Patel *et al.,* 2025). In clinical trials of liver diseases, E. prostrata has been reported to lower high liver enzymes dose-dependently and certain formulations have been reported to be as effective as pharmaceutical compounds references such as silymarin (milk thistle extract) (Arman *et al.,* 2022). Such clinical confirmations not only validate traditional knowledge but also guide dosing, the course of treatment for patients, and patient selection in modern medicinal use.

7. Cultural Significance and Contemporary Popular Practice

Outside official medicinal systems, *E. prostrata* holds a unique position in popular health culture in South and East Asia. Bhringraj oil is extremely common in India, found in grocery stores, markets, and pharmacies, in amounts equal to those of aspirin in Western medicine, and the home health regimen has become as familiar a habit as using soap and water. This historical relationship with the plant and hair health has provided it with a strong cultural narrative. The Bhringraj, the king of hair to the common imagination, is thus a natural component of hair products to prevent hair loss, gray hair or even just regular scalp care. Such extensive usage is addressed to the practical utility of the plant and to transpersonal flow of health information between generations in families. Traditional medicine practitioners in Nepal use drops of *E. prostrata* in sesame oil to treat sinusitis, migraine headache, and inflammation of the eyes, nose, and ears- uses that probably represent local medical knowledge rather than major classical systems (Das *et al.,* 2024). In Brazil, where the American *E. prostrata* has become naturalized, it is locally known as erva-botão and is used in folk medicine both to treat snakebites (envenomation) and to treat hepatoprotection and high blood pressure, classic uses that have now been scientifically studied, with anti-venom activity of the plant extract and wedelolactone (Antunes *et al.,* 2022).

8. Significance for Integration into Contemporary Healthcare Systems

8.1 Bridging Traditional and Biomedical Paradigms

This example of E. prostrata illuminates promising paths to the reconciliation of traditional and modern medicine, and it works. Instead of rejecting traditional systems as unscientific or accepting their claims as such, the ethnopharmacological approach treats traditional indications as hypotheses to be rigorously tested empirically. The plant is then eligible to be incorporated into evidence-based healthcare systems where there is evidence of its use that can be validated through pharmacological, clinical, and mechanistic research. And the converse is also true, in cases where contemporary research has detected functional activity in biological systems not involved in classical systems, there is a new pharmaceutical prospect. In the case of *E. prostrata*, contemporary science has confirmed the majority of significant traditional uses and has also identified other activities like anticancer, antivenom, and neuroprotective activities, that are not well documented in the traditional literature (Chung *et al.,* 2017).

8.2 Advantages of Plant-Based Medicines within Traditional Frameworks

There are some benefits of formal interaction with traditional medicinal systems as compared to individual phytochemical research. Traditional systems are the product of centuries or even millennia of accumulated clinical knowledge, including enormous observational studies that show that certain preparations are effective under what circumstances and in what types of populations, as well as the side effects they exhibit. Classical literature offers particularity on how to prepare (which parts of the botanicals to use, when to harvest the crop, and the methods of extracting the compound) as demonstrated empirically to create maximum therapeutic effect. Conventional recipes in which *E. prostrata* is used with other herbs could be synergistic when compared to an exclusive use of the plant itself. The dosage, contraindications, and the patient selection criteria incorporated in conventional texts are a result of the accumulated knowledge that could otherwise be costly to find back through clinical trials. Integration of validated traditional therapies reverses cultural inclinations in the health care systems that serve populations whose main health resource is traditional medicine, but at the same time, enhances treatment outcomes.

Conclusion

E. prostrata has an exceptional status as a vital medicinal plant of immense relevance across a variety of autonomously evolved traditional medical systems in South Asia, East Asia, and the Americas. Instead of being the ancient folk medicine of some isolated folk healer, the uses of *E. prostrata* are formalized in three major, formally recognized Indian medical systems, such as Ayurveda, Siddha, and Unani, all of which have elaborate theoretical foundations, standardized preparation techniques, and long histories of clinical experience. The overlap in therapeutic indications of these autonomous systems, i.e., hepatic illness, hair well-being, hemorrhagic issues, and inflammatory illness, provides the strongest epidemiological evidence of actual pharmacological action. The traditional uses have been repeatedly verified by modern ethnopharmacological studies, which also continue to provide mechanistic insights through phytochemical analysis and controlled clinical trials. The plant is a good example of how traditional medical knowledge is not a matter of historical interest but rather a rich source of empirically based therapeutic knowledge, ready for scientific verification and to become part of modern evidence-based medical practice.

In modern healthcare systems, especially in areas where traditional medicine systems remain the main providers of health care access, the identification of *E. prostrata* underscores the need to invest in advancing rigorous studies, quality control, and risk monitoring. In the case of pharmaceutical development, the traditional applications of the plant and its confirmed bioactivities make it a potential source of new therapeutic agents or adjunctive therapies for disease mechanisms, including liver disease and antimicrobial-resistant infections. *E. prostrata* provides an interesting case study of fruitful interaction between the traditional and the modern knowledge system for researchers whose expertise lies within botany, ethnobotany, and phytopharmacology. The experience that the plant has gone through in classical Sanskrit and Tamil texts to modern scientific validation is just an example of how the traditional medicinal plants themselves can perform significant roles in health worldwide without discrimination to the cultural and intellectual histories from which the knowledge is a product.

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KALLMANN SYNDROME: ETIOLOGY, CLINICAL MANIFESTATIONS AND DIAGNOSTIC STRATEGIES

Swathi M. and K. Lavanya
Department of Biochemistry,
PSG College of Arts & Science, Coimbatore – 14
Corresponding author E-mail: lavanya@psgcas.ac.in, swathimurugesan2895@gmail.com

Abstract

Kallmann syndrome is a rare genetic congenital disorder characterised by hypogonadotropic hypogonadism and anosmia or hyposmia. The syndrome is caused by a defect in the migration of Gonadotropin-releasing hormone (GnRH) neurons from the olfactory or the nasal placode to the hypothalamus due to the mutation in the gene KAL1, which produces the anosmin-1 protein, resulting in a defect in the secretion and stimulation of GnRH, which leads to hypogonadotropic hypogonadism. The prevalence of the Kallmann syndrome is estimated to be 1 in 30,000 and 1 in 125,000 in males and females, respectively. Boys during their neonatal stage of life show signs of micropenis and cryptorchidism, while in girls, it is less apparent. During the adolescent stage of life, both male and female patients affected with the syndrome show signs of the absence or incomplete development of secondary sexual characteristics. In adulthood, it leads to infertility, cardiovascular issues and neurological symptoms. Olfactory tests and hormonal analysis, assessing levels of gonadotropins, testosterone, FSH and LH, can be performed in diagnosing patients with Kallmann syndrome.

Keywords: Kallmann Syndrome, Hypogonadotropic Hypogonadism, *KAL1* Gene, Anosmin. Gonadotropin-Releasing Hormone.

Introduction

The hypothalamus plays a crucial role in the initiation and progression of pubertal development by stimulating the secretion of luteinizing hormone (LH) and follicular-stimulating hormone (FSH). GnRH is secreted from the arcuate nucleus in the hypothalamus through the blood vessels. It stimulates the synthesis of LH and FSH from the anterior pituitary gland. Both LH and FSH play a major role in the gonadal maturation in both men and women (Fanis *et al.,* 2023).

The reproducibility in humans is managed mainly by 4,000 GnRH-secreting neurons present in the hypothalamus (Bonomi *et al.,* 2016). GnRH neurons were unusual neuroendocrine cells derived outside the central nervous system from the progenitor cells in the medial olfactory placode, which then migrated across the nasal septum and entered the forebrain with the nervus terminalis and finally attached to the septal preoptic area and hypothalamus (Duan & Allard, 2020).

Puberty is the transition period between childhood and adulthood, which is characterised by the development of secondary sexual characteristics, gonadal maturation and attainment of reproduction (Abreu & Kaiser, 2016). The gonadal axis from the hypothalamus is activated from mid-trimester, restrained at the end of gestation, during the foetus's development and reactivated after birth. In early childhood, GnRH secretion is actively inhibited until the child reaches adolescence. Reactivation of GnRH initiates puberty in both men and women after this quiescent period. In boys, testicular and penile growth occurs, while in girls, enlargement of the uterus and breasts occurs (Bizzarri & Cappa, 2020).

Gonadotropin-releasing hormone regulates the mechanism of synthesis and release of FSH and LH during puberty. In boys, proliferation of immature Sertoli cells and spermatogonia is promoted by FSH, while LH produce testosterone by stimulating the Leydig cells, leading to initiation of spermatogenesis. In girls, FSH and LH are important for follicular maturation and ovulation (Koskenniemi *et al.,* 2017).

Among various hypogonadotropic hypogonadism conditions, the Kallmann syndrome is unique, in which there is a complete absence of the synthesis of GnRH, which affects the synthesis of FSH and LH, leading to the lack of puberty development in both men and women (Young *et al.,* 2019). This review mainly focuses on the Kallmann syndrome, primarily by discussing the recently developed treatments and techniques to overcome the defect in the synthesis of the gonadotropic hormones.

Kallmann Syndrome

Kallmann syndrome is characterised by rare, congenital, isolated, idiopathic hypogonadotropic hypogonadism and anosmia due to abnormal migration of olfactory axons and gonadotropin-releasing hormone-producing neurons (Hilman *et al.,* 2023)

Kallmann syndrome can occur sporadically as X-linked, KAL genes, autosomal dominant or autosomal recessive forms of inheritance. It is caused by a missense and frameshift mutation in the *KAL1* gene or *ANOS1* gene, which encodes anosmin protein, which is located on the X chromosome. Anosmin has a key role in the migration of GnRH neurons and olfactory nerves to the hypothalamus (Oliveira *et al.,* 2001; Arora *et al.,* 2023).

A deficiency in GnRH results in decreased levels of sex steroids, including FSH and LH, leading to a lack of sexual development with the absence of secondary sexual characteristics and maturity. The diagnosis occurs when a child fails to begin puberty and it can be identified earlier when associated congenital defects raise suspicion or when there is a family history of delayed puberty that suggests a heritable disorder (Sonne *et al.,* 2024).

Epidemiology

The prevalence of Kallmann syndrome is estimated to be approximately 1 in 30,000 in males and 1 in 125,000 in females. The incidence in males is high compared with females (Zak, 2024). Recent epidemiological, clinical and genetic features of Kallmann syndrome in Finland. The minimal incidence estimate of KS in Finland was 1 in 48,000, with a clear difference between males, 1 in 30,000 and females, 1 in 125,000 (Laitinen *et al.,* 2011).

Mechanism

Gonadotropin-releasing hormone neurons originate within the nasal placode. GnRH neurons migrate along the olfactory-derived vomeronasal axons to the nasal forebrain junction and then into the developing forebrain. Within the forebrain, GnRH neurons function in hormone signalling through the hypothalamic-pituitary-gonadal axis. Disruption of GnRH results in hypogonadism and anosmia, which is termed Kallmann Syndrome (Cho *et al.,* 2019).

Kallmann syndrome is genetic but can result from many different mutations. More than 30 genes have been associated with hypogonadotropic hypogonadism, including Kallmann syndrome, with slight variations in secondary characteristics (Chu *et al.,* 2023).

Kallmann syndrome is associated with the most common defect in the genes *KAL1 (ANOS1)* and *FGFR1*, but approximately 35% to 45% of cases are not explained by the currently identified genetic abnormalities. The list of other genetic mutations to cause Kallmann syndrome includes CCDC141, CHD7, DUSP6, FEZF1, FGF8, FGF17, HS6ST1, IL17RD, KISS1, NSMF(NELF), PROK2, PROKR2, SEMA3A, SEMA3E, SOX10, SPRY4, TAC3 and WDR11 (Sonne *et al.,* 2024).

X-linked recessive inheritance of Kallmann syndrome is due to mutations in the *KAL1* gene, located on the X chromosome, found in 8% of all patients with Kallmann syndrome. Mutations in the *KAL1* gene are associated with anosmia, cryptorchidism, decreased libido, erectile dysfunction, infertility, micropenis, one-sided renal agenesis and synkinesia (Yadav *et al.,* 2025).

The *ANOS1* gene is found to cause X-linked Kallmann Syndrome. It is located on the X chromosome (Xp22.3), which contains 14 exons, present adjacent to the pseudo-autosomal 1 region, which is a highly variable and unstable region on the chromosome found in 8% of all patients with Kallmann syndrome. The *ANOS1* gene encodes anosmin-1, an extracellular matrix protein. Anosmin-1 protein consists of a cysteine-rich region, a whey acidic protein-like domain, four consecutive fibronectin type III domains and a C-terminal region rich in basic histidines and prolines. Anosmin-1 promotes neurite outgrowth, neuronal cell adhesion, axon guidance and CNS projection neuron branching. It is also involved in the migration of neural precursors, including GnRH-producing neurons and oligodendrocyte precursors. In Kallmann syndrome patients with *ANOS1* mutations, the loss or dysplasia of cryptorchidism and the olfactory bulb is high (Sun *et al.,* 2025).

Kallmann syndrome is due to deficient GnRH production, caused by the failure in the differentiation and migration of GnRH-producing neurons that arise embryologically in the olfactory mucosa to reach the hypothalamus. Figure 1 shows the path of migration of GnRH neurons from the olfactory to the hypothalamus. In Kallmann syndrome, this process is associated with abnormal olfactory nerve development, that causes both anosmia and hypogonadotropic hypogonadism. Deficient hypothalamic GnRH secretion results in the abnormal production of gonadotropins, such as FSH and LH, which in turn results in hypogonadism, infertility, pubertal delay and other clinical symptoms associated with Kallmann syndrome (Sonne *et al.,* 2024).

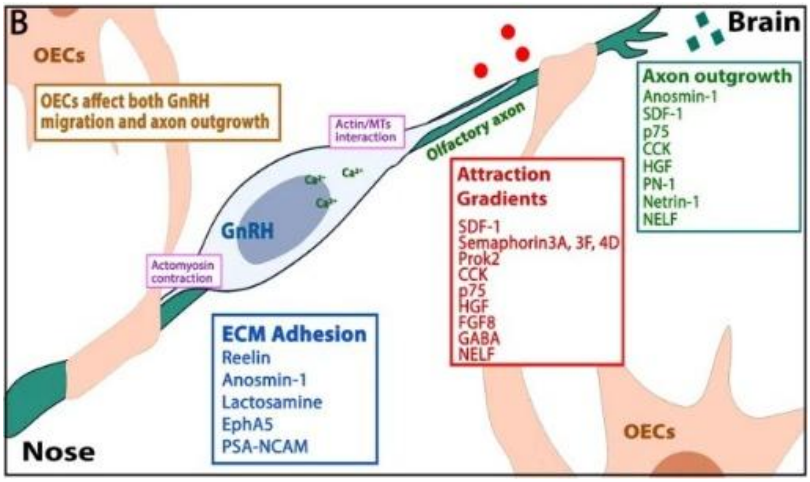

Figure 1: Migration of GnRH neurons

Figure 1 describes the GnRH neuron along the olfactory axon bundles. Actin/microtubule interactions and actomyosin contractions occur at the leading process and the trailing process of the migrating GnRH neuron to the brain (Cho *et al.,* 2019).

Clinical Manifestations

Neonatal boys with the syndrome may present earlier with micropenis and cryptorchidism due to a lack of GnRH stimulation. Signs and symptoms are recognised during adolescence, as patients with the syndrome have either a partial or a complete lack of secondary sexual characteristics, including the absence of testicular and penile enlargement. Lack of pubertal progression. Patients affected with this syndrome may also present later with concerns related to infertility, low libido, unilateral renal agenesis, synkinesia and erectile dysfunction (Millar *et al.,* 2021; Yadav *et al.,* 2025)

Diagnosis

The diagnosis of Kallmann syndrome is very challenging during childhood, as childhood is a physiologically hypogonadal period, with the quiescence of the GnRH stimulation and secretion. Delayed puberty is a diagnosis in adolescence. Patients can exhibit absent or partial puberty or male infants, micropenis with or without cryptorchidism, which can be suggestive of the diagnosis (Young *et al.,* 2019). Magnetic resonance imaging examination can show abnormalities in the olfactory bulb or tract agenesis, reduced depth, reduced curvature, or increased cortical thickness within the olfactory sulcus in patients (Lewkowitz-Shpuntoff *et al.,* 2012; Manara *et al.,* 2014). Anosmia is not recognised by the affected individuals, so olfactory screening tests and obligatory ENT examination are performed, even when the patient reports a normal sense of smell. It leads to early diagnosis of Kallmann Syndrome (Dzaman *et al.,* 2017). Preimplantation genetic testing during the in vitro fertilisation process helps to interrupt the transmission of the hereditary syndrome to offspring at an early stage. Genetic testing and counselling are recommended to patients with family histories, prenatal diagnosis and preimplantation genetic testing are considered to be useful options (Liu & Zhi, 2022).

Treatment and Management

Identification of the disorder in early stages is crucial, which allows for the timely initiation of appropriate hormonal therapy and psychological support to the patients, which improves the quality of life for patients affected by the Kallmann syndrome (Zak, 2024). For women affected with Kallmann syndrome, assisted reproductive technology treatment and selective embryo cryopreservation may be a reasonable and safe option. Assisted reproductive technology is considered when optimal ovulation induction therapy is difficult to perform or when several cycles of ovulation induction therapy have been performed and do not result in pregnancy (Shiraiwa *et al.,* 2022).

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WASTE TO WEALTH: VALORIZATION OF FRUIT AND VEGETABLE WASTE FOR NANOPARTICLE SYNTHESIS

Priyanka Jojo
Department of Chemistry,
Jagannath Nagar College, Ranchi, Jharkhand
*Corresponding author E-mail: priyankajojo07@gmail.com

Abstract

Fruit and vegetable waste constitutes a significant portion of organic waste produced worldwide at numerous points in food supply chain. These wastes, comprising peels, seeds, pulp, pomace, and other residues, are rich in bioactive compounds, namely organic acids, flavonoids, polyphenols, and vitamins. These phytochemicals, that act as natural reducing and stabilizing agents, are essential for production of green nanoparticles (NPs). Reduction of metal ions, nucleation, growth, and stabilization are important stages in the biosynthesis process that result in formation of metal nanoparticles with controlled physicochemical characteristics. The properties of the nanoparticles are influenced by the synthesis conditions. Owing to their special properties, these nanoparticles are being extensively investigated for biomedical, environmental, energy, food, and agricultural applications.

Keywords: Valorization, Fruit and Vegetable Waste, Nanofactory, Bioactive Compounds, Metal Nanoparticles.

1. Introduction

The world population is growing rapidly, driving up demand for food and agricultural products. This results in increased production and consumption of fruits and vegetables. Nearly 60% of these food products end up as waste, such as seeds, pulp, peels, or spoiled items, which adds to the amount of organic waste. However, they are often discarded when they change color, break, suffer frostbite, are exposed to heat, become infested with microbes, or reach a level of ripeness which makes them unacceptable to consumers. This waste could happen at any stage of food supply chain. According to latest FAO data from 2020, world produced 2015 million tons of fruits and vegetables. China as largest producer, accounted for 38% of global production with 709 million tons in 2020. Despite this, approximately 30% of fruits and vegetables are wasted during harvesting, transportation, sales, and processing (Wang *et al.,* 2022). These residues are often disposed of as waste, which harms the environment. Reducing fruit and vegetable waste could assist in easing pressures of rising food consumption and improve supply chain efficiency (Matharu *et al.,* 2016; Liu *et al.,* 2023). As a result, researchers are working to find better ways to use this plentiful and sustainable resource.

Therefore, to properly dispose of these wastes, there is an urgent need for a sustainable waste management system. Fruit and vegetable waste contains valuable bioactive components, including flavonoids, saponins, alkaloids, vitamins, polyphenols, tannins, minerals, phenolic acids, amino acids (AA), and polysaccharides. These compounds exhibit antimicrobial, anti-inflammatory, anticarcinogenic, and antioxidant properties that are beneficial for human health. The idea of waste valorization has attracted considerable attention because agricultural waste could be converted into high-value products for use in industries, namely food, agriculture, pharmaceuticals, and nanotechnology. When food waste is improperly handled and disposed of, it can seriously harm the ecosystem. Around 3.3 billion tonnes of carbon dioxide equivalent, or 4.4 kilotons, are emitted annually due to poor management of food waste and loss. This accounts for around 8% of total human-produced "greenhouse gas emissions (GHGs)" (Sharma *et al.,* 2020).

2. Fruit and Vegetable Waste as Natural Nanofactories: A Waste Valorization Approach for Green Nanoparticle Synthesis

Waste valorization refers to transformation of waste materials into value-added products, energy, or other useful resources rather than just discarding them. Waste valorization has garnered an effective, sustainable waste management system. The goal of this process is to use biological, chemical, and physical techniques to extract valuable components from waste material and turn them into high-value goods. It aligns with the waste-to-wealth concept and supports the circular economy, in which waste is reused, recycled, and regenerated to produce innovative products. Turning these wastes into resources instead of discarding them in landfills helps reduce pollution and supports sustainable development. Waste valorization is essential because it addresses both environmental and economic issues simultaneously. Improper handling of agricultural and food waste can pollute land, water, and air, leading to ecological imbalance. They are often disposed of by burning, landfilling, or composting. However, these methods may cause significant environmental harm, such as the release of toxic or greenhouse gases and increased microbial growth resulting from elevated moisture and leachate levels (Dessie *et al.,* 2018; Liu *et al.,* 2023).

Moreover, waste valorization facilitates advancement of green technologies, such as more sustainable nanoparticle production. Transforming fruit and vegetable waste into micro- or nanoscale materials or valuable products is proving to be a promising approach. This method uses bioactive compounds derived from waste, namely flavonoids, tannins, amino acids, alkaloids, polyphenols, namely vitamins, caffeic acid and gallic acid, minerals to create products which benefit both society and economy (Tarrahi *et al.,* 2021). Fruit and vegetable waste is useful for producing nanoparticles and can serve as a kind of nanofactory. This process avoids hazardous reducing agents, reduces nanoparticle toxicity, and allows production of nanoparticles with specific shapes, sizes, functional groups (Abu-Danso *et al.,* 2020). This method not only discourses waste management issues but also improves resource utilization, thereby promoting sustainable development by turning agricultural waste into valuable nanomaterials (Jeevanandam *et al.,* 2018; Gupta *et al.,* 2025). Therefore, waste valorization is essential for advancing sustainable resource management, economic value, and environmental protection, establishing it as a key strategy in contemporary scientific research and industrial practice.

3. Different Types of Fruit and Vegetable Wastes

3.1 Peels

Peels are the outer protective layer (skin or covering) of fruits and vegetables that are typically discarded as waste after being processed or consumed. The main constituents of peel involve proteins, minerals, flavonoids, pectin, cellulose, hemicellulose, antioxidants, their associated polysaccharides (Rakariyatham *et al.,* 2020; Hanna *et al.,* 2025). These compounds help reduce and stabilize metal ions in green synthesis of nanoparticles. Peels from papaya, tomato, orange, banana, gourd were utilized for synthesizing metallic NPs, comprising gold, copper, iron, silver, zinc oxide, titanium, palladium. These nanoparticles are appropriate for broad range of biological utilizations.

3.2 Seeds

Caffeic acid, cinnamic acid, chlorogenic acid, epicatechin gallate, ferulic acid, (epi) catechin, (epi) gallocatechin, essential amino acids, naringenin, "polyunsaturated fatty acids (PUFA)", coumaric acid, syringic acid, vanillic acid are among the highly valuable phytochemicals found in seeds. Seed extracts exhibit several bioactivities, comprising antibacterial, antimutagenic, antiviral, anticancer, cardioprotective activities. (Chakraborty *et al.,* 2017). In contrast to traditional plant-based extracts, phytochemicals derived from seed extracts can be obtained effectively without the need for complex reactor systems or regulated growth conditions. The linked follicles of seeds, one of most widely utilized plant components in traditional medicine, produce inflated capsule that contains significant amount of oil with bitter taste and strong odor (Sayed Nady *et al.,* 2025; Chakraborty *et al.,* 2017).

Several phytochemicals derived from seeds are used in nanoparticle synthesis, and these compounds have excellent biological uses in humans. Plant seeds are considered to be significant source of biogenic NPs (Khalil *et al.,* 2021; Hanna *et al.,* 2025).

3.3 Pomace Residues

Pomace residues are byproducts of the food processing and juice industries. Pomace is a rich source of dietary fibre, phytochemicals, polysaccharides, phenolic compounds, and natural antioxidants. These components contribute to improved metabolic and cellular functions in human health through their antioxidant, anti-inflammatory, anti-allergenic, and anticarcinogenic properties (Iqbal *et al.,* 2021).

In addition to peels, seeds, and pomace, fruit and vegetable wastes also include rinds, husks, stems, cores, stalks, pits, unharvested parts, and spoiled and overripe materials (Sarker *et al.,* 2024). They are rich in bioactive components that are important for nanoparticle synthesis and are a key resource for green nanotechnology.

4. Biosynthesis of Metal Nanoparticles from Fruit and Vegetable Waste Extract

4.1 Preparation of Extract

The process begins with preparing fruit and vegetable waste extract. Waste materials are collected and washed to remove impurities. It is then dried and ground into powder. Powdered material is later boiled or soaked in water or another solvent to obtain the extract. This step promotes the extraction of bioactive constituents, namely flavonoids, alkaloids, phenolic compounds, terpenoids from waste materials into the solution, which acts as reducing, capping, stabilizing agent in nanoparticle synthesis (Deka *et al.,* 2025; Hanna *et al.,* 2025).

4.2 Mixing with a Metal Salt Solution

Prepared extract is later added to metal salt solution. It enables reduction of metal ions and stabilization of the synthesized metal NPs. Several factors, namely pH, reaction duration, temperature, plant extract-to-metal salt ratio, significantly impact this stage. These factors are important for defining shape, size, overall characteristics of NPs produced along with reaction kinetics (Chen *et al.,* 2024; Hanna *et al.,* 2025).

4.3 Reduction and Nucleation

The metal ions are then reduced into neutral atoms by phytochemicals present in the extract. These atoms are then aggregated together to form stable clusters. These clusters are utilized for producing final NPs. Size and quantity of these initial clusters largely evaluate how final particles develop (Deka *et al.,* 2025; Hanna *et al.,* 2025).

4.4 Growth of Nanoparticles

As soon as the tiny seeds are created, metal atoms begin to build up on them. This is known as the growth period. Depending on several variables (including temperature, pH, type of biomolecules), the nanoparticles can form spheres, triangles, rods, other shapes. Furthermore, many plant compounds act as shape-directing agents, guiding the structure of particles as they expand (Gong *et al.,* 2024; Hanna *et al.,* 2025).

4.5 Stabilization of Nanoparticles

NPs tend to agglomerate after synthesis due to their high surface energy. Therefore, in order to maintain their size and avoid agglomeration, stabilization is important. Fruits and vegetable extracts contain phytochemicals that function as natural capping agents in green synthesis. Flavonoids, polyphenols, proteins, and organic acids are among the substances that adsorb onto nanoparticle surfaces to form a protective layer (Azad *et al.,* 2023; Hanna *et al.,* 2025).

Some of the metal nanoparticles synthesized from different fruit and vegetable wastes extract, and their application are listed below (Table 1):

Table 1: Fruits and Vegetable Waste Extract-Derived Nanoparticles and Their Application

Vegetable/Fruit waste	Part	Nanoparticle	Application	Reference
Abelmoschus esculentus	Seed	Au	Antifungal	Jayaseelan *et al.,* 2013
Actinidia deliciosa	Peel	Ti	Antibacterial, Antioxidant	Ajmal *et al.,* 2019
Alpinia katsumadai	Seed	Ag	Antioxidant, Antibacterial, Anti-cancer	He *et al.,* 2017
Banana	Pulp	Ag	Antibacterial Bioelectricity generation	Ohiduzzaman *et al.,* 2024
Carica papaya	Peel	Co_3O_4	Photocatalytic activity	Sharma *et al.,* 2026
Lagenaria siceraria	Peel	Ag	Antibacterial	Deepa *et al.,* 2022
Orange	Peel	ZnO	Antibacterial	Thi *et al.,* 2020
Pistachia atlantica	Seed	Ag	Antibacterial	Sadhegi *et al.,* 2015
Pisum sativum	Peel	Ag	Antibacterial	Deepa *et al.,* 2022
Vitas vinifera	Pomace	Ag	Anti-diabeticAntioxidant Antimicrobial	Saratale *et al.,* 2021

5. Characterization of Biosynthesized nanoparticles

Synthesized NPs are then characterized using FTIR, XRD, UV-Vis spectroscopy, SEM, and TEM to assess their functional groups, crystalline structure, optical properties, and morphology. Particle size distribution, surface charge, elemental composition, and thermal stability are also determined using additional procedures, involving “Dynamic Light Scattering (DLS)”, zeta potential analysis, “Atomic Force Microscopy (AFM)”, “Energy-Dispersive X-ray Spectroscopy (EDX)”, and “Thermogravimetric Analysis (TGA)”. These characteristics significantly influence nanoparticle performance and enable their utilization in agriculture, medicine, food technology, energy storage, and environmental remediation.

6. Application of Biosynthesized Nanoparticles

As a result of their small size, high “surface area-to-volume ratio ((SA/V)”, improved stability, biocompatibility, fruit and vegetable waste-synthesised NPs are appropriate for various applications. They have demonstrated significant potential in biological sectors, namely cancer treatments, drug-delivery systems, and antibacterial agents. In environmental research, these NPs are important for wastewater treatment, heavy metal removal, air purification. Additionally, they help boost plant growth in agriculture, control insects, and improve soil health. Additionally, they are utilised in food industry for food preservation, antimicrobial packaging, and vitamin fortification. These NPs also promote photocatalysis for energy conversion in the energy sector, function as green catalysts, and help produce biofuel (Gupta *et al.,* 2025).

7. Current Challenges and Future Opportunities of Fruit and Vegetable Waste as Nanofactories

7.1 Current Challenges

- The primary concern is that waste varies in composition. Fruits and vegetables contain various phytochemicals, depending on plant species, maturity, and environmental and cultivation conditions. These factors can alter their biochemical profile, thereby affecting efficiency and consistency of NPs synthesis (Piras *et al.,* 2019).
- There are often no clear, standardized techniques for the green synthesis of NPs from fruit and vegetable waste. Green synthesis of NPs is strongly influenced by several factors, namely pH, reaction, metal ion concentration, temperature, solvent type, extract composition. These variations may result in differences in size, shape, stability, yield of NPs. As a result, achieving consistent, reproducible results becomes difficult, limiting the large-scale application and industrial adoption of waste-based nanoparticle synthesis methods.
- Nanoparticles synthesized from biological sources are considered safe and environmentally friendly, but they can still pose potential toxicity when used in pharmaceutical, food, and agricultural fields. Environmental risks could arise when nanoparticles interact with the ecosystem. Extensive toxicity research and environmental effect assessments are essential to guarantee its safe and sustainable use (Patil & Chandrasekaran, 2020).
- Fruit and vegetable waste decomposes readily through microbial degradation. If extracts are improperly stored or kept for too long, their chemical composition can change, affecting their stability during nanoparticle formation.

7.2 Future Opportunities

- Reducing organic waste and pollutants from conventional disposal methods like landfilling and incineration can be achieved sustainably and in an environmentally friendly way by converting fruit and vegetable waste into nanoparticles.
- Biologically synthesized nanoparticles exhibit strong antimicrobial, antioxidant, and catalytic activities, offering a promising strategy for applications in drug delivery, food preservation, agricultural improvement, and environmental remediation (Hemmati *et al.,* 2020).
- Bioactive components present in fruit and vegetable wastes could serve as natural reducing as well as stabilizing agents through the synthesis of NPs. Therefore, it does not

require a hazardous chemical as a reducing agent, which makes the process cost-effective and environmentally benign.

- Fruit and vegetable waste valorization is consistent with circular economy principles, as waste materials are converted into value-added nanomaterials for various scientific and industrial applications, which improves resource efficiency and sustainability.
- Strengthening collaboration among academia, industry, and policymakers may accelerate the development and commercialization of nanoparticle products from fruit and vegetable-derived waste (Bhadauriya *et al.,* 2018).
- Advanced imaging and spectroscopic techniques, such AFM and X-ray diffraction, improve understanding of structure and function of biogenic nanoparticles and increase their potential application (Kumar *et al.,* 2018).

Despite several scientific and practical challenges, converting fruit and vegetable waste into NPs is promising and sustainable approach in nanotechnology. It is important to address issues of standardization, scalability, and safety to translate laboratory research into commercial applications and promote economic growth as well as environmental sustainability (Gupta *et al.,* 2025).

Conclusion

Waste from fruits and vegetables is a sustainable and significant resource for producing green nanoparticles. Bioactive substances, including alkaloids, flavonoids, polyphenols, and organic acids, serve as natural reducing, capping, and stabilizing agents, enabling the green production of NPs and avoiding the need for hazardous chemicals. This method promotes both biocompatibility and environmental safety. They have numerous uses in energy storage, food safety and preservation, the biomedical and pharmaceutical industries, sustainable agriculture, and environmental remediation technologies. While there are challenges related to standardization, scalability, and variability, this strategy provides a cost-effective, eco-friendly alternative to conventional approaches. Valorization of fruit and vegetable waste into NPs demonstrates significant potential for development of green nanotechnology and the promotion of a waste-to-resource strategy.

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RECENT PROGRESS IN CHEMICAL, BIOLOGICAL, AND PHARMACEUTICAL SCIENCE: A COMPREHENSIVE REVIEW

Anant V. Kardel
MVP Samaj's K K W Arts, Science and Commerce College,
Pimpalgaon (B.), Maharashtra, India 422209
Affiliated to Savitribai Phule Pune University, Pune (MH) – 411007
Corresponding author E-mail: anantkardel11@gmail.com

Abstract

The years 2025–2026 have seen revolutionary developments in the chemical, biological, and pharmaceutical sciences, marked by the convergence of quantum biology, artificial intelligence, and cutting-edge materials science. Recent developments in three related fields are summarized in this overview. Significant methodological and material developments in the chemical sciences include the development of solvent-free Nano fluids, uncertainty quantification for computational chemistry, and machine learning-driven molecular dynamics. The first successful engineering of quantum-enabled proteins, multiscale aggregation materials for therapy and nanoparticle-based vaccine platforms that allow customized immune regulation have all transformed biological sciences. The development of targeted protein degradation, the growth of covalent drug approvals, and notable advancements in weight management therapies and rare disease treatments are just a few examples of the exceptional advancements in pharmaceutical sciences therapeutic modalities. While acknowledging enduring issues like drug-induced hepatotoxicity and the consequences of research funding limitations, this review also looks at the changing landscape of drug discovery infrastructure, including the creation of transdisciplinary centers addressing region-specific healthcare challenges. When taken as a whole, these developments highlight a paradigm shift toward integration in which conventional disciplinary barriers vanish in favour of convergent approaches that use biological understanding, manufactured materials, and computational tools to address urgent healthcare needs.

Introduction

The sciences of chemistry, biology, and pharmacology are at a spectacular turning point. Convergent approaches that use the resources of each discipline to tackle difficult scientific and medical problems have emerged as a result of the conventional barriers dividing different fields becoming more and more porous. The development of "translational chemical biology," a framework that blends chemical principles with biological research to create verified probes and models that increase both fundamental understanding and clinical application, may be the most obvious example of this integration [4].

For a number of reasons, the time frame examined here (2025–2026) is especially significant. First, advancements in molecular dynamics modelling, chemical design, and uncertainty quantification that were unthinkable ten years ago have been made possible by the transformation of artificial intelligence and machine learning from supplementary tools to essential pillars of research technique [1]. Second, the successful engineering of quantum mechanical features in biological systems is a major advance that creates new opportunities for biomedical imaging and biotechnology. Third, therapeutic methods such as PROTACs and macrocyclic peptides have matured in the pharmaceutical industry, expanding the druggable target space beyond conventional small molecules [8].

The goal of this review is to present a thorough summary of current developments in these related fields. We analyse chemical, biological, and pharmacological advancements as parts of a completely scientific ecosystem rather than as discrete innovations. Innovations in chemical science methodology, such as new materials and computational developments [1], are examined in Section 2. Biological innovations, including quantum-enabled proteins, aggregation materials, and vaccination platforms, are examined in Section 3. The pharmaceutical industry is surveyed in Section 4, with particular attention paid to therapeutic modalities, approval trends, and new issues. The changing institutional and geographical context of drug discovery is examined in Section 5. Section 6 concludes by considering ongoing issues and potential paths forward.

2. Advances in Chemical Sciences

2.1 Computational Chemistry and Artificial intelligence

Recent reviews have shown how data-driven methods are revolutionizing our comprehension of complicated chemical processes, and the integration of machine learning with molecular simulation has accelerated significantly. Neural network potentials can attain quantum-level accuracy with classical computing efficiency [1], according to a thorough review published in Chemical Reviews that explores machine learning-driven developments in molecular dynamics of bulk and interfacial aqueous systems. These developments are especially important for pharmaceutical applications, since drug formulation and distribution are directly impacted by knowledge of solvation dynamics and interfacial phenomena.

The concurrent development of uncertainty quantification techniques for computational chemistry is equally significant. Tom Frömbgen and colleagues point out that our capacity to measure and convey uncertainty is crucial to the accuracy of in silico predictions; this problem is especially severe when computational results influence experimental design or regulatory choices. Computational chemistry has evolved from an exploratory tool to a prediction science with the creation of rigorous uncertainty models [1].

The concurrent development of uncertainty quantification techniques for computational chemistry is equally significant. Tom Frömbgen and colleagues point out that our capacity to quantify and explain uncertainty is crucial to the accuracy of in silico predictions. This difficulty is especially obvious when computational results influence experimental design or regulatory choices. Computational chemistry has evolved from an exploratory tool to a prediction science with the creation of rigorous uncertainty models.

Additionally, there has been significant advancement in the use of artificial intelligence in molecular design. The development of small molecule generation is surveyed in a recent study on molecular design with artificial intelligence, which emphasizes how generative models, reinforcement learning, and active learning are being integrated to explore chemical space more effectively than conventional high-throughput screening. These techniques are becoming more and more capable of integrating target-specific restrictions, ADME features, and synthetic accessibility into the design process [1].

The development of general-purpose models for the chemical sciences is perhaps the most notable. Large language models and foundation models are being modified for chemical applications, enabling tasks like literature synthesis and reaction prediction, according to a review published in February 2026. These models promise to speed up discovery cycles and democratize chemical knowledge because they were trained on enormous corpora of experimental data and chemical literature [1].

2.2 New Materials: Nano fluids without Solvents

The creation of solvent-free nanofluids (SFNs), which are thoroughly reviewed in the *Chemical Engineering Journal* [6], is one of the most important developments in materials. These new materials reflect a fundamental rethinking of nanoparticle formulations: SFNs attain intrinsic fluidity by surface engineering instead of dispersing nanoparticles in traditional solvents. By grafting thick organic ligands or polymer shells onto nanoparticle cores, the ongoing problems of volatility, sedimentation, and agglomeration that restrict traditional Nano fluids are resolved without the need for external solvents.

SFNs have a core-corona-canopy structural architecture. A corona of grafted molecules encircling the nanoparticle core, which could be metallic, inorganic non-metallic, polymeric, or composite ends in a canopy layer that offers fluidity [6]. Through logical ligand design, this architecture allows for exact control of functional features, interfacial compatibility, and rheological properties.

SFNs have been expanded to intelligent, responsive systems by recent developments. SFNs that respond to both electric and magnetic fields have been created and shown to be useful in gas adsorption, lubrication, composite engineering, and energy storage. Applications in precise lubrication and adaptive materials are made possible by the capacity to alter fluid characteristics in response to external stimuli.

SFNs have very intriguing uses in biomedicine. They are appealing for drug delivery systems that can adjust to physiological conditions because of their adjustable rheology and the functional characteristics of nanoparticle cores [6]. Concerns regarding solvent toxicity and volatility are eliminated by the solvent-free nature, and site-specific distribution is made possible by the direct incorporation of targeted ligands into the canopy layer.

2.3 Confinement Effects and Mechanochemistry

Through the lens of confinement effects, the role of mechanical force in chemical reactions has been re-examined. A recent review published in Chemical Reviews examines mechanochemistry triggered by molecular distortion caused by shear and confinement. This work demonstrates how reaction routes can be drastically changed by nanoscale confinement, allowing changes that are not conceivable in bulk solution. There are significant ramifications for the production of pharmaceuticals, especially when it comes to the synthesis of complicated compounds that need exact stereo chemical control [1].

3 Advances in Biology

3.1 Engineering Biology at the Quantum Frontier: Quantum-Enabled Proteins

In a historic breakthrough, scientists at the University of Oxford have successfully engineered quantum mechanical activity inside proteins for the first time, producing magneto-sensitive fluorescent proteins (MFPs) that can interact with radio waves and magnetic fields. This work, which was published in Nature, marks a paradigm change from studying quantum phenomena in the natural world to purposefully creating them for useful uses [2].

To create proteins with significantly increased magnetic sensitivity, the study team used directed evolution, a bioengineering process that involves repeated rounds of random mutation and selection. The scientists began with proteins found in common oats and used multiple cycles of screening and mutation to produce variations that, when exposed to the right light wavelengths, display strong quantum mechanical behaviour [2].

This discovery has implications that go beyond basic science. The group has already shown off a prototype-imaging device that uses a method similar to magnetic resonance imaging to find these modified proteins. However, in contrast to traditional MRI, this method allows molecule-specific imaging in living things, tracking particular gene expression or keeping an eye on molecular alterations within cancers. Assessment of focused drug administration and real-time tracking of genetic alterations in malignant tissues are examples of potential uses [2].

This accomplishment is notable for its interdisciplinary nature. The project requires knowledge of artificial intelligence, quantum science, and engineering biology—three fields that are becoming more and more acknowledged as essential to innovation. According to the first author, Gabriel Abrahams, "What blows me away is the power of evolution: we don't yet know how to design a really good biological quantum sensor from scratch, but by carefully steering the evolutionary process in bacteria, Nature found a way for us" [2].

3.2 Materials for Multiscale Aggregation in Therapy and Bioanalysis

A potent tactic for biomedical applications is the creation of aggregation materials that span several length scales. Recent developments in using aggregation materials for drug delivery, bioanalysis, and antimicrobial therapy are summarized in a thorough review published in Chemical Communications [5].

The multiscale approach acknowledges that biological activity manifests at various length scales, ranging from macroscopic tissue structure to molecular interactions. As a result, aggregation materials are designed at three different scales: mesoscale biological aggregation interfaces, nanoscale assemblies created by chemical synthesis or microfluidics, and small molecular-scale aggregates. The logical design of materials that can target certain tissues, overcome biological obstacles, and carry out intricate therapeutic tasks is made possible by this hierarchical approach [5].

Antimicrobial applications, where aggregation materials provide modes of action different from traditional small-molecule antibiotics, have received special attention. Enhanced binding to bacterial surfaces and interruption of biofilm formation are made possible by the capacity to generate multivalent contacts through controlled aggregation, which may make resistance development less likely [5].

3.3 Vaccines Enhanced by Nanomaterials

Promising developments in tailored immunization have resulted from the use of nanomaterials in vaccine design. By carefully presenting immunological targets, Michelle Teplensky and her colleagues at Boston University have created nanomaterial-based vaccine systems that improve both potency and customisation. This discovery, which was published in the Proceedings of the National Academy of Sciences, shows how nanomaterial design can adjust the immune response by varying the rate at which vaccine components are released within cells [7].

Rapid optimization of vaccination formulations for particular populations is made possible by the easier manipulation of nanomaterials compared to biological therapies. According to Teplensky, "We can manipulate nanomaterials a lot easier and quicker than manipulating biological therapeutics themselves but ultimately have the same effect on changing the potency of the immune response". This strategy is especially promising for customizing vaccinations to immunological status, age, and sex, including immunocompromised people who could react adversely to traditional formulations [7].

The Biomedical Engineering Society's Cellular and Molecular Bioengineering Rising Star Award for this work highlights the increasing significance of nanotechnology in tackling healthcare issues [7].

4 Innovations in Pharmaceuticals

4.1 Therapeutic Approaches: Increasing the Druggable Area

A notable diversity of therapeutic modalities can be seen in the pharmaceutical scene in 2025–2026. The range of targets that can be addressed has significantly increased due to the development of various new techniques, even though small molecules continue to be the most popular class of drugs [8].

4.1.1 Degradation of Targeted Proteins

With the first approval expected in 2026, PROTACs (proteolysis-targeting chimeras) and related degraders have become a significant medicinal class. Nearly 25 years after the ground-breaking PROTAC papers from the Crews and Deshaies laboratories, the vepdegestrant NDA submission represents a significant milestone. Instead of only blocking disease-causing proteins, these heterobifunctional compounds use the cell's ubiquitin-proteasome system to break them down [8].

Especially noteworthy is the growth beyond the original E3 ligases CRBN and VHL. Targets that were previously thought to be resistant to degradation may now be addressed thanks to FBXO22 (F-box Only 22), a promising substrate adapter for targeted degradation. Additionally, E3 ligases' tissue-specific expression creates opportunities for cell-type-specific degradation tactics.

4.1.2 Covalent Substances

A record number of covalent medications were approved by the FDA in 2025. Six of the thirty small molecule approvals were covalent, setting a new record for this class of drugs. Interestingly, just three of these were oncology medications; the remaining ones dealt with immunology, haematological, and pulmonary disease, indicating the growing therapeutic range of covalent methods [8].

The first cyan acrylamide cysteine-targeting warhead to be approved by the FDA is rilzabrutinib, which is approved for immune-mediated diseases. The approval of improved covalent compounds against recognized targets indicates that optimization beyond first in class can result in significant clinical benefits, even though the targets themselves, EGFR and BTK, are not new [8].

4.1.3 Peptide and Macrocyclic Medicines

Notable clinical results have been achieved with macrocycles. Through a macrocyclic structure, daraxonrasib, a tri-complex molecular glue pan-RAS inhibitor, binds RAS to the chaperone protein cyclophilin A. A target class RAS that has traditionally been thought to be undruggable is addressed by this unique mechanism of action. Similar to this, icotorkinra, an oral macrocyclic peptide that binds IL-23 has shown promising clinical outcomes in ulcerative colitis and plaque psoriasis, making it a possible rival to monoclonal antibody treatments [8].

When paired with permeation enhancers, enlicitide decanoate, a macrocyclic peptide PCSK9 inhibitor, shows promise for oral peptide administration despite its low bioavailability (about 2%). This implies that for some applications, exceptional potency can overcome absorption constraints [8].

4.2 Metabolic Disease and Weight Management:

With the release of Novo Nordisk's first licensed oral GLP-1 receptor agonist, the field of obesity treatment has experienced tremendous activity. This development demonstrates fierce competition in a therapeutic area with significant unmet need, as do late-stage assets from Lilly, Structure Therapeutics, Pfizer/YaoPharma, and Zealand/OTR [3].

Particularly promising are small chemical GLP-1 receptor agonists. Phase 3 success was attained by Lilly's orforglipron, a once-daily oral formulation with efficacy on par with peptide-based medications. However, there have been some difficulties in the field. Similar to previous problems with lotiglipron, hepatotoxicity concerns led to the discontinuation of Pfizer's danuglipron. Despite growing knowledge of structural signals, drug-induced liver damage (DILI) continues to be a problem, highlighting the difficulty of forecasting safety outcomes [8].

4.3 Orphan Drugs and Rare Diseases

The ongoing predominance of orphan medications is a notable aspect of 2025 drug approvals. Twenty-two (56%) of the 39 newly authorized novel medications were designated as orphan pharmaceuticals. This demonstrates sponsors' willingness to take on financial risk for mechanisms with small commercial markets as well as the scientific potential in uncommon diseases [8].

Notable approvals include cell treatment from Capricor Therapeutics, gene therapies from Fondazione Telethon and Novartis, and favourable clinical trial results for Kyverna Therapeutics, Encoded Therapeutics, and Kite. Despite more general industry difficulties, the concentration of approvals in rare diseases, which frequently represent first-in-class processes, shows how crucial the innovation pipeline is [3].

4.4 Developments in Gene and Cell Therapy

Numerous product approvals and encouraging clinical results demonstrated the potential of cell and gene therapy for uncommon disorders. Gene therapies for uncommon genetic illnesses were approved by Novartis and Fondazione Telethon, favourable trial results were published by Capricor Therapeutics, Kyverna Therapeutics, Encoded Therapeutics, and Kite [3].

The development of advanced therapy manufacturing platforms and regulatory processes is reflected in these approvals. The high cost of commodities, the complexity of manufacturing, and the requirement for specialized administrative infrastructure are still obstacles.

4.5 Innovative Delivery Methods

There has been substantial innovation in therapeutic delivery on several fronts. A calcium channel blocker nasal spray formulation for paroxysmal supraventricular tachycardia (PSVT) was approved by Milestone Pharmaceuticals, providing a quick, non-invasive substitute for intravenous delivery. Clinical trials for a doxorubicin microneedle array patch for basal skin cancer were advanced by Medicus Pharma, allowing for localized chemotherapy delivery with less systemic exposure [3].

The aforementioned therapeutic developments are complemented by these delivery technology advancements, which may enhance patient convenience, adherence, and safety.

5. Changing Drug Discovery Infrastructure

5.1 Transdisciplinary Research and Centers of Excellence

The University of Kentucky's Center of Biomedical Research Excellence in Pharmaceutical Research and Innovation (CPRI), which focuses on "translational chemical biology" the intersection of chemical biology and pharmaceutical science, is an example of this trend. The realization that drug discovery necessitates integrated approaches has led to the creation of centers specifically intended to bridge disciplinary boundaries [4].

In order to assist junior academics in three therapeutic areas cancer, infectious illness, and cardiovascular disease CPRI's organizational structure consists of administrative and research support cores (Translational Core, Computational Core, and Organic Synthesis Core). The specific objective of closing the "valley of death" that frequently obstructs translation is to close the gap between fundamental academic research and commercial/clinical use [4].

5.2 Regional Projects: The Center for Therapeutic Innovation in Africa

Infrastructure for locally relevant research is necessary to address healthcare issues unique to a certain location. Launched at Stellenbosch University in October 2025, the Africa Centre for

Therapeutics Innovation (ACTI) closes a significant gap in African drug discovery capacity. The epidemiology of the area is reflected in the center's emphasis on disease biology and mechanistic knowledge of therapeutic action, particularly for malaria and tuberculosis [9].

Since significant health innovation necessitates integration across knowledge disciplines, ACTI's framework places a strong emphasis on transdisciplinary collaboration spanning chemistry, biology, and pharmacology. "To translate drug discovery research to health innovations requires a multidisciplinary approach, with inputs from stakeholders in various knowledge areas," according to co-director Prof. Erick Strauss. Building sustainable capacity is shown in the center's collaborations with networks like GC ADDA (Global Challenges Accelerating African Drug Discovery Solutions) and RAFIKI Project [9].

5.3 Industry-Academic Cooperation

The 2025 regulatory approvals are the result of decades of consistent funding for fundamental research. For instance, the creation of PROTACs may be traced back to scholarly findings from about 25 years ago [8]. In a similar vein, scientists who had spent "decades studying how birds navigate using the Earth's magnetic field" were needed for the quantum-enabled protein discovery [2].

This longer time from discovery to implementation emphasizes how crucial it is to continue funding fundamental research. Concerns over the long-term innovation pipeline have been highlighted by recent reductions in government research funding in various jurisdictions. According to one analyst, "The recent short-sighted funding cuts to the NIH and NSF will undoubtedly lead to fewer medicines in the future [8]."

6. Difficulties and Prospects

6.1 Enduring Difficulties in Drug Development

Even with the progress discussed here, there are still many obstacles to overcome. Success rates for new medications are still persistently low, and the time and money needed to bring them to market keeps rising. In 2025, the job sector in pharmaceutical research saw hitherto unheard-of difficulties, which were a reflection of a larger industry reorganization [8].

ATR inhibitor gartisertib, apelin (APJ) receptor agonist azelaprag, and IL-17 inhibitor LY3509754 are only a few of the high-profile drug-induced liver damage (DILI) discontinuations. The persistence of hepatotoxicity in spite of advanced screening techniques points to serious flaws in our capacity to forecast human safety consequences [8].

6.2 Artificial Intelligence's Function

One commentator's query, "Will AI save us?" [8], encapsulates the potential and unpredictability of artificial intelligence in drug discovery. It is unclear how much AI will help with basic issues in safety prediction, clinical translation, and production, even if machine learning has clearly sped up several areas of molecular design and simulation.

The development of general-purpose models for the chemical sciences [1] indicates that AI will play a bigger part in the discovery process rather than only in specific applications. However, ongoing investment in data infrastructure, model development, and validation techniques will be necessary to fully realize these approaches' promise.

6.3 Prospects for Further Research

This review reveals a number of promising directions. The ongoing development of intelligent responsive materials and solvent-free Nano fluids in the chemical sciences has prospects for biomedical applications, including bio sensing [6] and medication delivery. In fields where experimental characterisation is still difficult, the combination of machine learning and molecular modelling will probably speed up discovery [1].

Quantum-enabled proteins mark a new frontier in the biological sciences at the nexus of quantum physics and biology [2]. Novel imaging modalities, sensors, and therapeutic interventions are made possible by the capacity to engineer quantum mechanical features into proteins. Nanomaterial vaccination platforms and multiscale aggregation materials provide avenues for personalized treatment strategies that can be customized to each patient's unique traits [5], [7].

The drug gable target space in pharmaceutical sciences will continue to grow as treatment modalities such as PROTACs, macrocycles, and covalent medicines are developed [8]. Diseases that were previously thought to be incurable may be treated by combining these methods with cutting-edge delivery systems and patient classification techniques.

Conclusion

Chemical, biological, and pharmaceutical sciences have advanced remarkably in 2025–2026, marked by the development of disruptive technologies and the convergence of disciplines. The creation of transdisciplinary research centers, the expansion of therapeutic modalities beyond conventional small molecules, the engineering of quantum properties into proteins, and the development of solvent-free Nano fluids all indicate a future in which conventional disciplinary boundaries are becoming more permeable.

This synthesis reveals a number of motifs. First, the integration of machine learning and artificial intelligence across all fields is facilitating previously unattainable methods and speeding up discoveries. Second, it takes decades of consistent investment to translate basic research into clinical application, which has an impact on research financing policy. Third, as demonstrated by the creation of ACTI in South Africa, solving global health issues calls for infrastructure that is suited for the region.

The field faces significant but manageable obstacles, such as the ongoing difficulty in predicting safety, the growing expense of development, and the consequences of research funding limitations. The innovations discussed here give hope that the scientific community will continue to create solutions that enhance human health.

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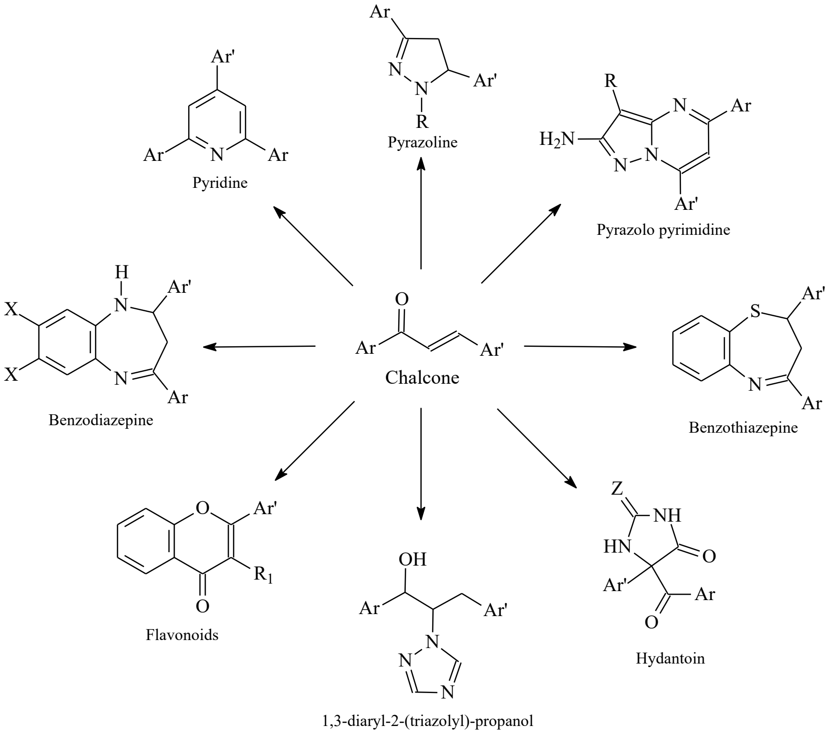
SHORT REVIEW ON SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME NEW HETEROCYCLIC COMPOUND

Shaikh Abdul Baseer*[1] and Mohammad Zameer Ahmed[2]
[1]Department of Chemistry,
Sir Sayyed College of Arts, Commerce and Science, Aurangabad CSN, M.S., India
[2]Organic Chemistry Research Laboratory,
Poona College of Arts, Science and Commerce Pune Maharashtra, India
*Corresponding author E-mail: basirssca@gmail.com

Several heterocyclic compounds bearing Nitrogen, Sulphur and Oxygen has attracted considerable attention from organic and medicinal chemists due to their considerable bioactivity. The heterocyclic compounds like, Pyrazoles, Pyrazolines, Pyridines, Pyrimidines, Pyrazolo pyrimidines and Benzodiazepines are shows broad spectrum of biological activities. The thiazole ring has been extensively studied and it forms a part of vitamin-B, Penicillin. The flavonoids have been reported to posses wide range of biological activities such antioxidant, anticancer, antihypertensive and enzyme inhibitory activities. Chalcones constitute an important group of natural products and have been reported to possess varied biological and pharmacological activities. Chalcone represent branch point intermediates in the bio-synthesis of various classes of flavonoids. Chalcones are also used as starting materials in the synthesis of many heterocyclic compounds. These wide biological applications of heterocyclic compound have promoted us to work on synthesis of new heterocycles. In this proposed work, new series of substituted heterocyclic compounds will be prepared from the reaction chalcones with different nitrogen nucleophiles in environmentally benign reaction media to accomplish the desired chemical transformation. The most common synthetic approach towards the synthesis of heterocyclic compounds involves the ring-closure reaction of chalcones with various cyclizing reagents (Scheme-1). The starting chalcones were synthesized by the Claisen-Schmidt condensation method. Furthermore, these compounds are treated with different cyclizing reagentsThere has been significant increase in the frequency of systematic fungal infection in humans. Patients undergoing organ transplants, anticancer chemotherapy, or long treatment with antimicrobial agents, and patients with AIDS, are immune-suppressed and very susceptible to life-threatening systematic fungal infections like *Candidiasis, Cryptococcosis*, and *Aspergillosis*. Resistance to antibacterial agents is a significant problem since last few decades. In addition to this, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immunocompromised patients (AIDS, cancer and transplants). Several reviews have appeared illustrating the problems encountered by today's infectious disease clinicians. To overcome this rapid development of drug resistance, new agents should preferably consist of chemical characteristics that clearly differ from those of existing agents. In the process of drug designing an essential component of the search for new leads is the synthesis of novel molecules, which are biologically active by the virtue of the presence of critical structural features. These wide biological applications of heterocyclic compound have promoted us to work on synthesis of new heterocycles. In this proposed work, new series of substituted heterocyclic compounds will be prepared from the reaction chalcones with different nitrogen nucleophiles in environmentally benign reaction media to accomplish the desired chemical transformation



Ar = Substituted aryl; Ar' = Heteroaryl; R = Sub.aryl; R1 = H, Br, Cl, OH; X = Br; Z = O, S;

Solvents are widely used in organic synthesis and have been a cause of major concern due to their associated environmental hazards. The major disadvantages are their pyrophoric nature, volatility, and poor recovery. To address some of these issues, attempts have been made to develop solvent-free chemistry, which to some extent has been successful for a few transformations. However, in performing the majority of organic transformations; solvents play a critical role in making the reaction homogeneous and allowing molecular interactions to be more efficient. To address the concerns raised by volatile organic solvents, we initiated a new to identify whether any available liquid polymers or low melting polymers can be used as solvents. Recently, polyethylene glycol (PEG) has been found to be an interesting green solvent system. The use of PEG as an environmentally benign protocol has proved to have many applications particularly, in substitution, oxidation and reduction reactions. A number of recent reviews have also covered PEG chemistry and its applications in biotechnology and medicine.

Electron-rich nitrogen heterocycles play an important role in diverse biological activities. Due to the emergence of new fungal pathogens, resistant to fluconazole, great efforts have been made to modify the chemical structure of fluconazole, in order to broaden its antifungal activity and increase its potency. As a part of our research programme chalcones are used as precursors in the synthesis of various biologically active molecules. Herein, I would like to synthesis some new heterocyclic compounds using chalcones as snythons. The synthesis of these derivatives can be achieved under the frame work of green chemistry. In addition to this, the organic compound containing pyrazole nucleus has wide applications in medicinal chemistry as well as considerable interest in the chemotherapeutic activity. Pyrazole and its synthetic analogues have been found to exhibit industrial, agricultural and some biological application. The ring system plays an important role in many biological processes, and many therapeutic agents contain pyrazole moiety. For example some alkyl, aryl substituted pyrazoles have pronounced sedative action on the central nervous system. Certain alkyl pyazoles also showed significant analgesic, antipyretic, bacteriostatic, bactericidal and fungicidal activities. Chalcones (1, 3-diaryl-2-propene-1-ones) are of a high interest due to their use as starting materials in the synthesis of a series of heterocyclic compounds. One of the key areas of green chemistry is the replacement of the hazardous solvent as with environmentally benign solvents like polyethylene glycol (PEG-400). In resent years polyethylene glycol (PEG-400) attracted the much more attention recently, liquid polymers or low melting polymers have emerged as alternative green reaction media with unique properties such as thermal stability, commercial availability, non-volatility, immiscibility with a number of organic solvents and recyclability. PEGs are preferred over other polymers because they are inexpensive, completely non-halogenated, easily degradable and of low toxicity Many organic reactions have been carried out using PEGs as solvent or co-solvent such as Heck reaction,asymmetric dihydroxylation,Suzuki cross-coupling reaction,oxydehydrogenation of alcohols and cyclic dienes, oxidation of sulfides and the Wacker reaction and partial reduction reaction of alkynes The use of PEG as a recyclable solvent system for the metal mediated radical polymerization of methyl methacrylate and styrene has also been reported the conventional condensation method of chalcones with *o*-phenylenediamine using few drops of piperidine PEG-400 as reaction solvent at mild reaction condition. This method has following merits.

1. IReaction is efficient and facile
2. It gives excellent yields of products.
3. Work up procedure and isolation is easier.
4. Procedure is green and environmentally benign methodology.
5. Shorter reaction time.

In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased Number of immuno compromised patients (AIDS, cancer and transplants). Various animals' experiments have been designed to study the effect of drug on living organism and isolated tissue. These gave an insight into where and how a drug acts. By knowledge of mode of action of a drug, its effect on various body systems and probable adverse/side effect are important. Thus the search for new drugs with improved antimicrobial activity and reduced toxicity is a continuous process. This was observed that the presence of phenolic (-OH) groups in 2 position which enhanced antibacterial and antifungal activity. Schiff bases, chalcones, pyrazolo [1, 5-a] pyrimidines, N-substituted pyrazolines, Isoxazolines, and benzodiazepines were exhibited stronger antifungal activity against more pathogens. The substitution of hydroxyl group in position 2 and presence of halo groups in 3 and 5 positions emerged as active in both antibacterial and antifungal screening.

When structure and activity relationships are investigated, we can infer from the results that R_2 substitution seem to be effective on antibacterial and antifungal activity. Furthermore, it can be concluded that compounds bearing with methyl group at R_3 position in combination with Br, I and Cl groups, then both groups enhanced the activity.

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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF 5-(4-NITROPHENYL)-1,2,4-TRIAZOLIDINE-3-ONE (4-NPTZ)

Vikas D. Jadhav
Department of Chemistry,
MVP Samaj's Arts, Science and Commerce College, Ozar (Mig),
Tal. Niphad, Dist. Nashik, Maharashtra, India – 422206
Corresponding author E-mail: vikasjadhav968@gmail.com

Abstract

A simple, rapid, and stability-indicating UV spectrophotometric method was developed and validated for the quantitative determination of 5-(4-nitrophenyl)-1,2,4-triazolidine-3-one (4-NPTZ). The analysis was performed using methanol as a solvent, and the maximum absorbance (λmax) was observed at 331 nm. The method exhibited excellent linearity over the concentration range of 10–100 µg/mL with a correlation coefficient (r^2) of 0.9994. Validation was carried out in accordance with ICH guidelines, including parameters such as accuracy, precision, robustness, ruggedness, limit of detection (LOD), and limit of quantification (LOQ). The recovery values ranged from 98.64% to 99.47%, confirming the method's accuracy. The %RSD values for precision studies were within acceptable limits, indicating good reproducibility. The LOD and LOQ were found to be 0.3211 µg/mL and 0.9731 µg/mL, respectively. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions demonstrated that the compound is susceptible to acid and base degradation, while stable under other conditions. The developed method was found to be reliable, economical, and suitable for routine analysis and stability studies of 4-NPTZ.

Keywords: 4-NPTZ; UV Spectrophotometry; Method Validation; ICH Guidelines; Forced Degradation; Stability-Indicating Method.

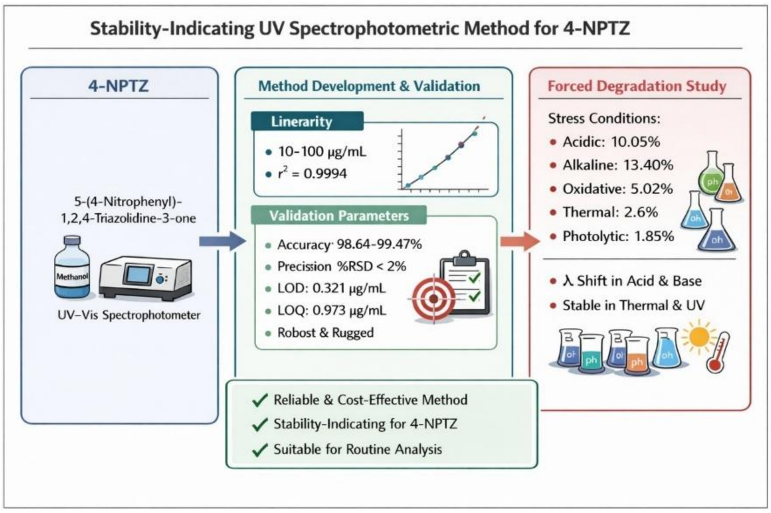

Figure 1: Graphical abstract

1. Introduction

The development of reliable analytical methods is essential for the quantitative determination of pharmaceutical and newly synthesized organic compounds. Among various analytical techniques, UV–visible spectrophotometry is widely preferred due to its simplicity, rapidity, cost-effectiveness, and applicability in routine quality control analysis.

Triazole and its derivatives represent an important class of heterocyclic compounds widely recognized for their diverse pharmacological activities, including antimicrobial, antifungal, anti-inflammatory, and anticancer properties. Due to their structural stability and ability to form strong interactions with biological targets, triazole-based compounds are frequently explored in drug design and development. Heterocyclic compounds, particularly triazole derivatives, have attracted significant attention due to their diverse pharmacological activities. 5-(4-Nitrophenyl)-1,2,4-triazolidine-3-one, Hereafter referred to as (4-NPTZ, is one such compound synthesised for potential biological evaluation. However, limited analytical methods are available for its quantitative estimation.

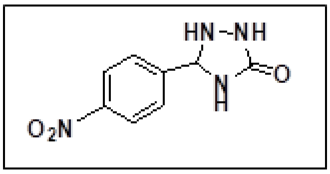

Figure 2: Structure of 5-(4-nitrophenyl)-1,2,4-triazolidin-3-one

Therefore, the present study aims to develop a simple and reliable UV spectrophotometric method for determining 4-NPTZ. The method was optimized using methanol as a solvent, and the analytical wavelength was selected based on maximum absorbance. The developed method was validated in accordance with International Conference on Harmonisation (ICH) guidelines for parameters such as linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ. In addition, forced degradation studies were carried out under various stress conditions including acidic, alkaline, oxidative, thermal, and photolytic environments to evaluate the stability-indicating capability of the method.

2. Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents used were of analytical grade(A.R). Methanol, hydrochloric acid, sodium hydroxide, hydrogen peroxide, and distilled water(Milli-Q). were used. The synthesized compound 4-NPTZ was prepared and characterized in the laboratory. The test samples were prepared from different batches than those used for the reference standards of the synthesised compounds, which were independently prepared in the laboratory.

2.2 Instrumentation

UV analysis was performed using a double-beam UV–visible spectrophotometer. Analytical weighing balance and ultrasonic bath sonicator were used for sample preparation. All instruments were calibrated prior to use.

2.3 Preparation of Standard Solution

A standard stock solution (1000 µg/mL) was prepared by dissolving 10 mg of 4-NPTZ in 10 mL methanol. Further dilutions were made to obtain working solutions.

2.4 Selection of Wavelength

A 100 µg/mL solution was scanned in the range of 200–400 nm, and λmax was found to be 331 nm.

3. Method Validation

3.1 Linearity

The method showed linearity in the concentration range of 10–100 µg/mL with $r^2 = 0.9994$.

3.2 Accuracy

Recovery studies at 80%, 100%, and 120% levels showed % recovery in the range of 98.64–99.47%.

3.3 Precision

Intraday and interday precision studies showed %RSD values within acceptable limits, indicating good reproducibility.

3.4 Robustness and Ruggedness

The method was found to be robust under small variations in temperature and rugged across different analysts.

3.5 LOD and LOQ

LOD and LOQ were found to be 0.321 µg/mL and 0.973 µg/mL, respectively.

4. Forced Degradation Studies

Forced degradation studies were carried out under acidic, alkaline, oxidative, thermal, and photolytic conditions. Significant degradation was observed under acidic (10.05%) and alkaline (13.40%) conditions, whereas minimal degradation was observed under photolytic, thermal, and oxidative conditions. These results confirm the stability-indicating nature of the method.

5. Results and Discussion

The developed UV spectrophotometric method demonstrated excellent linearity, accuracy, and precision. The low %RSD values indicate high reproducibility of the method. The sensitivity of the method is supported by low LOD and LOQ values.

Forced degradation studies revealed that the compound is highly sensitive to hydrolytic conditions, while it remains stable under environmental stress conditions. The method successfully detected changes in absorbance due to degradation, confirming its suitability as a stability-indicating method.

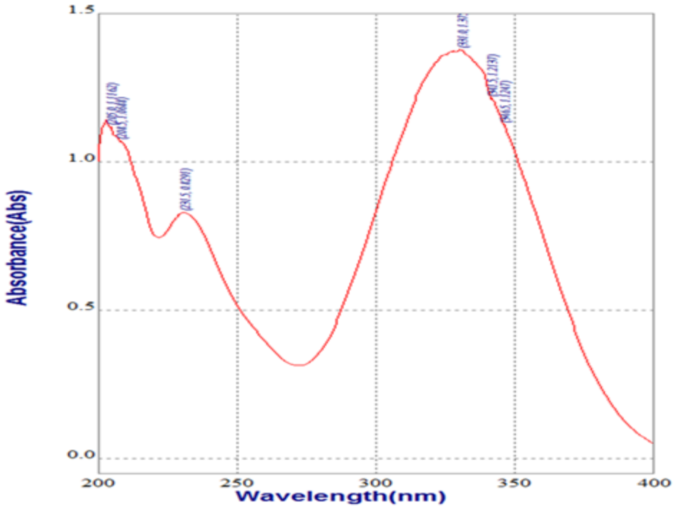

Figure 3: UV spectrum of 4-NPTZ

Table 1: Assessment of linearity for 4-NPTZ using working standard solutions

Sr. No.	Concentration (ppm)	Absorbance
1	10	0.152
2	20	0.279
3	30	0.428
4	40	0.566
5	50	0.682
6	60	0.821
7	70	0.949
8	80	1.069
9	90	1.228
10	100	1.369

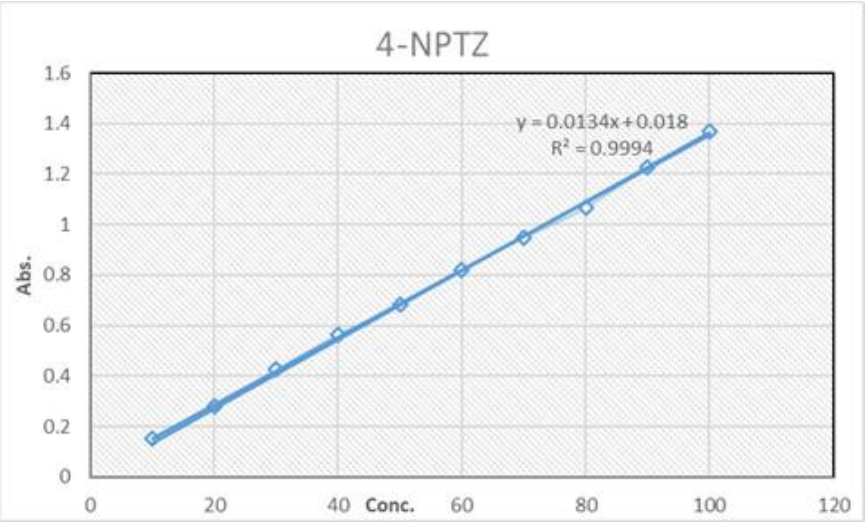

Figure 4: Standard calibration curve of 4-NPTZ

Table 2: UV method optical linearity characteristics of 4-NPTZ

Parameters	Result
Beer's law range (µg/mL)	10–100
Correlation coefficient (r^2)	0.9994
Regression equation (Y)	Y = 0.0134 x + 0.018
Slope (a)	0.0134
Intercept (b)	0.018

Accuracy The accuracy of the developed UV spectrophotometric method was evaluated according to ICH Q2(R1) guidelines. Stock solutions of 100 µg/mL were independently prepared from a synthesized test batch and a well-characterized standard batch. A working solution of 10 µg/mL was made from each stock solution. While the functioning solution from the test batch was regarded as the unknown test solution, the working solution from the standard batch was utilized as the reference (100%) standard solution.

for 80%:	for 100%:	for 120%:
100% = 10 µg/mL	100% = 10 µg/mL	100% = 10 µg/mL
80% = X	100% = X	120% = X
X = 8 µg/mL	X = 10 µg/mL	X = 12 µg/mL

Three concentration levels 80%, 100%, and 120% of the nominal test concentration were used to evaluate accuracy. Consequently, the test batch stock solution was used to prepare theoretical quantities of 8 µg/mL, 10 µg/mL, and 12 µg/mL. The absorbance of each solution was measured in triplicate at the chosen analytical wavelength after each concentration level was generated in triplicate.

The following formula was used to compare the absorbance of the test solution and the standard solution in order to determine the concentration found for each preparation:

$$\text{Concentration found (µg/mL)} = \frac{A_{\text{test}}}{A_{\text{standard}}} \times C_{\text{standard}}$$

where A_{test}= Absorbance of the test solution, A_{standard}= Absorbance of the standard solution (10 µg/mL), and C_{standard} = Standard solution concentration (10 µg/mL).

The percentage recovery was determined by comparing the concentration found with the corresponding theoretical concentration according to the equation:

$$\%\text{Recovery} = \frac{\text{Concentration found}}{\text{Theoretical concentration}} \times 100$$

The final results of the recovery were determined to be within the acceptable range of 98.64% - 99.47%. for all three concentration levels (80–120%). confirming that the proposed UV spectrophotometric method is useful for quantitative estimation and is reliable as well as accurate for the compound over the studied concentration range.

Table 3: Accuracy study of the UV method for 4-NPTZ

No. of Preparation	Concentration (µg/ml)		% Recovery	Mean
	Test solution	Standard solution		
S_1:80%	8	7.8	97.53	98.83%
S_2:80%	8	7.95	99.37	
S_3:80%	8	7.97	99.62	
S_1:100%	10	9.98	99.80	99.47%
S_2:100%	10	9.916	99.16	
S_3:100%	10	9.945	99.45	
S_1:120%	12	11.802	98.35	98.64%
S_2:120%	12	11.75	97.91	
S_3:120%	12	11.96	99.67	

3. Precision Precision was assessed by intraday and interday studies using three concentrations analyzed in triplicate. Mean absorbance, SD, and %RSD were calculated to evaluate reproducibility.

$$s = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \overline{X})^2}{n-1}}$$

Where,
X = individual value
X = arithmetic mean
n = number of samples or Coefficient of variation (C.O.V)

% Relative standard deviation (%R.S.D.) = SD/X x 100

Where,
SD= Standard Deviation
X= Mean

Table 4: Intra-day precision study of the UV method for 4-NPTZ

Conc. (µg/ml)	Absorbance (nm)			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
40	0.54992	0.55137	0.55003	0.55044	±0.000807	0.146
80	1.09250	1.10788	1.09740	1.09926	±0.007857	0.714
100	1.37410	1.38250	1.36962	1.375407	±0.006539	0.475
Average of % RSD= 0.445						

Table 5: Inter-day precision study of the UV method for 4-NPTZ

Conc. (µg/ml)	Absorbance (nm)			Mean	SD	% RSD
	Day 1	**Day 2**	**Day 3**			
4	0.05492	0.05674	0.05588	0.05585	±0.00092	1.65
8	0.11029	0.11362	0.11278	0.11223	±0.00168	1.50
12	0.16378	0.16762	0.16890	0.16676	±0.00264	1.58
Average of % RSD= 1.577%						

4. Robustness The method's robustness was assessed by conducting the analysis at two distinct temperatures: room temperature (25 ± 2 °C) and 18°C. The result was expressed as %RSD and the corresponding absorbances of 20 µg/ml were recorded.

Table 6: Robustness evaluation of the UV method for 4-NPTZ

Sr. no	Concentration (µg/ml)	Absorbance	
		Room temperature	**18^0C**
1	20	0.27562	0.28120
2	20	0.27733	0.28351
3	20	0.27647	0.28423
4	20	0.27912	0.28267
5	20	0.27796	0.28503
6	20	0.27834	0.28334
Mean		0.28714	0.28300
SD		0.00130	0.00133
%RSD		0.4690	0.4871
Average% RSD=0.4780 %			

5. Ruggedness Method ruggedness was evaluated by having the analysis performed by a second analyst. The absorbance of a 20 µg/mL solution was measured, and the results were expressed as %RSD.

$$s = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \overline{X})^2}{n - 1}}$$

Where,

X = individual value

X = arithmetic mean

n = number of samples

% Relative standard deviation (%R.S.D.) = S/X x 100

Where,

S= Standard Deviation, X= Mean

Table 7: Ruggedness study of the UV method for 4-NPTZ

Sr. No.	Conc. (µg/ml)	Absorbance		
		Analyst 1	Analyst 2	Analyst 3
1	20	0.279	0.281	0.279
2	20	0.281	0.279	0.283
3	20	0.276	0.286	0.281
4	20	0.281	0.278	0.283
5	20	0.276	0.288	0.279
6	20	0.283	0.281	0.284
Mean		0.2794	0.2821	0.2815
SD		0.002875	0.003971	0.002168
% RSD		1.0292	1.40	0.770
Average% RSD= 1.066%				

6. Limit of detection (LOD) The limit of detection (LOD) was calculated from the standard deviation of the response and the slope of the calibration curve. The standard deviation of the y-intercept was used as the measure of response variability. LOD is computed using the following formula:

$$\boxed{\textbf{LOD = 3.3 X S.D/ S}}$$

Where,

Where:

LOD = Limit of Detection

3.3 = Statistical factor

SD = Standard deviation of the response

S = Slope of the calibration curve

$$\mathrm{LOD} = \frac{3.3 \times \mathrm{SD}}{S}$$

$$\mathrm{LOD} = \frac{3.3 \times 0.001304}{0.0134} = 0.321\ \mu\mathrm{g/mL}$$

Thus, the limit of detection for the proposed method was found to be 0.321 µg/mL.

7. Limit of quantification (LOQ) The limit of quantification is the lowest concentration of analyte that can be measured with acceptable precision and accuracy. It was calculated using the standard deviation of the response and the slope of the calibration curve.

$$\mathrm{LOQ} = \frac{10 \times \mathrm{SD}}{S}$$

Where:

LOQ = Limit of Quantification, 10 = Statistical factor

SD = Standard deviation of the response, S = Slope of the calibration curve

$$\mathrm{LOQ} = \frac{10 \times 0.001304}{0.0134} = 0.9731\, \mu g/mL$$

Thus, the LOQ for the proposed method was found to be 0.9731 µg/mL.

Table 8: Overview of UV method validation for 4-NPTZ

Sr. No.	Parameter	Result
1	Linearity (correlation coefficient, r^2)	0.9994
2	Linear regression equation	Y = 0.0134x + 0.018
3	Linearity range	10–100 µg/mL
4	Intraday precision (% RSD)	0.445
5	Interday precision (% RSD)	1.577
6	Limit of Detection (LOD) µg/mL	0.3211
7	Limit of Quantification (LOQ) µg/mL	0.9731
8	Robustness (%RSD)	0.4780
9	Ruggedness (%RSD)	1.066

2.3.5. UV Spectrophotometric Method of Stress Degradation Studies For 4-NPTZ

To perform forced degradation studies, ICH QIA (R2) and ICH QIB guidelines were followed. Stress testing must be done to clarify the intrinsic stability properties of the synthesized organic compounds in accordance with the International Conference on Harmonization (ICH) standards titled Stability Testing of Novel Compounds and Products. This work's objective was to conduct impurity analysis on the 4-NPTZ

A Stress degradation study is a crucial part of pharmaceutical research to understand the stability of a compound under various stress conditions. Here's how to conduct a forced degradation study for 4-NPTZ**:**

1. Acid-induced degradation

In separate volumetric flasks, 2 mL of standard stock solution of 4-NPTZ (1 mg/mL in methanol) was transferred. To each flask, 3 mL of hydrochloric acid (0.1 N, 0.5 N, 1 N, 1.5 N, and 2 N) was added. The flasks were kept at room temperature for 3 hours. At 1-hour intervals, aliquots were withdrawn, neutralized with equimolar sodium hydroxide, and diluted with methanol to make the volume up to 10 mL. Further dilution was carried out to obtain a final concentration of 20 µg/mL, and UV analysis was performed at the λmax of 4-NPTZ. Noticeable degradation was observed in 0.1 N HCl after 1 hour.

2. Base-induced degradation

To separate volumetric flasks containing 2 mL of 4-NPTZ stock solution, 3 mL of sodium hydroxide (0.1 N, 0.5 N, 1 N, 1.5 N, and 2 N) was added. The solutions were kept for 3 hours at room temperature. At 1-hour intervals, samples were withdrawn, neutralized with equimolar hydrochloric acid, diluted with methanol to 10 mL, and further diluted to 20 µg/mL for UV analysis. Degradation was observed in 0.1 N NaOH after 1 hour.

3.Photolytic degradation Solid 4-NPTZ was spread in a petri dish and exposed to UV light (254 nm) for 24 hours. After exposure, 20 mg of the sample was dissolved in methanol, volume adjusted to 10 mL, diluted to 20 µg/mL, and analyzed by UV spectrophotometry.

4.Thermal degradation

Solid 4-NPTZ was exposed to 70°C for 4 hours in a hot air oven. The sample was allowed to cool for 1 hour at room temperature. Then, 20 mg of the degraded sample was dissolved in methanol, volume adjusted to 10 mL, diluted to 20 µg/mL, and analyzed.

5. Oxidative degradation- In separate volumetric flasks, 2 mL of 4-NPTZ stock solution was mixed with 3 mL of hydrogen peroxide (1%, 3%, 6%, and 10% v/v). The solutions were kept at room temperature for 3 hours. Aliquots withdrawn at 1-hour intervals were diluted with methanol to obtain 20 µg/mL and analyzed by UV spectroscopy. Significant oxidative degradation was observed with 6 % v/v hydrogen peroxide.

Table 9: UV spectral comparison of pure and stressed samples

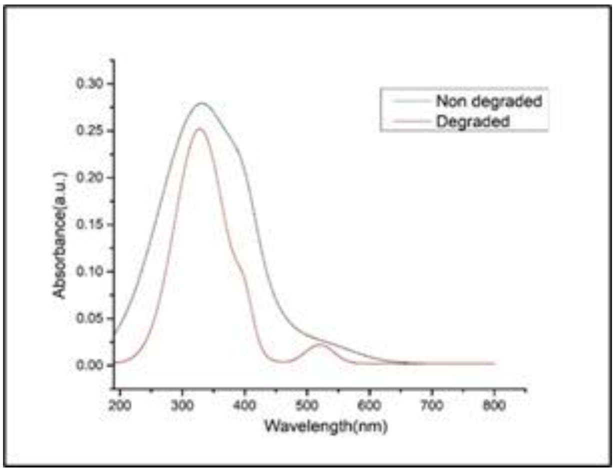

Figure 5: UV spectra of pure and acid-degraded 4-NPTZ

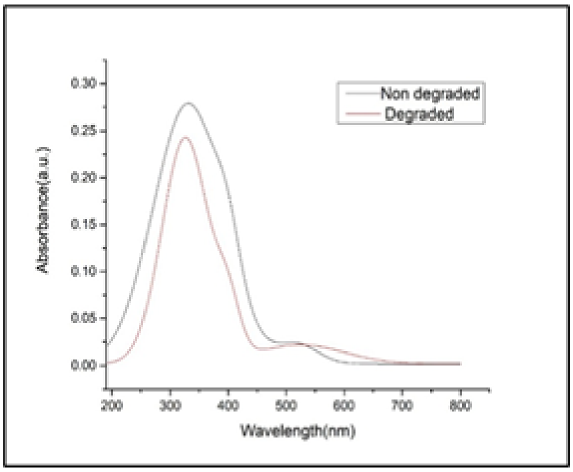

Figure 6: UV spectra of pure and alkaline-degraded 4-NPTZ

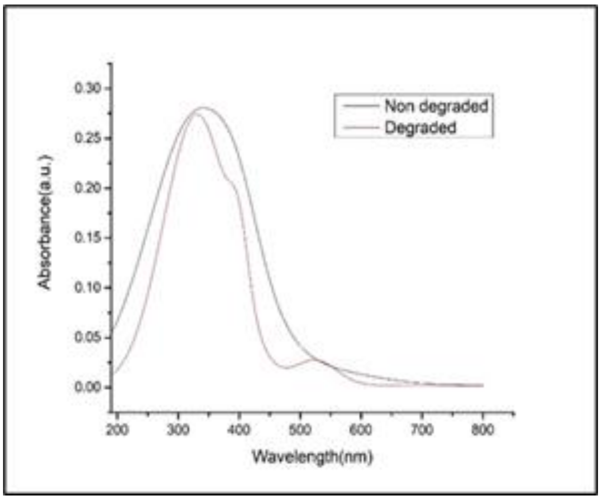

Figure 7: UV spectra of pure and photolytically degraded 4-NPTZ

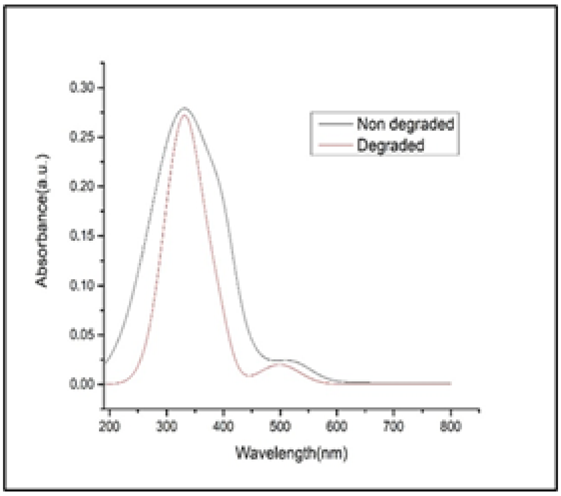

Figure 8: UV spectra of pure and thermally degraded 4-NPTZ

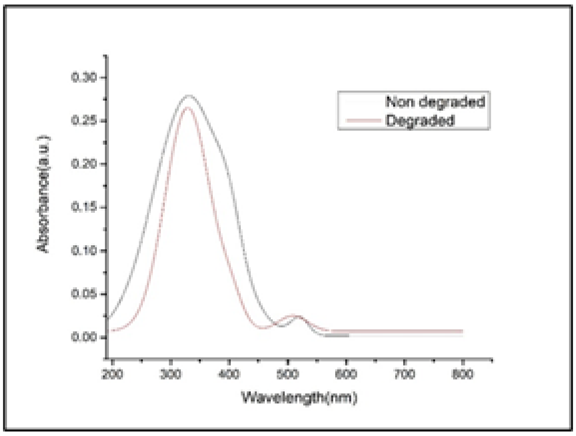

Figure 9: UV spectra of pure and oxidatively degraded 4-NPTZ

Table 10: Summary of UV method forced degradation study for 4-NPTZ

Stress condition	**Time**	**Observation**	**λ max / Concentration of triazole derivative degraded (µg/mL)**	**% Degradation**	**Absorbance**
Undegraded	-	331 nm	-	-	0.279
Acidic Degradation	Reflux 1 hours	(328 nm) λ max shifted	2.01	10.05	0.252
Alkali Degradation	Reflux 1 hours	(326 nm) λ max shifted	2.68	13.4	0.243
Photo Degradation	24 hours	No λ max shifted	0.37	1.85	0.274
Thermal degradation	48 hours	No λ max shifted	0.52	2.6	0.272
Oxidative degradation	RT 1 hours	No λ max shifted	1.04	5.02	0.265

General Observations

The compound is most sensitive to acidic and alkaline conditions, with significant degradation and observable structural changes. Thermal, photolytic, and oxidative conditions result in negligible degradation, indicating good stability under these stressors.

The results highlight the necessity for controlled pH conditions during the formulation and storage of 4-NPTZ. The compound's stability against photolytic, thermal, and oxidative stressors is favorable, reducing concerns about environmental factors during standard handling and storage.

Conclusion

A simple, precise, and stability-indicating UV spectrophotometric method was successfully developed and validated for the determination of 4-NPTZ. The method complies with ICH validation requirements and is suitable for routine quality control and stability studies. The results of forced degradation studies confirm the stability-indicating capability of the method.

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PHYTOCHEMICAL EVALUATION AND MOLECULAR DOCKING ANALYSIS OF *BORASSUS FLABELLIFER LINN*. BARK EXTRACTS AGAINST FUNGAL SPECIES

Ranjith S
College of Pharmacy, Madurai Medical College, Madurai – 625020, Tamil Nadu
Corresponding author E-mail: ranjithsenp@gmail.com

Abstract

Objective: To comparatively evaluate the phytochemical constituents of ethanolic and aqueous bark extracts of *Borassus flabellifer L.*, to investigate the interaction of GC-MS identified compounds with the fungal target protein Lanosterol 14-α-demethylase (CYP51) through molecular docking and to asses their antifungal activity against *Trichophyton rubrum* and *Candida albicans.*

Materials and Methods: The bark of *B. flabellifer* was collected, authenticated and extracted using 70% ethanol and aqueous water by maceration. Preliminary phytochemical screening was performed using standard qualitative tests. Reported GC-MS compounds of *B. flabellifer* were analyzed through molecular docking using AutoDock Vina against the fungal target protein Lanosterol 14-α-demethylase and the antifungal activity was assessed by the agar well diffusion method at concentrations of 50 µL, 100 µL and 150 µL. Terbinafine (10 µg/mL) and 1% DMSO were used as negative control respectively.

Results: Phytochemical screening revealed the presence of flavonoids, tannins, phenols, and saponins in the bark extracts of *B. flabellifer.* Molecular docking analysis showed that β-sitosterol exhibited the highest binding affinity toward the fungal target protein Lanosterol 14-α-demethylase (CYP51), followed by 9,12-Octadecadienoic acid and n-Hexadecanoic acid. The compound 2-methoxyphenol, a water-soluble phenolic compound, may contribute to the antifungal activity observed in the aqueous bark extract indicating potential inhibition of fungal ergosterol biosynthesis and in vitro antifungal studies revealed that only the aqueous extract showed significant inhibitory activity against *Trichophyton rubrum* and *Candida albicans*, whereas the ethanolic extract showed no inhibition.

Conclusion: The antifungal activity of the aqueous bark extract may be attributed to water-soluble phenolic compounds such as guaiacol (2-methoxyphenol), along with phytosterol and fatty acids. Molecular docking showed strong binding of β-sitosterol with Lanosterol 14-α-demethylase (CYP51), supporting its potential antifungal role.

Keywords: *Borassus flabellifer* Bark, Molecular Docking, B-Sitosterol, Guaiacol, Fatty Acids, *T. rubrum, C. albicans.*

Introduction

The palmyra tree (*Borassus flabellifer* L.), popularly referred to as the toddy palm or sugar palm, is among the oldest indigenous tree species of India[1]. Palmyra has been firmly rooted in traditional practices, with different parts of the tree serving a wide range of utilitarian and medicinal purposes. It's leaves are extensively used for roof thatching, mat viewing and basket preparation[2]. Borassus is the genus name and flabellifer refers to the species classified under the family *Arecaceae.* Palmyra palm is a slow growing, perennial, dioecious tree with a single that reaches 30 meter in height and has big fan shaped leaves. The lifespan of the wood tree is estimated to be around 100 years[3]. Across South and Southeast Asia, the palmyra palm forms an important component of dry-zone and semi-arid ecosystems, occurring naturally in countries including India, Bangladesh, Myanmar in addition to Sri Lanka, Thailand, and Malaysia. Sri Lanka supports a large population of palmyra palms, estimated at nearly ten million trees, with a major concentration in the northern Jaffna region. Similarly, extensive palmyra growth is observed in the central plains of Myanmar, where the tree is well adapted to local climatic conditions, while significant populations are also present in the central regions within Cambodia, playing a role in regional biodiversity and rural landscape[4]. Extracts obtained from different parts of the tree, including sprouts, tubers, roots, sap, leaves have antioxidant, anti-inflammatory, and antidiabetic effects[5]. The tree mainly contains gums, albuminoids, fats, glycosides, and carbohydrates. Seed coat derived extract of *Borassus flabellifer* Linn. It has been reported as an antimicrobial activity. Male inflorescences show anti-inflammatory property[6]. The juice from flowering stalks used for diabetes[7]. The fruit pulp had used to treat dermatitis like skin diseases[8].

Table 1: Phytochemical composition (%) and distribution across different plant parts

Phytochemical Class	Assumed Composition %	Seed Coat	Sprouts/ Tubers	Root	Fruit Pulp
Phenols	22-26%	+	+	+	+
Fatty Acid	18-20%	Nil	Nil	Nil	Nil
Terpenoids	12-14%	+	+	+	Nil
Saponins	11-13%	+	+	+	+
Tannins	7-9%	+	+	+	-
Alkaloids	5-7%	+	+	-	-
Flavonoids	4-6%	+	+	+	+
Glycosides	1-3%	+	+	-	+

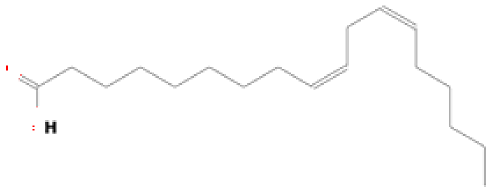
+: Presence; -; Absence

Available literature demonstrates that Ethanolic extracts of different parts of Borassus flabellifer show antimicrobial activity due to the presence of rich phytochemical composition of phenols, flavonoids, saponins, alkaloids, tannins, terpenoids, and glycosides (Table 1)[7,8,9,10,11]. Bark of

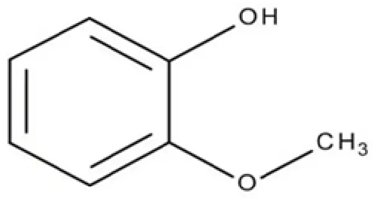
Borassus flabellifer is used as mouthwash is made from a decoction of bark, combined with salt and charcoal from the same bark, which also used as dentifrice[12,13].Gas chromatography–mass spectrometry (GC–MS) analysis of different parts of *Borassus flabellifer* (tubers, roots, and fruit pulp) revealed a assumed composition of Secondary metabolites that presence of fatty acids such as n-hexadecanoic acid was consistently detected across all solvent fractions, including ethanol, aqueous, hexane, chloroform, and methanol extracts, indicating its broad distribution in the plant matrix. 9,12-Octadecadienoic acid (linoleic acid) was predominantly identified in non-polar and semi-polar fractions, particularly in hexane and chloroform extracts, reflecting its lipophilic nature. β-Sitosterol also identified in hexane, chloroform, methanol and aqueous extracts are observed. In contrast 2-methoxyphenol (Guaiacol) was exclusively observed in the cold extraction method within the polar fraction. Phytochemical classification confirmed that guaiacol belongs to the phenolic class, whereas n-hexadecanoic acid and 9,12-octadecadienoic acid are categorized as fatty acids[11,14,15,16,17,18]. Notably, all four compounds have been reported to exhibit antifungal activity. However, despite these extensive studies, the bark of *Borassus flabellifer* has not been specifically investigated for its antifungal activity. Therefore, the present study was undertaken to examine the antifungal potential of bark of *Borassus flabellifer* extracted using different solvents of Ethanol and Aqueous. Since there are no sufficient scientific reports available on the antifungal activity of the bark, this study aims to explore and establish its potential therapeutic value. This work may provide new insights into the medicinal importance of the bark and contribute to the development of novel antifungal agents from natural sources (fig. 1) of palmyra tree.

Isolated Compounds

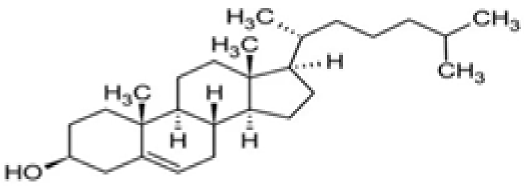
n- Hexadecanoic acid (Palmitic acid)



9,12 Octadecaenoic acid (linoleic acid)



2- methoxyphenol (Guaiacol)



β-Sitosterol

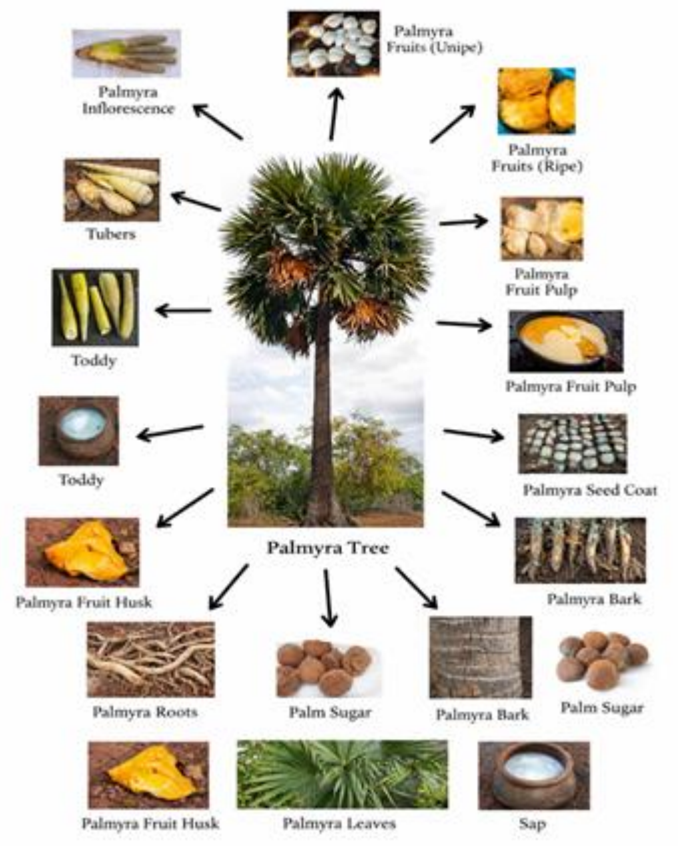


Figure 1: Parts of *Borassus flabellifer*

Material and Methods

1. Collection and Authentication of Plant Material

The bark of *Borassus flabellifer L.* was collected from Erode District, Tamil Nadu, India, during the month on 30th Dec 2025. The plant specimen was botanically authenticated by Dr. K. Devanathan, Assistant Professor, Department of Botany, The American College at Madurai, Tamil Nadu, India. A voucher specimen was verified and identified as belonging to the family Arecaceae, and the authenticated specimen was preserved for future reference.

1.1 Bark

The bark of *Borassus flabellifer* (fig. 2) is a fibrous and hard outer covering that protects the trunk of the palm tree. It is traditionally used in folk medicine for its potential therapeutic properties.

Figure 2: Bark of *Borassus flabellifer*

1.2 Bark Extract Process

After collection the bark material was thoroughly cleaned to remove adhering impurities and then shade-dried for 3 days at room temperature to prevent deterioration of bioactive constituents. After complete drying, the bark was coarsely powdered using a mechanical grinder. The dried bark powder of *Borassus flabellifer* (50 g) was extracted separately using 70% ethanol and aqueous. Each extraction was carried out by maceration with the solvent volume made up to 500 ml. The mixtures were kept for 7 days at room temperature with occasional shaking. After completion of maceration, the extracts were filtered using sterile filter paper to remove the plant residue. The filtered extract is evaporated to dryness by being kept in a water bath at 50°C. The obtained dried extracts were stored in a sterile capped container under refrigeration at 4 °C for further study. The weight of the extract was measure and the percentage yield of the bark material was calculated[19].

2. Phytochemical screening of Ethanol and Aqueous Bark Extracts of *Borassus flabellifer*

After completion of the bark extraction process, the obtained ethanolic and aqueous extracts were subjected to preliminary phytochemical screening. Both extracts were analyzed using standard qualitative chemical tests to identify the presence of various bioactive constituents. The screening was carried out to detect flavonoids, alkaloids, saponin, tannins, terpenoids and phenols. Each test was performed according to established procedures to confirm the presence or absence of these phytochemicals. The results of the phytochemical analysis help to correlate the bioactive compounds with the antifungal activity of the bark extract.

2.1 Shinoda test for flavanoids[20]: A few drops of magnesium chloride and concentrated hydrochloric acid were added to 1 ml of bark extract and observed after 5 Minutes. The presence of flavonoids is confirmed by observing red or pink colour.

2.2 Mayers test for alkaloids[21]: Few drops of Mayers reagent were added to 2 ml of bark extract. The presence of alkaloids is indicated by creamy/white precipitate.

2.3 Frothing test for saponin[22]: 3 ml of bark extract were added to 10 ml of distilled water and strongly shaked for 5 minutes. Later, it was permitted to stand for 30 min. The presence of saponins was indicated by frothing or foaming appearance.

2.3.4. Ferric chloride test for tannins[23]: 1 ml of bark extract was mixed with a few drops of 0.1 % ferric chloride. The presence of gallic tannins and catechol tannins was confirmed through the observation of blue-black and green-black colors, respectively.

2.4 Salkowski's test for terpenoids[24]: 2 ml of chloroform was added along with little drops of concentrated sulphuric acid to 1 ml of bark extract. Indicated by the appearance of a reddish-brown ring at the interface confirms the activity of terpenoids.

2.5 Ferric chloride test for phenol[25]: A few drops of 5 % ferric chloride were added to 1 ml of bark extracts and noticed for dark green or bluish black color indicating the activity of phenols.

3.Molecular Docking

3.1 Protein preparation: The three-dimensional crystal structure of 7P1Q is a PDB structure from *Trichophyton rubrum* and it is related to Heat shock protein 90 (Hsp90). and Lanosterol 14-α-demethylase (CYP51) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/) with PDB ID: 4LXJ (fig. 3). The prepared protein structure was then used for molecular docking analysis. In order to align the mesh box prior to docking, the position must be determined.

→ **Primary target (mechanism study)**	• 4LXJ
→ **Dermatophyte Protein**	• 7P1Q

CYP51 was selected because it is a conserved antifungal drug target involved in ergosterol biosynthesis in dermatophytes including *Trichophyton rubrum*[26].

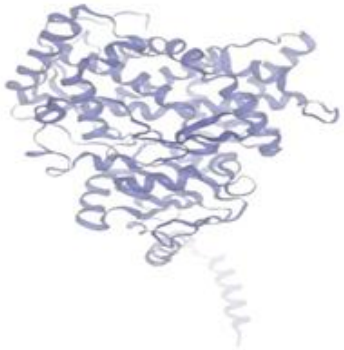


Figure 3: Structural model of Lanosterol 14-α-demethylase (CYP51) with PDB ID: 4LXJ

3.2 Ligand preparation: The chemical structures of the selected GC–MS identified compounds are

→2-Methoxyphenol and it’s smiles are COC1=CC=CC=C1O

→n-Hexadecanoic acid and it’s smiles are CCCCCCCCCCCCCCCC(=O)O

→9,12-Octadecadienoic acid and it’s smiles are CCCCC/C=C\C/C=C\CCCCCCCC(=O)O

→β-Sitosterol and it’s smiles are CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C.

Along with the reference antifungal drug Terbinafine (fig. 4) and it’s smiles are CC(C)(C)C#C/C=C/CN(C)CC1=CC=CC2=CC=CC=C21 were obtained from the PubChem (https://Pubchem.ncbi.nlm.nih.gov) database in SDF format. The ligand structures were converted to PDB format and prepared for docking using the ligand preparation tools available in AutoDock Vina[27].

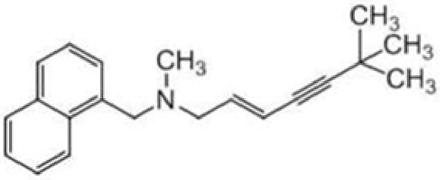


Figure 4: Structure of Terbinafine

3.3 Molecular docking simulation

Molecular docking analysis was performed using AutoDock Vina to predict the binding affinity and interaction between ligands and the target protein, lanosterol 14-α-demethylase (CYP51) (PDB ID: 4LXJ). AutoDock Vina employs a scoring function and an efficient optimization algorithm to estimate ligand–protein binding modes. The grid box for docking was centered at coordinates X = 9 , Y = 9, Z = 18 covering the active site region of the protein. Docking simulations were performed with default parameters, and the best binding conformations were selected based on the lowest binding energy values (kcal/mol)[28].

4. Microorganisms used for Analyzing Antifungal Activity

After phytochemical screening, the microorganism used for antifungal activity of *Borassus flabellifer* bark extracts were analyzed using agar well diffusion method against some fungal species.

- ***i.*** *Trichophyton rubrum*
- ***ii.*** *Candida albicans*

5. Antifungal Activity

5.1 Agar well diffusion assay against *Trichophyton rubrum*

Antifungal activity against *Trichophyton rubrum* was evaluated by the agar well diffusion method on Muller Hinton Agar + 2 percentage Glucose plate was inoculated with a fungal strain (*T. rubrum*) under aseptic conditions. The ethanol and aqueous bark extracts (10 mg/mL) stock prepared in 1% DMSO diluted in distilled water (100 µL DMSO diluted in 10 ml distilled water). Wells were filled with 50 µL, 100 µL and 150 µL concentration of the test Sample. Terbinafine (10 µg/mL) diluted with DMSO was used as positive control. The 1% DMSO was used as a negative control. The plates were then incubated at 28°C for 72 hours. After incubation, the zone of inhibition against the tested fungi was observed.

5.2 Agar well diffusion assay against *Candida albicans*

Antifungal activity against *Candida albicans* was evaluated by the agar well diffusion method on Potato Dextrose Agar (PDA) medium was seeded with 72 hours culture of fungal strain (*C. albicans*) under aseptic conditions. The ethanol and aqueous bark extracts (10 mg/mL) stock prepared in 1% DMSO diluted in distilled water (100 µL DMSO diluted in 10 ml distilled water). Wells were filled with 50 µL, 100 µL and 150 µL concentration of the test Sample. Terbinafine (10 µg/mL) diluted with DMSO was used as positive control. The 1% DMSO was used as a negative control. The plates were then incubated at 28°C for 72 hours. After incubation, the zone of inhibition against the tested fungi was observed.

Results

1. Percentage Yield of Bark Material

The percentage yield of the Ethanol and Aqueous extract of palmyra palm bark was around 4.6% and 4.2% w/w respectively. Weight of ethanol and aqueous extracts are 2.3 and 2.1 grams.

Yield % = $(w_1/w_2) \times 100$

W1= The weight of the dried extract (g).

W2= The weight of the dry plant material (g).

2. Phytochemical screening from bark extracts of *Borassus flabellifer.*

The phytochemical screening of the ethanolic and aqueous extracts of *B. flabellifer* revealed the presence of certain phytochemicals like flavonoids, saponins, tannins, and phenols, confirming that these bioactive compounds are abundantly present in the plant material (Table 2).

Table 2: The phytochemical screening of the ethanolic and aqueous extracts of *B. flabellifer*

Phytochemical constituent	Ethanol	Aqueous
Flavonoids	+	+
Alkaloids	-	-
Saponin	+	+
Tannins	+	+
Terpenoids	+	-
Phenol	+	+

+: Presence; -; Absence

Alkaloids were absent in both extracts. The ethanol extract showed a positive result for terpenoids, Whereas the aqueous extract showed no such reaction, indicating that terpenoids are more soluble in moderately polar solvents like ethanol. The presence of phenolic compounds and flavonoids suggests strong antifungal and antioxidant potential, while saponins and tannins indicate possible antimicrobial activity. flavonoids, phenol, saponin, these compounds were detected may account for the antifungal activities of these extracts against *Trichophyton rubrum and Candida albicans* which have been tested in this study.

3. Results of the interactions between the 5 selected ligands and the protein Lanosterol 14-α-demethylase

Molecular docking analysis was carried out using AutoDock Vina to evaluate the binding affinity of selected GC–MS compounds with the target protein Lanosterol 14-α-demethylase (CYP51). The docking scores indicated different levels of interaction between the phytochemicals and the enzyme active site (Table 3). Among the tested compounds, standard drug Terbinafine showed the strongest binding affinity with a docking energy of (−9.9 kcal/mol), which followed by β-sitosterol showed strongest binding affinity (−9.4 kcal/mol) equal to standard drug Terbinafine. 9,12-Octadecadienoic acid also exhibited good binding with a score of (−7.3 kcal/mol), followed by n-hexadecanoic acid (−6.6 kcal/mol). 2-Methoxyphenol showed comparatively lower binding affinity with (−5.8 kcal/mol). The interactions between ligands and the protein were mainly stabilized through hydrogen bonding, hydrophobic interactions, and van der Waals forces within the active binding pocket. These findings suggest that the identified phytochemicals, particularly β-sitosterol, may possess potential antifungal activity by inhibiting the CYP51 enzyme.

Table 3: Molecular docking analysis

Compound Name	Pubchem CID	Docking Energy (Kcal/Mol)
2-methoxyphenol	460	-5.8
n-Hexadecanoic acid	985	-6.6
9,12-Octadecadienoic acid	5280450	-7.3
β-Sitosterol	222284	-9.4
Terbinafine	1549008	-9.9

4. Antifungal Activity

4.1 Ethanolic and Aqueous bark extract of antifungal activity against *Trichophyton rubrum* and *Candida albicans.*

The antifungal activity of ethanolic and aqueous bark extracts was evaluated against *Trichophyton rubrum* and *Candida albicans* using the agar well diffusion method at different concentrations (50, 100 and150 µL). Against *Trichophyton rubrum*, the aqueous extract showed clear antifungal (fig. 5,6) activity at all tested concentrations, producing zones of inhibition of approximately 10 mm at 50 µL, 16 mm at 100 µL, and 11 mm at 150 µL. The ethanolic extract showed no inhibition. The negative control showed no zone of inhibition against *Trichophyton rubrum* while the standard drug Terbinafine produced the maximum inhibition (17–18 mm)**.** In the case of *Candida albicans*, the aqueous extract again demonstrated effective antifungal (fig. 7,8) activity, with zones of inhibition measuring about 12 mm, 13 mm, and 15 mm at 50 µL, 100 µL and 150 µL, respectively, showing a concentration-dependent response. The ethanolic extract showed no inhibition against *Candida albicans*. The negative control showed no activity whereas the standard antifungal drug about 11–12 mm. Overall, the results indicate that the aqueous bark extract exhibits better antifungal activity against both *Trichophyton rubrum* and *Candida albicans* Compared to the ethanolic extract, suggesting that water-soluble phytochemicals may play a key role in the observed antifungal effect.

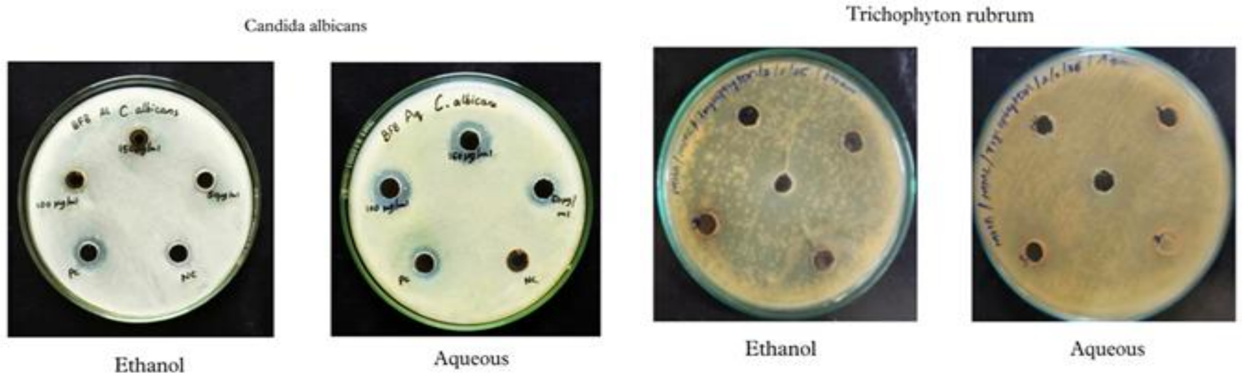


Figure 5: Antifungal activity against *C. albicans* and *T. rubrum*

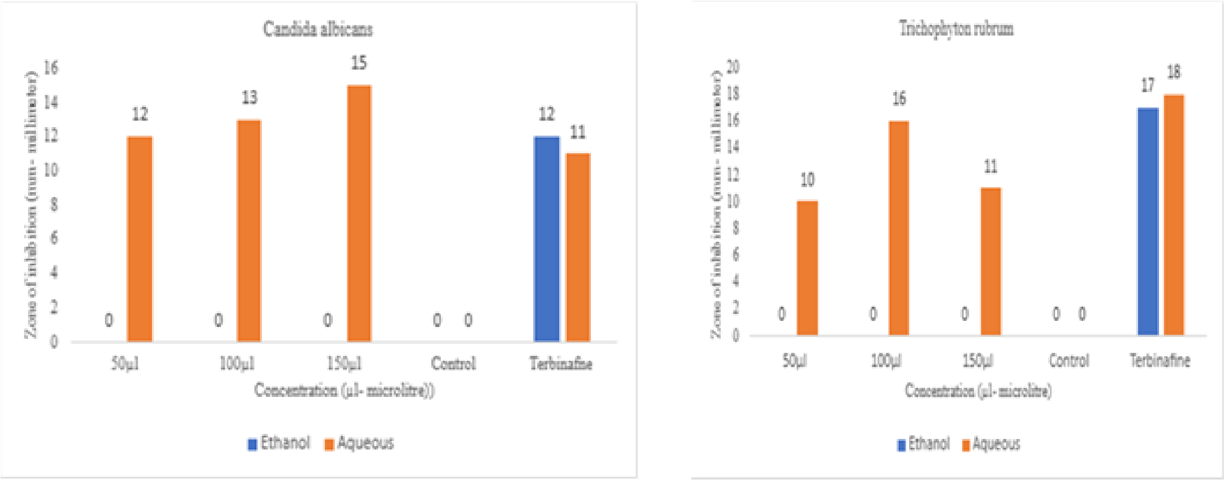


Figure 5: Antifungal activity against *C. albicans* and *T. rubrum*

This study investigates the phytochemical, molecular docking and antifungal activity of *Borassus flabellifer* bark against ringworm and candidiasis infection. Ringworm (tinea) is a common contagious fungal skin disease caused mainly by dermatophytes such as *Trichophyton rubrum* and *Microsporum canis*. *Trichophyton rubrum* is a major dermatophyte fungus responsible for ringworm (tinea) infections of the skin. In this study, *Trichophyton rubrum* is identified as one the primary causative agents of tinea corporis, tinea pedis, and other superficial fungal infections. The fungus thrives in warm and moist conditions and infects the keratinized tissues of the skin, leading to red, scaly, ring-shaped lesions[29]. The aqueous bark extract of *B. flabellifer* demonstrated significant inhibitory activity against *T. rubrum*, indicating its potential role in controlling ringworm infections caused by this organism. *Candida albicans* was selected as it is a widespread fungal infection affecting people of all ages causing oral thrush, genital infections like vulvovaginal candidiasis. It is also a normal commensal of our human body. Infection occurs when the fungus overgrows due to weakened immune system[30]. Terbinafine, an allylamine antifungal agent, exhibits potent fungicidal activity against dermatophytes by inhibiting squalene epoxidase, leading to disruption of fungal cell membrane synthesis. It is effective via both oral and topical administration and shows superior activity against *Trichophyton* and *Microsporum* species compared to several conventional antifungal drugs. Against *Candida albicans*, Terbinafine demonstrates dose-dependent activity, though it is generally less effective than azole antifungals, indicating its primary suitability for dermatophyte infections[31]. Aqueous extract shows good antifungal activity against *Trichophyton rubrum and Candida albicans.* The zone of inhibition of standard drug Terbinafine has 17-18 mm and 11-12 mm (10 µg/ml). When (10 mg/ml) taken aqueous extract shows highest inhibition of 16 mm at 100 µL against *Trichophyton rubrum* and 15 mm at 150 µL against *Candida albicans.* Aqueous extract of lowest concentration shows highest inhibition of antifungal activity against *Trichophyton rubrum* and *Candida albicans species.* n-Hexadecanoic acid exhibits notable antifungal activity against *Candida albicans*, *Candida tropicalis, Aspergillus species*[32].

Conclusion

The present study demonstrated that the aqueous bark extract of *Borassus flabellifer* exhibits significant antifungal activity against *Trichophyton rubrum* and *Candida albicans*. This activity may be attributed to water-soluble phenolic compounds such as guaiacol along with phytosterols and fatty acids present in the extract. Molecular docking analysis using AutoDock Vina showed strongest binding affinity of β-sitosterol with the fungal target protein Lanosterol 14-α-demethylase (CYP51), suggesting inhibition of fungal ergosterol biosynthesis in fungi. The compound β-Sitosterol demonstrated interactions comparable to the standard antifungal drug Terbinafine, indicating their potential as promising antifungal candidates. However, the exact bioactive compound responsible for the antifungal activity of the bark has not yet been isolated. Therefore, future studies should focus on isolation and purification of active phenolic compounds, β-Sitosterol, fatty acids and toxicity assesment. Such investigations may lead to the development of novel plant-derived antifungal agents from *B. flabellifer* bark with potential therapeutic application against fungal species.

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ARTIFICIAL INTELLIGENCE IN DRUG DISCOVERY AND DEVELOPMENT: RECENT ADVANCES AND FUTURE PERSPECTIVES

Nikita Walunjkar and Jaymala Kumawat*
School of Pharmacy,
Vishwakarma University, Pune, Maharashtra, India
*Corresponding author E-mail: jaymala.kumawat@vupune.ac.in

Abstract

Drug discovery is a complex and resource-intensive process that traditionally requires more than a decade and significant financial investment. Artificial intelligence (AI) has emerged as a transformative technology capable of accelerating pharmaceutical research by improving the efficiency of drug discovery and development. AI techniques such as machine learning, deep learning, and natural language processing enable researchers to analyze massive biomedical datasets, predict molecular interactions, and identify potential drug candidates with higher accuracy. Applications of AI span multiple stages of drug discovery, including target identification, drug design, virtual screening, toxicity prediction, and clinical trial optimization. Recent breakthroughs such as AI-driven protein structure prediction and generative molecular design have significantly improved the efficiency of drug development. Despite challenges such as data quality, regulatory issues, and interpretability, AI holds great promise for transforming pharmaceutical research. This review discusses the principles of AI, its major applications in drug discovery, recent technological advances, limitations, and future perspectives.

Keywords: Artificial Intelligence, Machine Learning, Drug Discovery, Pharmaceutical Research, Computational Drug Design, Deep Learning.

1. Introduction

Drug discovery is a multidisciplinary process involving chemistry, biology, pharmacology, and clinical sciences. Traditional drug development involves sequential stages such as target identification, hit discovery, lead optimization, preclinical testing, and clinical trials. This process typically requires 10–15 years and substantial financial resources, with many drug candidates failing during clinical trials due to safety or efficacy issues.

Artificial intelligence (AI) has emerged as an innovative technology capable of transforming pharmaceutical research by enabling the analysis of large biological datasets and accelerating drug discovery processes. AI involves computational methods that simulate human intelligence, allowing machines to learn from data, identify patterns, and make predictions. In pharmaceutical sciences, AI technologies such as machine learning (ML), deep learning (DL), and natural language processing (NLP) are increasingly used to analyze chemical structures, predict biological activity, and identify potential therapeutic molecules [1].

Recent advances in computational power and the availability of large biological datasets have significantly increased the application of AI in drug discovery. AI-driven computational methods enable researchers to screen millions of chemical compounds and identify promising drug candidates more efficiently than traditional experimental approaches [2].

2. Artificial Intelligence and Machine Learning in Pharmaceutical Sciences

Artificial intelligence refers to computer systems capable of performing tasks that typically require human intelligence, such as pattern recognition, learning from data, and decision-making. Machine learning is a subset of AI that involves algorithms capable of learning from large datasets and improving their predictions over time.

In pharmaceutical sciences, AI integrates computational chemistry, bioinformatics, pharmacology, and systems biology to analyze complex biomedical datasets. Machine learning algorithms can identify relationships between chemical structures and biological activities, allowing researchers to predict the therapeutic potential of molecules before experimental testing [3].

Major AI techniques used in drug discovery include:

2.1 Machine Learning

Machine learning algorithms analyze large datasets to identify patterns and relationships between chemical structures and biological activity. ML methods are commonly used in quantitative structure–activity relationship (QSAR) studies to predict drug efficacy and toxicity.

2.2 Deep Learning

Deep learning is a specialized branch of machine learning that uses artificial neural networks with multiple layers. Deep learning models can analyze complex datasets such as molecular structures, genomic sequences, and protein interactions.

2.3 Natural Language Processing

Natural language processing allows computers to extract relevant information from scientific literature, clinical reports, and biomedical databases. NLP techniques help researchers identify potential drug targets and therapeutic strategies by analyzing large volumes of published data.

These AI technologies enable pharmaceutical scientists to analyze biological systems more efficiently and accelerate the identification of new therapeutic molecules.

3. Role of Artificial Intelligence in Drug Discovery

3.1 Target Identification

Target identification involves discovering biological molecules such as proteins or genes that play a critical role in disease development. AI algorithms analyze genomic, proteomic, and transcriptomic datasets to identify potential drug targets.

Machine learning techniques can analyze biological networks and identify associations between genes, proteins, and diseases, helping researchers prioritize targets for drug development [4].

3.2 Virtual Screening and Hit Identification

Virtual screening is a computational technique used to evaluate large libraries of chemical compounds and identify potential drug candidates. AI-based virtual screening methods significantly reduce the time and cost associated with experimental screening.

Deep learning models can predict binding affinity between drug molecules and biological targets, enabling the identification of promising drug candidates with higher accuracy [3].

3.3 Lead Optimization

Lead optimization involves modifying chemical structures to improve drug efficacy, selectivity, and pharmacokinetic properties. AI-based predictive models can analyze chemical structures and suggest modifications that enhance drug activity.

Generative AI models are capable of designing entirely new chemical structures with desired pharmacological properties, enabling researchers to explore previously unknown chemical space [2].

3.4 Drug Repurposing

Drug repurposing involves identifying new therapeutic uses for existing drugs. AI systems analyze clinical data and biomedical databases to identify drugs that may be effective against other diseases.

AI-based drug repurposing approaches gained significant attention during the COVID-19 pandemic, where computational methods were used to identify existing drugs that could potentially treat viral infections [3].

3.5 Prediction of Drug Toxicity and Safety

Drug toxicity is one of the major causes of drug failure during clinical trials. AI models can predict toxicity and adverse effects early in the drug development process by analyzing chemical structures and biological data.

These predictive models help pharmaceutical researchers identify potentially harmful compounds and reduce the risk of late-stage drug failure.

3.6 Clinical Trial Optimization

Clinical trials represent one of the most expensive stages of drug development. AI technologies can improve clinical trial design by identifying suitable patient populations, predicting treatment outcomes, and monitoring patient responses.

Predictive analytics tools help researchers optimize clinical trials and reduce development costs by improving trial efficiency.

4. Recent Advances in AI-Driven Drug Discovery

4.1 Protein Structure Prediction

One of the most significant breakthroughs in AI-based drug discovery is protein structure prediction. Understanding the three-dimensional structure of proteins is essential for rational drug design.

The AI system AlphaFold has demonstrated remarkable accuracy in predicting protein structures from amino acid sequences, significantly improving our understanding of molecular interactions and facilitating structure-based drug design [5].

4.2 AI-Driven Molecular Generation

Generative deep learning models can design new chemical molecules with specific biological properties. These models analyze large chemical datasets and generate novel compounds that meet predefined pharmacological criteria.

This approach enables researchers to rapidly identify potential drug candidates and optimize their properties before experimental testing.

4.3 Graph Neural Networks in Drug Discovery

Graph neural networks (GNNs) represent molecules as graphs, where atoms are nodes and chemical bonds are edges. These models can analyze molecular structures and predict chemical properties with high accuracy.

GNNs are increasingly used in drug discovery to predict molecular interactions, toxicity, and pharmacokinetic properties.

5. Advantages of Artificial Intelligence in Drug Discovery

Artificial intelligence provides several advantages in pharmaceutical research:

- Faster identification of drug candidates
- Reduced drug development time and cost
- Improved prediction accuracy for drug properties
- Ability to analyze large biological datasets
- Facilitation of personalized medicine

AI-driven computational models can screen millions of compounds in a short period of time, significantly accelerating drug discovery processes [1].

6. Challenges and Limitations

Despite its significant potential, AI-based drug discovery faces several challenges.

6.1 Data Availability and Quality

AI models require large datasets for training. Incomplete or biased data can reduce the accuracy of predictions.

6.2 Algorithm Transparency

Many AI models function as “black boxes,” making it difficult to interpret how predictions are generated.

6.3 Regulatory Challenges

Regulatory agencies must establish guidelines for evaluating AI-generated drug candidates and ensuring patient safety.

6.4 Ethical Concerns

The use of AI in healthcare raises concerns regarding patient privacy, data security, and algorithmic bias.

7. Future Perspectives

Artificial intelligence is expected to play an increasingly important role in pharmaceutical research. Future developments may include:

- Integration of AI with genomics and precision medicine
- Development of AI-driven personalized therapies
- Automated robotic laboratories for drug discovery
- Advanced predictive pharmacology models

The pharmaceutical industry is increasingly investing in AI-driven drug discovery platforms to accelerate therapeutic innovation.

Conclusion

Artificial intelligence has emerged as a powerful technology capable of transforming pharmaceutical research. By enabling rapid analysis of complex biological datasets, AI significantly accelerates drug discovery and improves the efficiency of drug development. Applications of AI in target identification, drug design, toxicity prediction, and clinical trial optimization demonstrate its potential to revolutionize pharmaceutical sciences. Although challenges such as data quality, algorithm transparency, and regulatory issues remain, continued advancements in AI technologies are expected to enhance drug discovery processes and improve healthcare outcomes.

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Recent Progress in Chemical, Biological and Pharmaceutical Sciences

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About Editors

Dr. Ravindra Raghunath Mahajan is a Post-Doctoral Fellow in the Department of Chemical Engineering at the National Institute of Technology, Warangal, Telangana. He completed his Ph.D. from North Maharashtra University, Jalgaon. He is an active researcher in chemical, biological, pharmaceutical, and polymer sciences, with a focus on coatings, microencapsulation, and self-healing materials. He has published five research papers in recognized journals and holds two patents, reflecting his interest in innovative and practical solutions. He has also contributed two book chapters and edited two academic books, demonstrating his involvement in scholarly publishing. His research bridges fundamental science with real-world applications, particularly in advanced material systems. He was honored with the International Best Researcher Award in 2025 and continues to work on developing functional, application-oriented materials.

Dr. Uttam B. Chougale is an Associate Professor in the Department of Chemistry at Mouni Vidyapeeth’s Karmaveer Hire Arts, Science, Commerce and Education College, Gargoti, affiliated with Shivaji University, Kolhapur. He has around 15 years of teaching experience and 10 years of administrative experience as Head of the Chemistry Department. He completed his B.Sc. in Chemistry with distinction from Shivaji University and M.Sc. in Organic Chemistry with distinction from Solapur University. He qualified NET-JRF in Chemical Sciences and obtained his Ph.D. in Heterocyclic Chemistry from Shivaji University. He has published 17 research papers in reputed journals and presented over 10 papers at national and international conferences. His research interests include Heterocyclic Chemistry and Medicinal Chemistry with conventional and green synthetic approaches.

Dr. (Mrs.) Pranjali T. Maskar, M.Sc., B.Ed., Ph.D. (University of Mumbai), is an Associate Professor and recognized Research Guide at B.K. Birla College of Arts, Science and Commerce (Empowered Autonomous Status), Kalyan (West). She is recognized as a Ph.D. guide and postgraduate teacher by the University of Mumbai and is a member of the university syllabus committee. She has successfully guided one Ph.D. scholar (2023), with two research scholars currently pursuing doctoral work under her supervision, and has guided 30 M.Sc. students. She has 15 years of permanent and 5 years of ad-hoc teaching experience. She has published 11 academic books and chapters, 15 research papers in national and international journals, and presented 26 papers at conferences and seminars.

Dr. Pravin Nandkumar Muli is an accomplished academician and researcher with 14 years of teaching experience in Chemistry. He currently serves as an Assistant Professor in the Department of Chemistry at Smt. Sindhutai Jadhao Arts and Science Mahavidyalaya, Mehkar, Dist. Buldhana. He holds M.Sc. degrees in Organic Chemistry and Mathematics, along with M.Phil., Ph.D., B.Ed., M.Ed., and has qualified SET. He has published 12 research papers in reputed journals and authored 4 books, along with securing 2 patents in his field. He has actively participated in national and international conferences, presenting research through oral and poster sessions and receiving recognition. He has also delivered several guest and expert lectures at seminars organized by various institutions.

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