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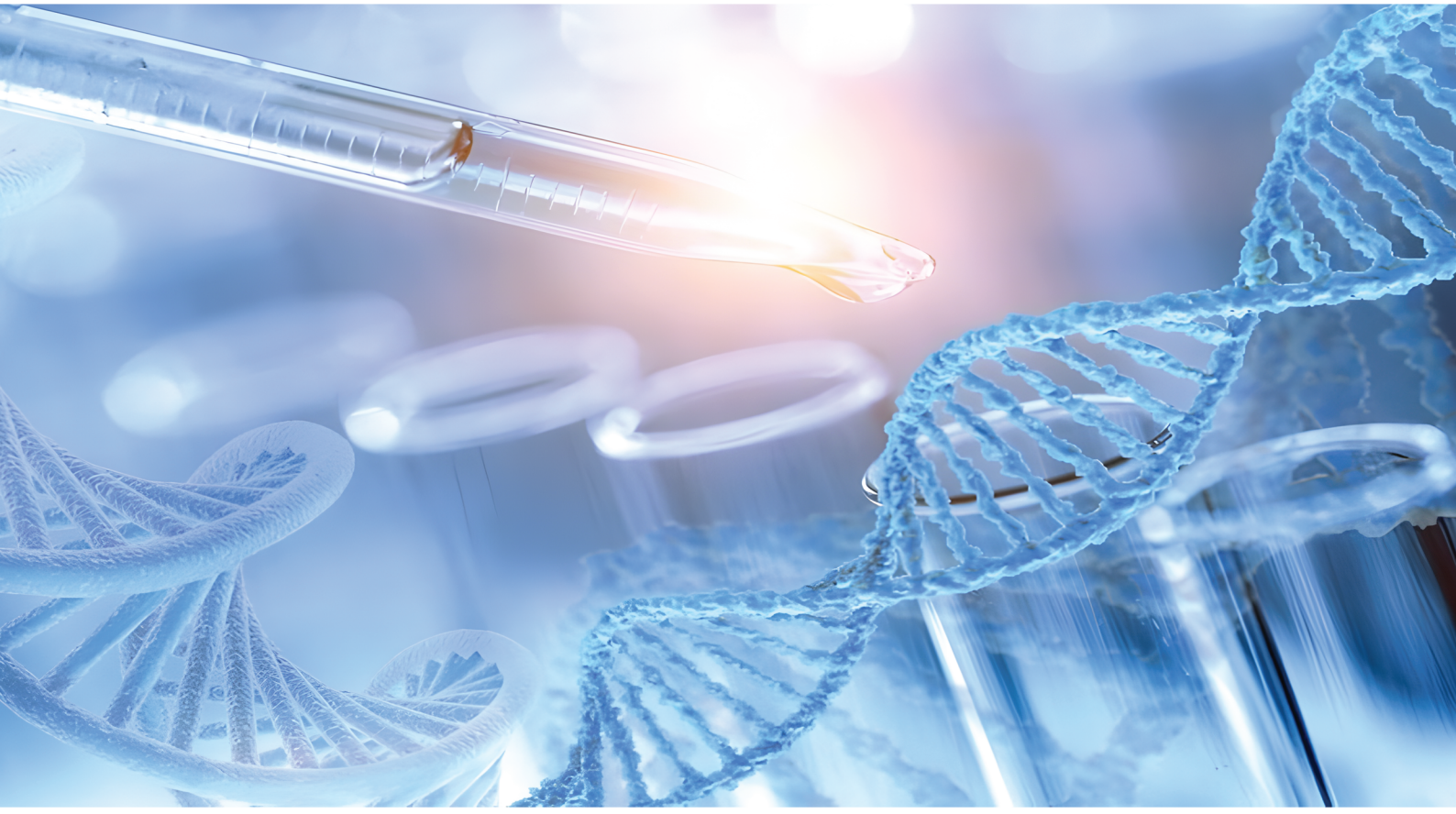

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Introduction

The **Journal of Pharma Innovative Research (ISSN Code: 2350-1332)** publishes high caliber articles each year that promote the cutting edge and useful applications of numerous pharmaceutical science disciplines. Publications include contributions to theoretical research as well as applications. The goal of the journal is to give academics and researchers in the pharmaceutical sciences a top-qualified medium where they may publish their original research and review articles.

The primary goal of the Journal of Pharma Innovative Research (JPIR) is to encourage timely publication in all areas of pharmaceutical sciences. Pharmaceutical Chemistry, Industrial Pharmacy, Pharmacology, Pharmacognosy, Phytochemistry, Pharmacodynamics, Pharmacokinetics, Pharmacogenomics, Biopharmaceutics, Physical Pharmacy, Drug Design, Pharmaceutical Analysis, Drug Stability, Quality Control and Assurance, Pharmaceutical Engineering, Hospital and Clinical Pharmacy are among the specific scientific topics that the journal is interested in.

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ARTIFICIAL INTELLIGENCE: TRANSFORMING PHARMACEUTICAL TECHNOLOGY, DRUG DISCOVERY AND DRUG DELIVERY SYSTEMS

Dr. Smitha K Nair, Edveena K Thomas, Elise P A

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ABSTRACT

Artificial intelligence (AI) has become a vital technology for addressing complex challenges in the pharmaceutical field. By integrating machine learning (ML) tools, AI enables rapid analysis of large biological datasets, helping identify disease-related targets and predict interactions between drugs and biomolecules. This approach streamlines drug discovery, enhances accuracy, and improves the chances of successful drug approval. AI also supports cost reduction by refining experimental design and forecasting pharmacokinetic and toxicity profiles, thus minimizing the need for animal testing. Furthermore, AI-driven analysis of patient data promotes personalized therapies that improve treatment outcomes and adherence. Overall, AI applications in formulation design, process optimization, and PK/PD evaluation are transforming pharmaceutical research and advancing patient care.

Keywords: Artificial intelligence; Pharmaceutical Technology; Drug Delivery Design and dosage.

1. INTRODUCTION

The pharmaceutical industry plays a crucial role in tackling global health challenges and responding to emergencies such as pandemics. Continuous innovation in drug development, manufacturing, and marketing is essential to meet growing healthcare demands. Research focuses on developing more stable and potent molecules while addressing the toxicity concerns of new drugs. Despite progress, the sector still faces technological and workforce-related challenges that require ongoing skill development and training.

Supply chain disruptions, particularly during the COVID-19 pandemic, have severely affected production, logistics, and clinical trials. Factors such as natural disasters, price fluctuations, cyberattacks, and transport issues further complicate operations, impacting profitability and customer trust. To overcome these issues, the adoption of artificial intelligence (AI) and digital platforms is being explored to improve supply chain efficiency and enable virtual clinical trials. However, cybersecurity threats and patient data breaches remain major obstacles, highlighting the need for more secure and innovative clinical trial models. [1]

2. CURRENT PHARMACEUTICAL SITUATION AND THE ROLE OF AI

In the pharmaceutical field, continuous research on small molecules has led to the development of improved and more effective products. These molecules are preferred due to their simple chemical synthesis, cost efficiency, and ability to form stable formulations. However, competition from generic versions and the need for extensive data and clinical trials—especially for rare diseases—create financial challenges for pharmaceutical companies,

pushing them toward greater innovation. To address these limitations, the biomolecular drug sector, which focuses on proteins and nucleic acids, has grown rapidly, producing therapies such as insulin and adalimumab. Despite their potential, biomolecules face challenges in stability, pharmacokinetics, and delivery methods, demanding advanced technological solutions.

Artificial intelligence (AI) has become an essential tool in enhancing drug discovery and formulation. By using techniques like machine learning, deep learning, and natural language processing, AI can predict drug interactions and optimize development processes. However, AI outcomes depend on data quality and still require human oversight to prevent bias, ensure accuracy, and interpret complex results. [2]

3. AI FOR DRUG DISCOVERY

- **Target Identification:**
  AI analyzes genetic, proteomic, and clinical data to detect disease-related targets and pathways for new drug development.
- **Virtual Screening:**
  It quickly screens large chemical libraries to find compounds with strong binding potential, saving time and cost.
- **Structure–Activity Relationship (SAR):**
  AI links chemical structures with biological activity, helping design more potent and selective molecules.
- **De Novo Drug Design:**
  Through generative algorithms, AI creates new drug-like molecules from existing data.
- **Optimization of Drug Candidates:**
  AI evaluates efficacy, safety, and pharmacokinetics, enabling refinement of compounds to improve effectiveness and reduce side effects. [3]

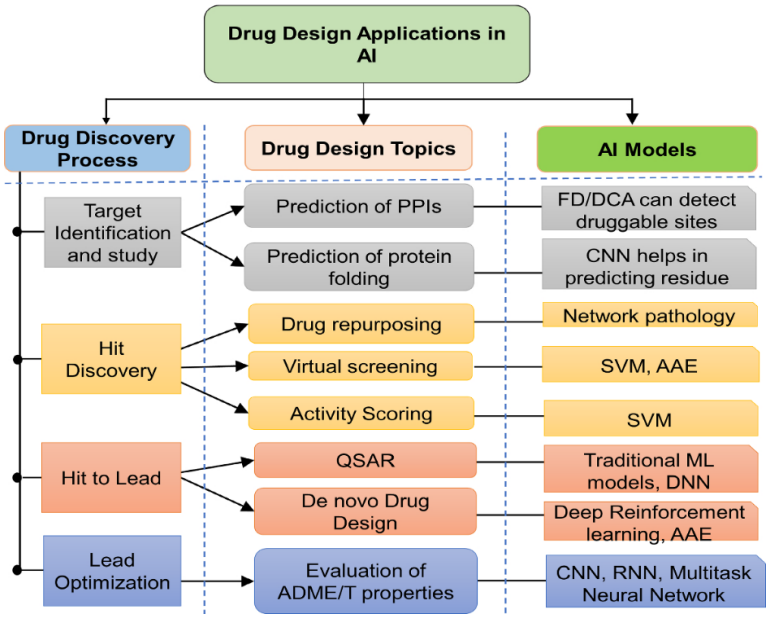

Fig 1: AI models in drug discovery

4. AI IN DRUG DELIVERY SYSTEMS

4.1. AI in Oral Solid Dosage Form Development

Artificial intelligence (AI) uses advanced computational tools to mimic human decision-making and has become increasingly valuable in pharmaceutical product development. Its implementation in solid dosage forms, particularly tablets, helps optimize formulations, predict drug release, and assess the impact of critical manufacturing parameters, saving time, cost, and resources across the production and supply chain.

Studies have demonstrated the application of machine learning in tablet development. For instance, Run Han et al. applied AI to predict the six-month stability of solid dispersions, while Hanlu Gao et al. used a random forest algorithm to classify dissolution profiles and maintain supersaturation with high accuracy and sensitivity. AI tools, including artificial neural networks (ANNs), fuzzy logic, and genetic algorithms, support the prediction of formulation outcomes, enabling better control over inputs and outputs in manufacturing processes.

Tablet production involves combining active pharmaceutical ingredients with excipients, followed by compression or molding. Excipients regulate key properties such as disintegration, dissolution, and drug release, while glidants and lubricants aid processing. AI is also employed to detect defects, ensure consistent quality, and guide systematic drug delivery. By integrating AI, pharmaceutical manufacturers can enhance efficiency, product reliability, and the overall quality of oral solid dosage forms. [4][5]

4.2. Prediction of Drug Release Using AI

Predicting drug release is crucial for maintaining consistent quality in oral solid dosage forms. Traditionally, drug release is evaluated using in vitro and in vivo methods, considering both critical material attributes and processing parameters. Factors such as tablet hardness, compaction pressure, geometry, and drug loading significantly influence release profiles. Conventional methods require repeated batch preparation and testing to achieve optimal formulations, making the process time-consuming and resource-intensive.

Artificial intelligence (AI) has emerged as an effective tool to predict drug release, reducing the number of experimental runs and associated costs. AI can forecast drug dissolution profiles, disintegration times, and overall release behavior, enabling the selection of optimal batches for scale-up. Techniques such as artificial neural networks (ANNs), support vector machines (SVM), and regression analysis have been successfully applied, particularly for hydrophilic matrix sustained-release tablets. Process analytical technology (PAT) data, including critical material attributes like particle size distribution, are used to train AI models. Among these, ANN-based models have shown high accuracy in predicting dissolution profiles, helping streamline formulation development and improve efficiency in the production process. [6] [7]

Applications of AI in Solid Dosages Forms (Since 2015)

Dosage Forms	Applications	Algorithms
Tablet	Predicting drug release	ANN, SVM, Ensemble of Regression Trees, and decision tree
	Developing 3D-printed tablets	ANN, self-organizing maps, RF, SVM, and CNN
	Detecting tablet defects	CNN, You Only Look Once v5 (YOLOv5)
	Estimation of disintegration rate	RF, XGBoost, ANN, and CNN
	Drug particle size inspection	Pattern recognition neural network
Powders	Process control of powder engineering	ANN
	Designing dry powder for inhalation	RF, XGBoost, LightGBM, SVM, KNN, ANN, and CNN
	Predicting particle size distribution of spray-dried powder	Unspecified
	Improving spray-dried powder compatibility	SVM and ANN

Fig 2: AI models in drug release

5. CONCLUSION

Artificial intelligence (AI) is transforming drug delivery and pharmaceutical development by enabling precise, personalized, and adaptive therapies. It improves drug effectiveness, minimizes side effects, and enhances patient outcomes. AI applications in pharmacokinetics and pharmacodynamics allow prediction of drug behavior, optimization of dosing, and reduction of reliance on animal studies and extensive clinical trials.

When combined with big data, AI also supports formulation optimization, regulatory compliance, and risk management. Overall, these technologies make drug development more efficient, cost-effective, and data-driven, advancing the pharmaceutical industry toward a smarter and more automated Era 5.0.

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Q-ABSORBANCE RATIO METHOD FOR THE SIMULTANEOUS ESTIMATION OF NORFLOXACIN AND TINIDAZOLE IN TABLET DOSAGE FORM BY UV SPECTROSCOPY

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ABSTRACT

In present research work a simple, specific, precise, accurate and economical Q absorbance ratio method for simultaneous estimation of Norfloxacin and Tinidazole using UV Spectroscopy was developed and validated. In the present work 50% Glacial acetic acid and 50% Methanol (HPLC Grade) blend was used as solvent. Two wavelengths 323 nm (Isosbestic point) and 308 nm (λ max of Tinidazole) were selected for the estimation of Norfloxacin and Tinidazole by Q absorbance ratio method. The analytical method was validated as per ICH guidelines in terms of linearity and range, accuracy, precision and Ruggedness. The percentage for the drugs estimated in tablet formulation for Norfloxacin and Tinidazole was found to be 98.63 and 99.78 respectively. The % recovery of Tinidazole was between 100-103% and Norfloxacin was between 98-100%.LOD of Norfloxacin and Tinidazole was found to be 0.107 and 1.022. LOQ of Norfloxacin and Tinidazole was found to be 0.326 and 3.097. Based on the results obtained the proposed method can be regarded as simple, precise, accurate, cost effective and eco-friendly for simultaneous estimation of Norfloxacin and Tinidazole.

KEYWORDS: Norfloxacin, Tinidazole, Q- absorbance ratio method, UV Spectroscopy.

INTRODUCTION

Norfloxacin is a kind of fluoroquinolone antibiotic and active against both Gram positive and Gram-negative micro-organism i.e. bactericidal in action, commonly used in treatment of urinary tract infections, infectious diarrhoea and gonococcus urethritis. Norfloxacin, chemically 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.

The antibacterial activity of norfloxacin is due to the presence of ketonic and carboxylic group and its potency is due to the presence of substituent on 1 and 7 position of fluoroquinolones.[1] Norfloxacin is extensively used in various applications in veterinary welfare and human medicine. The bactericidal action of Norfloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination.[2]

Fig No: 1 Structure of Norfloxacin

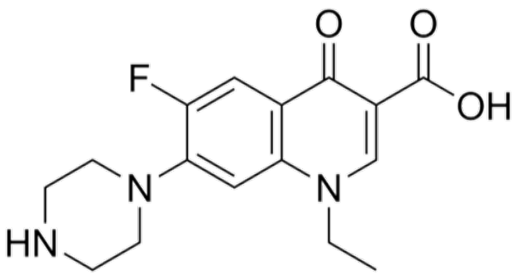
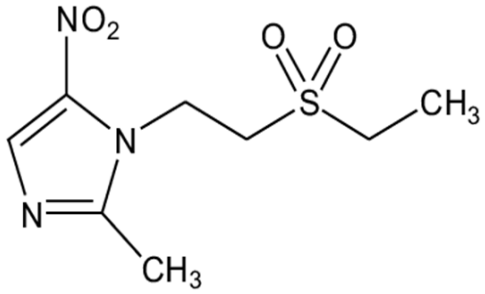


Fig No: 2 Structure of Tinidazole



Tinidazole is chemically 1-[2-(ethyl sulfonyl)ethyl]-2-methyl-5-nitro-1H-imidazole,a medication used against protozoan infections. It is widely known throughout Europe and the developing world as a treatment for a variety of anaerobic amoebic and bacterial infections. It was developed in 1972 and is a prominent member of the nitroimidazole antibiotic class.[3] The nitro group of tinidazole is reduced in Trichomonas by a ferredoxin-mediated electron transport system. The free nitro radical generated as a result of this reduction is believed to be responsible for the antiprotozoal activity.[3][2]

The aim of the present work was to develop a Q- Absorption Ratio spectrophotometric method for simultaneous estimation of Norfloxacin and Tinidazole in combination. There are several methods for estimating the amount of drug in a tablet by UV Spectroscopy. In this study, we selected the Q absorbance ratio method for simultaneous estimation of Norfloxacin and Tinidazole in tablet dosage form by UV Spectroscopy.[4] Absorption ratio method is the ratio of the absorption at two selected wavelengths, one of which is isosbestic point and other being the λ max of one of the two components. e. Hence, to achieve this aim an accurate Q-Absorption ratio method has been developed and successfully applied to synthetic mixture.

MATERIALS AND METHODS

Apparatus

A Shimadzu UV-1700, software UV Probe, UV-Visible double-beam spectrophotometer with two matched 1 cm path-length quartz cells. The subsequent statistical manipulation was performed by transferring the spectral data to Microsoft Excel program.

Chemicals and Reagents

Pure drugs Norfloxacin and Tinidazole, along with the selected solvent system (Glacial Acetic Acid and Methanol (HPLC Grade) in a 50:50 ratio), were obtained from St. James' College of Pharmaceutical Sciences, Chalakudy. Combined tablet dosage form contain Norfloxacin and Tinidazole were obtained from retail shop.

Solvent System

The combination of Methanol (HPLC-grade) and glacial acetic acid in the ratio 50:50 was selected as the solvent system for further studies.

Preparation of standard stock solution

Accurately weigh 10 mg of both Norfloxacin and Tinidazole, then transfer them into separate 10 ml standard flasks and make up to the mark with the selected solvent system. The resultant solutions will have a concentration of 1000 µg/ml. From these prepared solutions, transfer 1 ml into a separate 10 ml standard flask and dilute with the solvent system. Then, take 1 ml from this solution and dilute it to 10 ml using the selected solvent system.

Determination of λ max of norfloxacin

Working standard solution of Norfloxacin (10 µg/ml) was prepared and scanned in the UV range of 200nm – 400 nm utilizing a blank.

Determination of λ max of tinidazole

Working standard solution of Tinidazole (10 µg/ml) was prepared and scanned in the UV range of 200nm – 400nm utilizing a blank.

METHODOLOGY

Absorption ratio method is the ratio of the absorption at two selected wavelengths, one of which is isosbestic point and other being the λ max of one of the two components. At 323 nm, solutions of both drugs of same concentration exhibit identical absorbance and consequently with zero difference. Such wavelengths of equal absorptivity of the two species are called isobestic or iso-absorptive points. From overlay spectra of two drugs, it was evident that Norfloxacin and Tinidazole have an iso-absorptive point at 323 nm (λ1). The second wavelength used was 308 nm (λ2) of λ max of Tinidazole.

$Cx = \{(QM-Qy)/(Qx-Qy)\} * (A1/ax1)$

$Cy = \{(QM-Qx)/(Qy-Qx)\} * (A1/ay1)$

Where, A1 and A2 are absorbance of mixture at 323nm and 308nm;

ax1 = A (Absorptivity, 1 %, 1 cm) of Norfloxacin at 323 nm

ay1 = A (1 %, 1 cm) of Tinidazole at 308 nm

ax2 = A (1 %, 1 cm) of Norfloxacin at 323 nm

ay2 = A (1 %, 1 cm) of Tinidazole at 308 nm;

Cx and Cy are the unknown concentration of Norfloxacin and Tinidazole respectively in sample solution.

QM =A2/A1, QX =ax2/ ax1 and QY= ay2/ay1

Preparation of sample solution for assay

Determination

Twenty tablets of the sample, each containing 400 mg of Norfloxacin and 600 mg of Tinidazole, were weighed and powdered. A quantity of powder equivalent to 5.5 mg of the sample was weighed and diluted to 100 mL with a mixture of Methanol (HPLC grade) and glacial acetic acid in the ratio of 50:50. Further dilutions were made to obtain the final concentration in the ratio of 2:3. The absorbance of the resulting solutions was measured at 308 nm and 323 nm. The amount and percentage purity were calculated using the Q-absorbance ratio method.

METHOD VALIDATION

Linearity

A stock solution was prepared by dissolving 10 mg of both Norfloxacin and Tinidazole separately and making each solution up to 10 mL with the solvent system. 10 µg/mL solutions of both Norfloxacin and Tinidazole were prepared. Concentrations were then prepared for each drug as follows: Norfloxacin: 2, 4, 6, 8, 10, 12 µg/mL Tinidazole: 5, 10, 15, 20, 25, 30 µg/mL The absorbance of the prepared solutions of Norfloxacin and Tinidazole was measured at 278 nm and 308 nm. A calibration curve was plotted by correlating concentration with absorbance.

Accuracy

10 mL of a sample solution with a concentration of 0.1 µg/mL was taken and denoted as (a). Sample solutions of 80%, 100%, and 120% concentrations were prepared from the above solution. The absorbance was measured, and the concentration was denoted as (b). Then, 80%, 100%, and 120% solutions were added to the 0.1 µg/mL sample solution. The absorbance was recorded, and the concentration was denoted as (c). % recovery and % RSD were calculated using the following equation: % Recovery = (c - a) / b.

Precision

Intra Day and Inter Day precision Intraday: carried out at different intervals on the same day. Inter day: carried out in different days. Transfer 0.4 mL of Norfloxacin and 0.6 mL of Tinidazole to separate 10 mL standard flasks. The volume was adjusted to the mark with the solvent system. The absorbance of the solutions was measured spectrophotometrically 6 times. The % RSD (Relative Standard Deviation) of each sample was calculated.

Ruggedness

4 µg/mL of Norfloxacin and 6 µg/mL of Tinidazole were prepared by different analysts using the same equipment, the same procedure, and under the same laboratory conditions. The experiment was repeated three times, and the absorbance of Norfloxacin was measured at 278 nm, while the absorbance of Tinidazole was measured at 308 nm.

LOD and LOQ

The LOD (Limit of Detection) and LOQ (Limit of Quantification) are estimated from a set of three calibration curves used to determine the method's linearity.

LOD = 3.3 * (σ./S)

LOQ = 10 * (σ/S)

Where, σ is Standard deviation of Y intercept and S is Slope of Regression lines calculated from the linearity study. Standard deviation was calculated from Microsoft word excel STYEX function.

RESULTS AND DISCUSSIONS

Norfloxacin:

λ max of Norfloxacin was found to be 278nm.

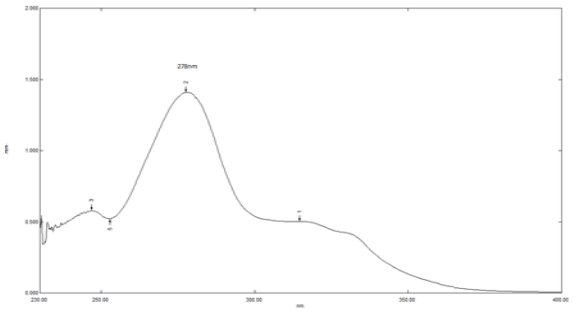


Fig no: 3 The λ max of Standard solution of Norfloxacin

Tinidazole:

λ max of Tinidazole was found to be 308nm.

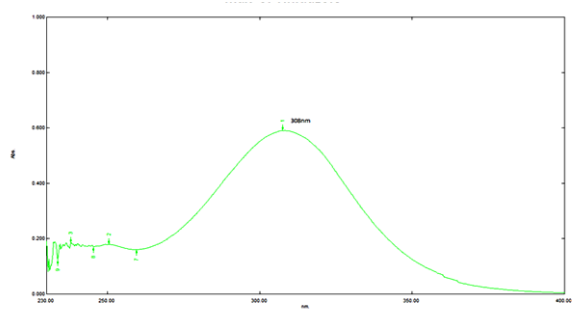


Fig no: 4 The λ max of Standard solution of Tinidazole

Determination of isosbestic point:

Isosbestic point of Norfloxacin and Tinidazole was found to be 323nm.

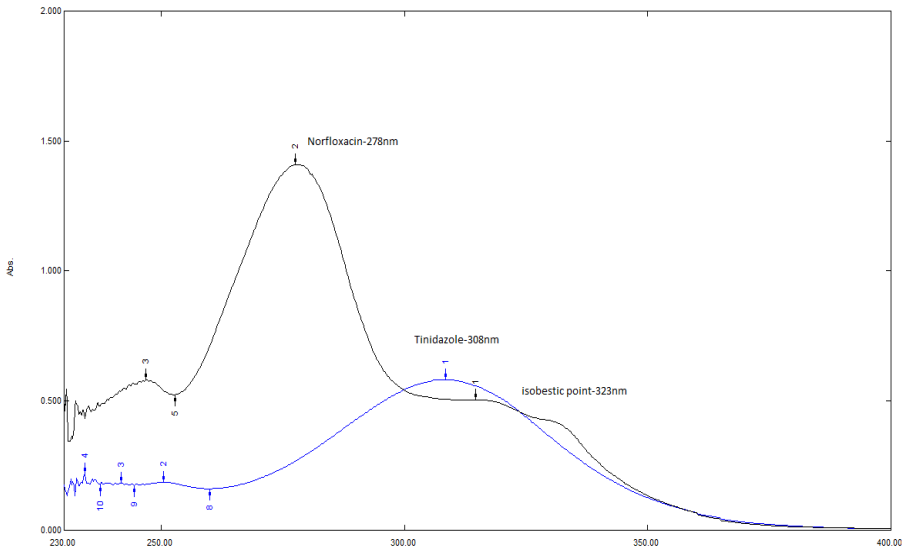


Fig no: 5 Isosbestic point of Norfloxacin and Tinidazole

Linearity

Aliquots of standard solution were applied in the concentration range 2-12 µg/ml and 5-30 µg/ml for Norfloxacin and Tinidazole respectively. The calibration curve obtained by the least square regression analysis between average absorbance and concentration showed linear relationship with a correlation coefficient R2 nearer to 0.999 for Norfloxacin and Tinidazole. The linear regression equation obtained were y = 0.1375x+0.00455 and y = 0.0321x-0.0052 for Norfloxacin and Tinidazole respectively.

Concentration (µg/ml)	Absorption at 278nm
2 (µg/ml)	0.320
4 (µg/ml)	0.590
6 (µg/ml)	0.874
8 (µg/ml)	1.151
10 (µg/ml)	1.420
12 (µg/ml)	1.691

Table no: 1 Linearity of Norfloxacin

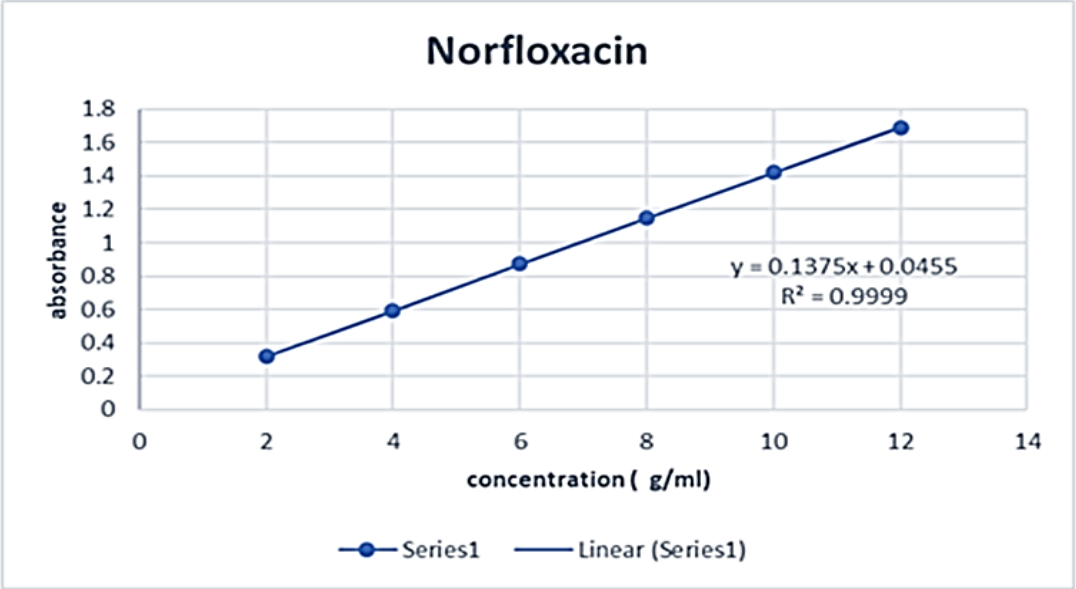


Fig no: 6 Calibration curve of Norfloxacin

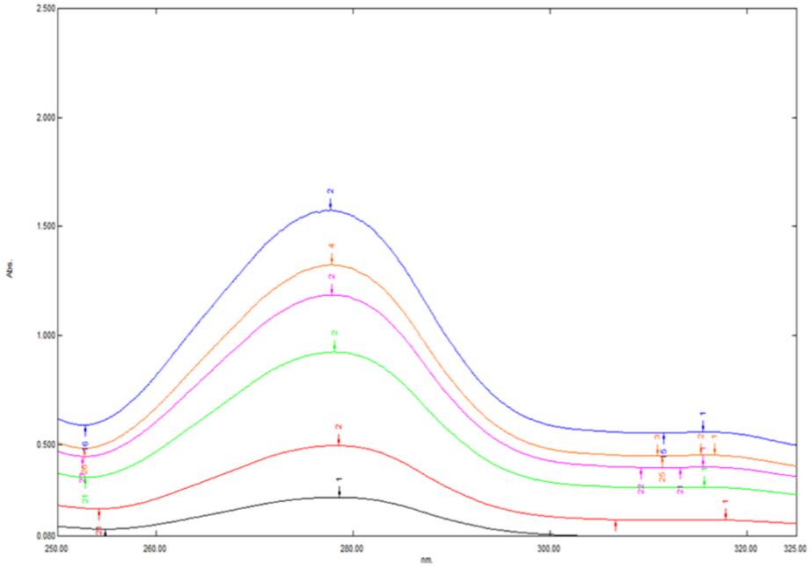


Fig no: 7 Overlay spectrum of Norfloxacin

Concentration (μg/ml)	Absorbance at 308nm,
5 (μg/ml)	0.162
10 (μg/ml)	0.319
15 (μg/ml)	0.470
20 (μg/ml)	0.630
25 (μg/ml)	0.788
30 (μg/ml)	0.973

Table no: 2 Linearity of Tinidazole

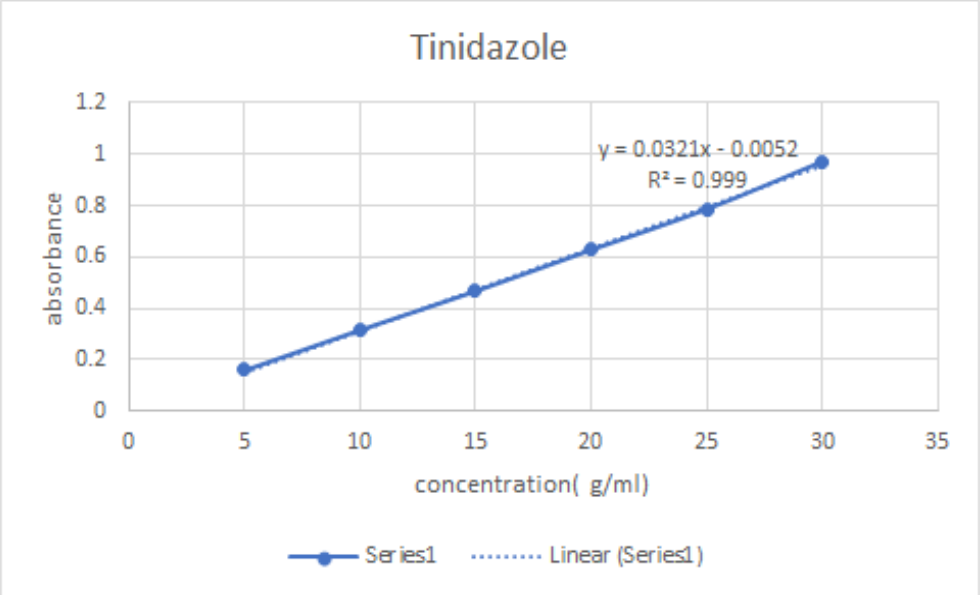


Fig no: 8 Calibration curve of Tinidazole

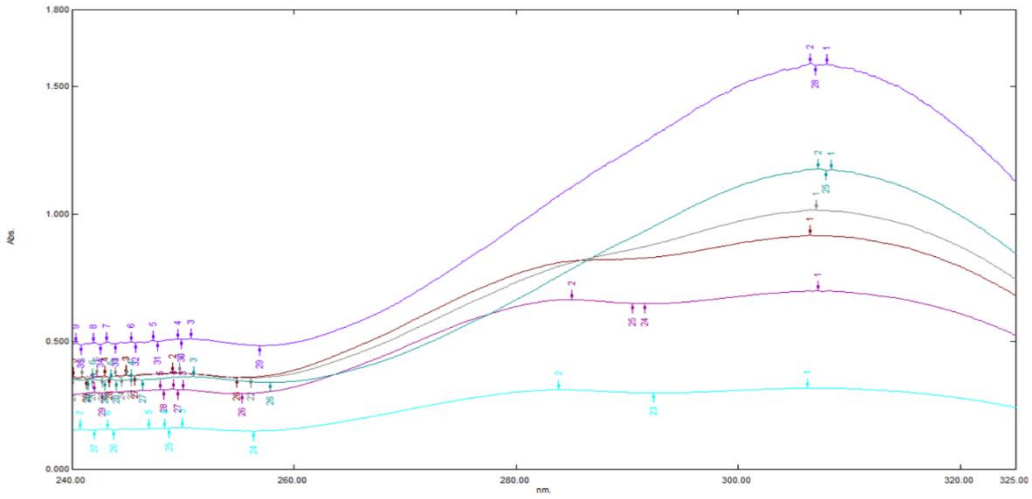


Fig no: 9 Overlay spectrum of Tinidazole

Preparation of test solution for assay

Determination

20 Tablets of the sample containing 400mg of Norfloxacin and 600mg Tinidazole were weighed, average weight was calculated and powdered. Weighed 5.5mg of sample which is equivalent to 400mg norfloxacin and 600mg Tinidazole, then diluted it to 100ml with selected solvent. Further dilutions were made to obtain final concentration 5 μg/ml which contain 0.4g norfloxacin and 0.6g Tinidazole. Absorbance were measured at 308nm (λ max) and 323 nm (isosbestic point).Amount and percentage purity were calculated using the Q- absorbance ratio method.

Analyte	Norfloxacin	Tinidazole
% Estimated	98.63	99.78
Amount found in mg	394.5	596.7
SD	0.004482	0.009952
%RSD	0.00113	0.001668

Table no: 3 Assay of Norfloxacin and Tinidazole

Accuracy

Percentage recovery shows the accuracy of the method.

%Recovery = (amount of drug found after the addition of standard drug) – (amount of drug before the addition of standard drug)/ amount of standard drug added*100

Recovery studies were carried out at 80%, 100%, 120%. The % recovery and its %RSD were calculated.

Contents	% level of addition	Concentration (μg/ml)		Total concentration	Drug recover (μg/ml)	% Recovery	Mean	SD	%RSD
		Std	Addition						
Norfloxacin	80	10	7.84	17.84	7.84	99.08	99.27	0.085	0.028
		10	7.84	17.84	7.84	99.25			
		10	7.84	17.84	7.84	99.18			
	100	10	10.05	20.05	10.05	100.16	100.25	0.060	0.020
		10	10.05	20.05	10.05	100.26			
		10	10.05	20.05	10.05	100.27			
	120	10	11.62	21.62	11.62	98.29	99.28	0.005	0.026
		10	11.62	21.62	11.62	98.28			
		10	11.62	21.62	11.62	98.28			
Tinidazole	80	10	8.04	18.04	8.04	100.23	100.22	0.005	0.005
		10	8.04	18.04	8.04	100.22			
		10	8.04	18.04	8.04	100.23			
	100	10	10.30	20.30	8.04	100.36	100.47	0.095	0.094
		10	10.30	20.30	8.04	100.52			
		10	10.30	20.30	8.04	100.53			

	120	10	12.27	22.27	8.04	102.21	102.21	0.011	0.011
		10	12.27	22.27	8.04	102.21			
		10	12.27	22.27	8.04	102.19			

Table no: 4 % Recovery of Norfloxacin and Tinidazole

Precision

The precision of the method was demonstrated by Intraday precision, Inter day precision and Repeatability.

Intraday Precision

It was found to the analysis of the Norfloxacin and Tinidazole concentration in tablet dosage form were performed six times on the same day and %RSD was calculated.

Interday Precision

It was found out by carrying out the analysis of the Norfloxacin and Tinidazole concentration in tablet dosage form were performed six days over one week and %RSD was calculated.

Norfloxacin

Concentration (μg/ml)	Absorbance		SD		%RSD	
	Intra-Day	Inter-Day	I	II	I	II
4	0.590	0.571				
4	0.586	0.588				
4	0.591	0.591	0.001	0.007	0.311	1.315
4	0.588	0.588				
4	0.590	0.590				
4	0.591	0.591				

Table no: 5 Precision of Norfloxacin

Tinidazole

Concentration (μg/ml)	Absorbance		SD		% RSD	
	Intra-Day	Inter-Day	I	II	I	II
6	0.478	0.477	0.003	0.002	0.806	0.547
6	0.468	0.471				
6	0.477	0.476				
6	0.478	0.478				
6	0.475	0.476				
6	0.477	0.478				

Table no: 6 Precision of Tinidazole

Intra Day Precision

Sample	Observed % RSD	Accepted % RSD	Result
Norfloxacin	0.3114	Not more than 2	Pass
Tinidazole	0.806321		

Table no: 7 Intraday precision

Inter-Day Precision

Sample	Observed % RSD	Accepted % RSD	Result
Norfloxacin	1.315	Not more than 2	Pass
Tinidazole	0.5478301		

Table no: 8 Inter-day precision

Ruggedness

Norfloxacin (4 μg/ml) at 278 nm.

	1	2	3	SD	RSD
Person A	0.590	0.586	0.592	0.003055	0.518391
Person B	0.589	0.594	0.593	0.002646	0.446917

Table no: 9 Ruggedness of Norfloxacin

Tinidazole (6 μg/ml) at 308 nm.

	1	2	3	SD	RSD
Person A	0.306	0.30	0.297	0.004583	1.52245
Person B	0.301	0.310	0.304	0.004583	1.502484

Table no: 10 Ruggedness of Tinidazole

Limit of Detection and Limit of Quantification

- LOD = $3.3\sigma / S$
- LOQ = $10\sigma / S$

Where, σ = standard deviation of the response

S = slope of calibration curve

	Norfloxacin	Tinidazole
SD	0.004482	0.00952
LOD	0.107595	1.022247
LOQ	0.326047	3.097719

Table No: 11 LOD & LOQ of Norfloxacin & Tinidazole

CONCLUSION

The combination of Norfloxacin and Tinidazole is commercially available in tablet forms to control gastrointestinal infections caused by bacterial or amoebic infections. Here, the Q absorbance ratio method for the simultaneous estimation of Norfloxacin and Tinidazole was developed for their analysis. The method was found to be simple, fast, and accurate, with minimal interference from other components in the formulation. The validation results confirmed that the method adheres to the required analytical criteria, such as linearity, precision, and accuracy, making it suitable for routine analysis of these drugs in combined dosage forms. This method offers a significant advantage for quality control in pharmaceutical industries, providing a cost-effective alternative to more complex methods of analysis.

ABBREVIATIONS

LOD : Limit Of Detection

LOQ : Limit Of Quantitation

RSD : Relative Standard Deviation

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GENETIC VACCINE TECHNOLOGIES

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ABSTRACT

Genetic vaccines, encompassing DNA and RNA platforms, represent a major advancement in the prevention and treatment of infectious diseases. Unlike conventional vaccines based on inactivated or attenuated pathogens, these vaccines deliver nucleic acid sequences encoding antigenic proteins, enabling host cells to synthesize the antigens and induce both humoral and cellular immune responses. Recent technological progress—particularly in messenger RNA (mRNA) and plasmid DNA vaccines has enabled rapid design, scalable manufacturing, and high adaptability against emerging pathogens, as exemplified during the COVID-19 pandemic. Innovations such as self-amplifying RNA, genetic adjuvants, nanomaterial-based delivery systems, and computational epitope prediction are enhancing vaccine efficacy, stability, and immunogenicity. Despite challenges related to delivery efficiency, storage stability, duration of immunity, and equitable access, these platforms hold immense promise for combating infectious agents including influenza, Zika, HIV, tuberculosis, and rabies. Ongoing research into universal and therapeutic vaccine strategies, improved thermostability, and needle-free delivery methods underscores their transformative potential in global health preparedness and infectious disease control.

Keywords: genetic vaccines, infectious diseases, vaccine technology, immune response

INTRODUCTION

Genetic vaccines refer primarily to platforms that deliver nucleic acids (DNA or RNA) encoding antigen(s) of interest into the host cells; the host then expresses these antigens and induces an immune response. These contrast with traditional approaches that deliver inactivated/attenuated pathogens or recombinant proteins. Because the recent pandemic accelerated use of these platforms (notably mRNA vaccines), there is growing interest in their broader applicability to other infectious diseases.[1]

Types of Genetic Vaccines

1. mRNA-based vaccines [2]

- mRNA vaccines deliver a messenger RNA encoding a pathogenic antigen (e.g., viral spike protein). The host cell translates it, presents antigen and triggers immune responses (humoral + cell-mediated).
- Advantages: fast design (once sequence known), scalable manufacture, non-integrating, no live pathogen/
- Key challenges: delivery (e.g., lipid nanoparticles), stability (cold chain), durability of immunity, reactogenicity.

2. DNA-based vaccines [3]

- DNA vaccines use plasmid DNA encoding antigen, delivered into host cells (often via electroporation, gene gun, nanoparticles) so that host cells express antigen.
- Advantages include stability, easier storage/handling relative to some RNA, ability to target various antigens.
- Challenges: efficient delivery into nucleus, often lower immunogenicity compared to RNA or viral-vector platforms.

3. Genetic adjuvants and advanced delivery [4]

- The review on "genetic adjuvants" highlights how including genes for immunomodulators (e.g., cytokines, chemokines) in the vaccine vector can enhance immune responses, especially in challenging populations (elderly, immunocompromised) by targeting antigen-presentation, T cell activation and memory formation.
- Nanoparticle / nanomaterial platforms: These are often used for delivery of nucleic acids and can also serve as vaccine scaffolds/adjuvants.

Recent Developments in Infectious Disease Applications [5]

- There are numerous review articles focusing on applying mRNA and DNA vaccines to a range of pathogens beyond SARS-CoV-2: e.g., influenza, Zika, rabies, HIV, TB.
- A recent 2025 review (published August 2025) in *European Journal of Medical Research* discusses the research progress of mRNA vaccines for infectious diseases, covering mechanisms, delivery systems, and challenges.
- The review titled "Infectious disease mRNA vaccines and a review on epitope prediction for vaccine design" (2021) in *Briefings in Functional Genomics* examines mRNA vaccine advances and the computational design of epitopes.
- The "Development of therapeutic vaccines" review (2022) in *Molecular Biomedicine* covers therapeutic vaccines (including DNA/RNA) for infectious diseases and non-communicable diseases.

Strengths & Advantages of Genetic Vaccine Technologies [6]

- **Speed & adaptability**: Once the antigen sequence is known, mRNA/DNA platforms allow rapid development and iteration (important for emerging pathogens). For example, the SARS-CoV-2 pandemic demonstrated how quickly mRNA vaccines could be deployed.
- **Customizability**: Genetic vaccines allow easy modification of antigen sequences (e.g., to adapt to variants), or multiplexing of antigens.
- **Induction of both humoral and cell-mediated immunity**: Since host cells produce the antigen in situ, there is potential for better activation of T-cells (especially $CD8^+$) compared to some subunit vaccines. The genetic adjuvant review emphasises this.

- **Manufacturing & scalability**: Particularly for mRNA, the cell-free lipid nanoparticle manufacture allows faster scale-up compared to traditional live-attenuated or viral-vector vaccines.
- **Safety**: Absence of live pathogen reduces risk of reversion or infection; DNA/RNA platforms avoid many safety concerns of live vaccines.

Challenges and Limitations [7,8]

- **Delivery and stability**: Ensuring efficient delivery of nucleic acids into the appropriate host cells (and for DNA, into nucleus) remains challenging. Cold-chain logistics for mRNA (especially early generations) are non-trivial.
- **Immunogenicity in certain contexts**: Though mRNA vaccines have been highly efficacious for SARS-CoV-2, for many other pathogens the immune correlates are less well defined, and achieving robust long-term immunity remains a challenge.
- **Durability of response**: The longevity of immunity and memory responses from these platforms are still being fully established for many pathogens.
- **Cost and access**: While manufacturing is scalable, global access (especially in low-resource settings) depends on cost, storage, and distribution infrastructure.
- **Safety concerns / reactogenicity / regulatory hurdles**: New platforms pose regulatory and safety monitoring challenges (though so far mRNA vaccines have held up well).

Emerging Trends & Future Directions [10]

- **Self-amplifying RNA (saRNA)**: An advanced mRNA format where the RNA replicates inside the cell, thus producing more antigen for a given dose. This could reduce required dose and cost.
- **Genetic adjuvants**: Encoding immunomodulators in the vaccine vector to enhance and tailor immune responses (especially for populations with weaker responses).
- **Nanomaterial platforms**: Use of nanoparticles not just for delivery, but also as antigen scaffolds/adjuvants—for example presenting antigen in multimeric form or controlling antigen presentation.
- **Therapeutic vaccines**: Not just prophylactic but vaccines for treatment of chronic infectious diseases (e.g., HIV, HBV) or non-infectious diseases (cancer).
- **AI/Computational design**: Use of epitope prediction, machine learning, and bioinformatics to design antigen sequences optimal for immune responses.
- **Improved global access**: Technologies aimed at lower cost, better storage/transport (e.g., thermostable formulations), and novel delivery routes (e.g., intradermal, microneedles).

CONCLUSION

The genetic vaccine technologies (DNA, mRNA, and their derivatives) represent a **transformative advance** in vaccinology, especially for infectious disease control where speed, adaptation and scalability are vital. The future expectation of genetic vaccine technologies are; More universal/multiplex vaccine strategies, Improved delivery technologies and formulations (for example, thermostable, needle-free). Expansion of therapeutic vaccine approaches for chronic/prolonged infections. More local manufacturing in diverse geographies (including India), which will help reduce inequities.

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MICROBIOME: TARGETED DRUG DELIVERY SYSTEM

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ABSTRACT

The human microbiome comprising the collective genomes of trillions of microorganisms inhabiting the body—plays a pivotal role in health, disease, and drug response. Recent advances have revealed that microbiome-drug interactions can profoundly affect pharmacokinetics, bioavailability, and therapeutic efficacy. This insight has led to the emergence of microbiome-based and microbiome-responsive drug delivery systems (DDS), representing a novel paradigm in targeted and personalized therapy. This review explores the current understanding and applications of microbiome science in drug delivery. Microbiome-targeted strategies include colon-specific delivery systems utilizing microbial enzymatic triggers, live microbial and engineered bacterial carriers for local or systemic drug transport, and microbiome-responsive materials designed to release drugs upon exposure to microbial metabolites or enzymes. Additionally, pharmacomicrobiomics—linking individual microbiome profiles to drug response, offers potential for personalized DDS optimization. These approaches have shown promise in treating gastrointestinal diseases, metabolic disorders, infections, and cancer.

Future directions emphasize integrating omics technologies for deeper microbiome-drug carrier understanding, developing smart materials responsive to microbial cues, expanding applications beyond the gut (e.g., vaginal or skin microbiomes), and establishing standardized regulatory frameworks. Ultimately, the integration of microbiome science with advanced DDS holds immense potential to revolutionize precision medicine and therapeutic delivery.

Keywords: microbiome, drug delivery system, targeted therapy

INTRODUCTION

The term *microbiome* refers to the complex community of microorganisms (bacteria, archaea, viruses, fungi) plus their genes and metabolic products that inhabit a particular environment such as the human gut, skin or vagina. In recent years, it has become clear that the human microbiome plays a major role in health and disease influencing metabolism, immunity, and drug responses.[1] At the same time, drug delivery system technologies are evolving beyond conventional carriers, towards more targeted, smart, responsive delivery. The interplay of microbiomes with these systems opens exciting new opportunities: (1) using microbial metabolism or microbial carriers as delivery vehicles, (2) designing carriers that exploit microbiome-mediated cues, (3) integrating knowledge of microbial–drug interactions (pharmacomicrobiomics) to optimise delivery and efficacy.[2,3] The aim of this article is to review this intersection: how microbiome science is being harnessed in drug delivery systems.

Microbiome in human health and drug response

The human microbiome influences many aspects of physiology including nutrient metabolism, immune modulation, and xenobiotic (drug) metabolism. As outlined by Pryor et al., the microbiome can both positively and negatively affect drug action — altering bioavailability, toxicity, and efficacy. Thus, appreciating the microbiome's role is important not just for delivery design, but for the whole pharmacokinetic-/pharmacodynamic (PK/PD) profile of therapeutics.[4]

Mechanistic pathways relevant to drug delivery

From a drug-delivery point of view, key features of the microbiome (especially the gut microbiome) relevant for system design include:

- Microbial enzymatic metabolism of drug molecules or carrier materials (e.g., colon-specific activation)
- Microbial regulation of local physiological microenvironment (pH, mucus layer, metabolite gradients)
- Microbe–host interface interactions (mucosal uptake, immune modulation)
- Potential for use of microbes (or engineered microbes) themselves as delivery agents.[5,6,7]

APPLICATIONS OF MICROBIOME-BASED / MICROBIOME-INSPIRED DRUG DELIVERY SYSTEMS

Colon / Gut-targeted drug delivery

The colon harbours a dense microbiome and distinct metabolic activities — making it a promising 'activation site' for colon-specific delivery. For example, the review by Bakshi et al. ("Exploiting the Metabolism of the Gut Microbiome as a Vehicle for Targeted Drug Delivery to the Colon") describes how microbial metabolism can transform carrier materials (e.g., polysaccharides degraded by colonic bacteria) into triggerable release systems Thus, carriers that resist upper GI digestion but degrade under colonic microbiota can enable site-specific release of drugs (e.g., for ulcerative colitis, Crohn's disease, colorectal cancer).[8]

Live microbial or engineered microbial carriers

Another exciting avenue is to use microbes (probiotics, genetically engineered bacteria) as active delivery vehicles. For instance, the review "Harnessing the Human Microbiome for Innovative Drug Delivery Systems: Exploring Pharmacomicrobiomics and Targeted Therapies" describes approaches such as bacteria-encapsulated nanoparticles, virus-based delivery, and bio-hybrid microbes for targeted therapy.
Such systems might colonise or transiently reside in specific niches and secrete therapeutics, or carry drug payloads to target tissues leveraging microbial tropism or engineered specificity.[9]

Microbiome-responsive or microenvironment-responsive carriers

Carriers can be designed to respond to microbiome-derived cues (e.g., microbial enzymes, metabolites) or the microenvironment shaped by the microbiome (e.g., pH shifts, redox changes). For example, polysaccharide coatings that are degraded by colonic bacteria, allowing

drug release in the colon. Also, nanomedicine strategies for modulating the gut microbiome to enhance barrier function, immune modulation and delivery are described in the "Oral nanomedicine for modulating immunity, intestinal barrier functions, and gut microbiome" review. Thus, the microbiome provides a "trigger" or a "gatekeeper" that can enhance specificity of delivery systems.[10]

Localised mucosal microbiome-based delivery (e.g., vaginal, skin)

Beyond the gut, other microbiome niches are also being tapped. For instance, a recent review on drug delivery systems for women's reproductive health highlights the vaginal microbiome (predominantly Lactobacillus species) and how localized delivery systems (gels, films, nanoparticles). This shows the concept is generalisable to other microbiome-rich sites for local therapeutic or prophylactic delivery.[11]

"Pharmacomicrobiomics" & personalised delivery

Another application area is the tailoring of drug delivery and dosage based on an individual's microbiome profile — acknowledging that different microbiomes metabolise and respond to drugs differently. The term "pharmacomicrobiomics" has been used to describe the study of the interplay between drugs, microbiome and host, which naturally links to delivery system design (e.g., adjusting carrier design, release kinetics, targeting) for a given microbiome state.[12]

Advantages and Potential Benefits [13]

The integration of microbiome-based strategies into drug delivery systems offers several potential benefits:

- **Enhanced targeting and site specificity**: By leveraging microbial environments (such as colon microbiota) or microbial carriers, delivery systems can be directed to specific anatomical or microenvironmental niches, reducing systemic exposure.
- **Triggerable/smart release**: Microbial enzymes or metabolites provide natural "switches" for drug release, enabling responsive delivery (e.g., only release when microbial trigger is present).
- **Improved bioavailability and reduced dose**: Targeted delivery can improve local concentrations of the drug, possibly reducing required dose and side-effects.
- **Exploiting endogenous systems**: Using microbes or microbiome cues may complement or exploit natural biological pathways, potentially improving biocompatibility and reducing immunogenicity compared to purely synthetic carriers.
- **Personalisation potential**: Incorporating knowledge of a patient's microbiome allows tune-making of delivery systems (release kinetics, targeting) for optimized therapy.

Challenges, Limitations & Safety Considerations [14]

Microbiome variability & complexity

Human microbiomes vary widely between individuals (and over time). This creates difficulty in standardising microbiome-based delivery systems: what works in one microbiome context may fail in another. The complexity of microbial interactions, competition, and adaptation also complicates predictions.

Safety and regulatory concerns

Using live microbes (or engineered microbes) as delivery vehicles raises biosafety issues: potential for pathogenicity, gene transfer, unintended persistent colonisation, immune activation. Rigorous safety and containment strategies are needed. PubMed+1 Regulatory frameworks (for live biotherapeutics) are still evolving and may be slower than conventional carriers.

Manufacturing, stability & scale-up

Microbiome-based systems (especially involving live cells or microbially responsive materials) may pose manufacturing challenges: ensuring reproducibility, viability, storage stability, shelf-life, and scale.

Delivery barriers & host interaction

Even with microbial targeting, the host environment may pose barriers: mucosal clearance, immune response, variability in microbial colonisation, changes in microbiome composition (e.g., due to antibiotics or diet) which may alter delivery behaviour. Also, unintended microbial metabolism of the drug or carrier (leading to inactive metabolites, toxic by-products) is a risk.

Lack of translation & clinical data

Although many proof-of-concept studies exist, translation to clinical use remains limited. Complex interactions and regulatory hurdles slow progress. Ensuring robust, reproducible human data is critical.

Future Perspectives & Research Directions [15]

Several key avenues for future research are emerging:

- **Better characterisation of microbiome–drug–carrier interactions**: Improved mechanistic understanding (metagenomics, metabolomics) will help design delivery systems tailored to specific microbiome profiles. For example, the pharmaco-microbiomics field is poised to inform such designs.
- **Smart materials and biosensing carriers**: Carriers that sense microbial metabolites, pH, enzyme activity and respond accordingly (release or activate) will become more sophisticated.
- **Personalised delivery systems**: Based on an individual's microbiome signature, carriers may be customised (e.g., for colon disease, based on microbial enzyme profile).
- **Integration with probiotics/prebiotics**: Combining therapeutic delivery with microbiome modulation (e.g., delivering a drug + probiotic) could synergise therapy and microbiome health.
- **Expansion beyond the gut**: As seen in vaginal microbiome drug delivery reviews, other niches (skin, oral, lung, reproductive tract) offer opportunities.

- **Clinical translation and regulation**: Developing standardised production, safety protocols, regulatory guidelines for live-microbe based or microbiome-responsive delivery systems will be vital.
- **Ethical and ecological considerations**: Manipulating microbiomes or using microbial carriers raises questions about long-term ecological impact (within host or environment), antibiotic resistance, gene transfer.
- **Interdisciplinary approaches**: Collaboration between microbiologists, materials scientists, pharmacologists, bioengineers and clinicians will accelerate progress.

CONCLUSION

The convergence of microbiome science and advanced drug delivery system design presents a rich and relatively under-explored frontier in therapeutic development. By treating the microbiome not just as a backdrop but as a dynamic partner — or even a delivery vehicle — researchers are creating novel strategies for site-specific, smart, and personalised drug delivery. The benefits are many: enhanced targeting, smarter release, leveraging endogenous biology. But the challenges are also substantial: microbiome variability, safety/regulatory concerns, manufacturing complexity, and translational barriers. Nonetheless, the literature indicates there is momentum: from colon-targeted systems exploiting microbial metabolism, live microbial vehicles, microbiome-responsive materials, to mucosal niche delivery strategies. As these technologies mature, and as our understanding of microbiome-pharmacology deepens, we can expect microbiome-based delivery systems to play an increasing role in the next generation of therapies.

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QUALITY CONTROL TESTING APPROACHES FOR PHARMACEUTICAL COSMETIC PRODUCTS

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ABSTRACT

Quality control (QC) is essential to ensure the safety, efficacy, and consistency of pharmaceutical and cosmetic products. Although regulated under different frameworks, both product classes share overlapping analytical requirements such as identity, purity, potency, stability, and microbiological safety. This review summarizes key QC tests used across pharmaceuticals and cosmetics, regulatory expectations, commonly used analytical techniques, approach to method validation, and modern trends including Quality by Design (QbD) and green analytical methods. The review highlights practical considerations for laboratories and manufacturers to maintain compliance and protect consumer safety.

Keywords: Quality control, pharmaceuticals, cosmetics, GMP

INTRODUCTION

Quality control ensures that manufactured products meet predefined specifications and are safe for use. Pharmaceuticals are typically intended to diagnose, cure, mitigate, treat, or prevent disease and therefore face stringent regulatory standards. Cosmetics, although primarily intended for cleansing, beautifying or altering appearance, also require strict QC to prevent harm and ensure product quality.[1] Both sectors employ similar analytical tests (chemical, physical, microbiological) but differ in end-point requirements and regulatory pathways. This review organizes QC tests into core categories and provides guidance on methods and best practices.[2,3]

Regulatory and quality framework [4,5,6,]

- **Pharmaceuticals:** International Council for Harmonisation (ICH) guidelines (e.g., stability: ICH Q1A), pharmacopeias (USP, Ph. Eur., BP), Good Manufacturing Practice (GMP) and country-specific regulations (FDA, EMA). Critical requirements include validated analytical methods, stability data supporting shelf-life, and strict impurity and sterility standards where applicable.
- **Cosmetics:** Regulations vary by region (EU Cosmetic Regulation (EC) No 1223/2009, FDA's FD&C Act in the U.S. for cosmetics, ASEAN cosmetic directives). ISO 22716 provides guidance on GMP for cosmetics. Key requirements include safety assessments, preservative efficacy, labeling, and limits for impurities (e.g., heavy metals, prohibited substances).

CORE QUALITY CONTROL CATEGORIES AND TYPICAL TESTS

Identity and composition [7, 8]

- **Tests:** Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, mass spectrometry (MS), nuclear magnetic resonance (NMR), chromatographic fingerprinting (HPLC/GC).
- **Purpose:** Confirm raw material and final product identity and assay active ingredients or claim substantiation (e.g., 1% w/w vitamin C).

Assay and potency [9,10]

- **Pharmaceuticals:** Quantitative assays (HPLC, UHPLC, LC–MS) to determine active pharmaceutical ingredient (API) content and degradation products.
- **Cosmetics:** Assay of actives (e.g., sunscreen filters, antioxidants) and percentage claims; often less stringent but must be accurate for labeling and safety.

Impurities and related substances [11, 12]

- **Tests:** HPLC with diode-array detection, LC–MS/MS for low-level impurities; GC for volatile impurities; headspace GC for residual solvents.
- **Considerations:** Set acceptance criteria based on toxicological risk; follow pharmacopeial or regulatory thresholds for genotoxic impurities, residual solvents (ICH Q3C), and degradation products.

Microbiological quality [14, 15]

- **Bioburden and Total Viable Count (TVC):** Plate count methods for non-sterile products.
- **Pathogen testing:** Tests for Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans (common in cosmetics and non-sterile pharmaceuticals).
- **Sterility:** For sterile pharmaceuticals, sterility tests per pharmacopeial methods (membrane filtration or direct inoculation).
- **Preservative Efficacy Test (PET) / Challenge test:** Demonstrates preservative system efficacy in products (log reduction criteria over defined timepoints).

Physical and organoleptic tests [16,17]

- **Tests:** pH, viscosity/rheology, particle size and distribution (laser diffraction), appearance, color, odor, homogeneity, spreadability for topical forms.
- **Purpose:** Ensure consumer acceptability, stability, and correct dosing for topical/transdermal systems.

Stability testing [18,19]

- **Accelerated and long-term stability studies:** Conditions defined by ICH (e.g., 25°C/60% RH, 40°C/75% RH) for pharmaceuticals; cosmetics often follow similar protocols adapted to formulation type and intended market.
- **Photostability:** Exposure to light to assess degradation (ICH Q1B for pharmaceuticals; cosmetics require evaluation for photoactive ingredients such as sunscreens).

- **Container-closure interaction:** Extractables and leachables testing, adsorption of actives to packaging.

Safety-related testing [21, 22]

- **Skin irritation and sensitization:** In vitro assays (e.g., reconstructed human epidermis tests) are preferred to animal testing where validated alternatives exist; human repeat insult patch tests (HRIPT) are used cautiously.
- **Toxicological profiling:** For new cosmetic ingredients or impurities, assess systemic toxicity, genotoxicity, phototoxicity, and endocrine activity as required.

3.8 Heavy metals and contaminants [23, 24]

- **Tests:** ICP-MS or ICP-OES for arsenic, lead, mercury, cadmium; PAHs and pesticides screening using GC–MS/MS or LC–MS/MS where relevant.

4. Common analytical techniques and platforms [25, 26, 27]

- **Chromatography:** HPLC/UHPLC, GC, TLC for separation and quantitation.
- **Spectroscopy:** UV–Vis for assay and content uniformity; FTIR and Raman for identity; NMR for structural elucidation.
- **Mass spectrometry:** LC–MS/MS for sensitivity and specificity in impurity profiling, trace analysis.
- **Thermal analysis:** Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA) for polymorph and compatibility studies.
- **Particle sizing & microscopy:** Laser diffraction, dynamic light scattering (DLS), scanning electron microscopy (SEM) for particulate systems and emulsions.

5. Method validation and system suitability [28, 29, 30]

Validated analytical methods are mandatory for regulatory compliance. Validation parameters typically include:

- Specificity/selectivity
- Accuracy
- Precision (repeatability and intermediate precision)
- Linearity and range
- Limit of detection (LOD) and limit of quantitation (LOQ)
- Robustness and ruggedness
- System suitability criteria to be run with each analytical batch (e.g., theoretical plates, tailing factor, %RSD for replicate injections)

6. Good Manufacturing Practice (GMP) and documentation [31, 32]

Robust QC is inseparable from GMP. Key elements include:

- Documented standard operating procedures (SOPs)
- Batch records and release criteria
- Change control and deviation management
- Stability protocols and shelf-life justification

- Supplier qualification and raw material testing
- Environmental monitoring for microbiological control in production areas

7. Challenges and modern trends [33, 34, 35]

- **Quality by Design (QbD):** A science-based approach to design quality into products and processes, using design of experiments (DoE) and risk assessment to set meaningful specifications.
- **Process Analytical Technology (PAT):** Real-time monitoring techniques (NIR, Raman) to control critical quality attributes during manufacturing.
- **Green analytical chemistry:** Minimize solvent use, miniaturize methods, and adopt less hazardous reagents.
- **Advanced analytics and data integrity:** Use of chemometrics, multivariate analysis, and secure LIMS to manage large datasets and ensure data integrity per regulatory expectations.
- **Alternative methods and animal testing replacement:** Adoption of validated in vitro and in silico models for safety testing, driven especially in cosmetics by regulatory bans on animal testing in some regions.

CONCLUSION

Quality control for pharmaceutical and cosmetic products shares many common analytical and managerial elements, but the degree of regulatory stringency differs depending on intended use and market. A modern QC program integrates validated analytical methods, GMP, QbD principles, and up-to-date safety assessments. Continuous improvement, adoption of greener and more efficient analytical techniques, and maintaining compliance with evolving regulations are essential to protect consumers and ensure market access.

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COMPARISON OF PHYTOCHEMICAL INVESTIGATION OF PLANTS WITH SIMILAR PHARMACOLOGICAL ACTIVITY FROM DIFFERENT FAMILIES

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ABSTRACT

Plants have long served as a valuable source of natural compounds with diverse therapeutic applications. Their medicinal properties are primarily attributed to the presence of bioactive phytoconstituents such as volatile oils, resins, tannins, terpenoids, alkaloids, and glycosides. In the present study, two medicinal plants with reported pharmacological activities-*Hemigraphis colorata* (Acanthaceae) and *Emilia sonchifolia* (Asteraceae)-were subjected to solvent extraction for the separation of active constituents. Comparative phytochemical screening and antioxidant assays were conducted to evaluate and correlate their physicochemical and phytochemical profiles. The findings provide insight into the therapeutic potential of these species and support their traditional use in herbal medicine.

KEY WORDS

Phytoconstituents, antioxidant assay, extraction, solvent

INTRODUCTION

Medicinal plants are of great value in the field of treatment and cure of diseases. The medicinal use of plants are based on the active constituents present in it. Most commonly present chemical constituents are volatile oils, resins, tannins, terpenoids, alkaloids, glycosides etc[1]. To improve the ease of administration handling, stability and biological effectiveness, these constituents are separated by various means. In pharmacy the extraction process has been widely employed for the isolation of active constituents from plant.

Hemigraphis colorata (Acanthaceae) is an excellent garden plant which is a native of tropical Malaysia. It is a versatile tropical low-creeping perennial herb that reaches a height of 15-30 cm[2]. This is a prostate herb with rooting branches opposite broad cordate and toothed leaves and terminal heads of small white flowers. The leaves are 6-10 cm long and shimmering silvery violet and red purple. The capsules are small, slender, oval, linear and light green in colour[3]. Seeds are small flat and white in colour and this contain secondary metabolites, phenols, saponins, flavonoids, terpenoids, coumarins, carbohydrates, carboxylic acid, xanthoproteins, tannins, proteins, alkaloids and steroids[4]. In this, the leaves contain flavonoids, poly phenols, and tannins. The stem contains tannins, saponins and the root contains flavonoids and poly phenols.

Emilia sonchifolia is traditionally used medicinal plant seen mostly in tropical and subtropical regions worldwide[5]. Various parts of plant are used for treatment of diseases like asthma, intermittent fever, breast cancer, ophthalmia etc. Decoction of it is used for diarrhoea and whole plant is used as an analgesic[6]. Extractions are reported to have antioxidant, anticancer, wound healing activity[7]. The main chemical constituents present are fumaric acid, succinic acid, esculetin, quarcetin, isorhamnetin, stigmasterol, senkirkine, doronin, luteolin etc.

Therefore, the present study primarily aimed to prepare hexane, chloroform, ethanol, and aqueous extracts of the leaves and stems of the above two plants, and to evaluate their phytochemical profiles and pharmacological activities. The antioxidant potential of the ethanolic extracts was further assessed *in vitro* using the DPPH radical scavenging assay.

MATERIALS AND METHODS

Collection Of Plant Materials

Hemigraphis colorata (Acanthaceae) and ***Emilia sonchifolia*** (Asteraceae) were collected from natural populations in Pampady, Thrissur District, Kerala, India. The plant specimens were brought to the Department of Botany, NSS College, Ottapalam, Palakkad, for further study. Taxonomic identification and authentication of the species were carried out and certified by Prof. Dr. V. Venugopalakrishna Kurup, Head of the Department of Botany, NSS College, Ottapalam.

Extraction of Plants

Leaves and stems of the plants were separated and washed thoroughly with running tap water, separately shade dried at room temperature. After complete drying, the leaves and stems were separately powdered well using a mixer. The powdered drug materials were weighed and kept in air tight containers. Both powdered samples of leaves (50g) and stems (100g) were macerated with solvents of increasing polarity - hexane, chloroform, ethanol and water.

Phytochemical Screening of Extracts

1.Steroids

10mg of extract was dissolved in dry chloroform. Few drops of acetic anhydride were added followed by 1 ml of concentrated sulphuric acid. Appearance of blue colour in the chloroform layer which changes to green.

2. Alkaloids

a) Dragendroff's test- 10mg of extract was dissolved in methanol and a few drops of Dragendroff's reagent were added. Orange red precipitate shows the presence of alkaloids.

b) Mayer's test-10mg of extract was dissolved in methanol and a few drops of Mayer's reagent were added. Yellow precipitate shows the presence of alkaloids.

3.Flavonoids

Shinoda test -10 mg of extract dissolved in methanol. Magnesium turnings were added into this followed by few drops of concentrated hydrochloric acid. A magenta colour shows the presence of flavonoids.

4. Coumarins

10mg extract was dissolved in methanol and alcoholic potassium hydroxide was added. Appearance of yellow colour which decolourises while adding concentrated hydrochloric acid showed the presence of coumarins.

5. Saponins

Extract was dissolved in water and shaken well. Froth formation, which lasts for a long time, shows the presence of saponins.

6. Carbohydrates

a) Molisch's test - About 10mg of the extract was dissolved in 1ml water. Add Molisch's reagent. Then carefully add 1ml conc. sulphuric acid along the sides of the test tube. Deep violet colour at the junction of two liquids indicated the presence of carbohydrates.

b) Fehling's test- To 3ml extract add Fehling's solution A and Fehling's solution B. Mix well and heat it on a water bath for 10 minutes. Appearance or brick red colour indicates the presence of reducing sugar.

Phytopharmacological Screening

Antioxidant Assay - DPPH Radical Scavenging Method

Radical scavenging activity was determined by spectrophotometric method based on the reduction of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH).

Different dilutions of *Hemigraphis colorata* (acanthaceae) and *Emilia sonchifolia* (Asteraceae) were added at equal volumes to a methanolic solution of DPPH. After 15 minutes, incubate at room temperature and the absorbance measured was recorded at 517 nm.

Percentage activity was calculated using formula = $[(A_{control}-A_{sample}) / A_{control}] \times 100$.

Where ascorbic acid was used as positive control.

RESULT AND DISCUSSION

Common Name	Murikotty	Muyalcheviyan
Botanical Name	*Hemigraphis colorata*	*Emilia sonchifolia*
Family	Acanthaceae	Compositae
Image of plant		
Habit	Versatile tropical low-creeping perennial herb.	Annual herb, erect , tropical plant
Height	15-30 cm	10-15cm
Root		Deep rooting and purplish green color.
Leaves	Opposite broad cordate and toothed leaves, 2-8cm long, 4-6cm wide scalloped or lobed margines.	Lyrate-pinnatilobate, sessile, green, alternate and mainly on stems. lower leaves have narrow winged petioles and fine, blunt teeth or deep, rounded.
Flower	Terminal head of small white flowers. (1-1.5cm)	Pink or purplish red and small.
Capsule	Capsules are small, slender oval, linear and light green in color.	Narrow and bell shaped.
Seed	Seeds are small flat and white in color.	

Phytochemical Screening of Extracts: *Hemigraphis colorata* (Acanthaceae)

Class of compounds		Hexane extract		Chloroform extract		Ethanol extract		Aqueous extract	
		S	L	S	L	S	L	S	L
Carbohydrates	Molisch's reagent	+	+	+	+	–	–	–	–
	Fehling's reagent	+	–	+	+	–	–	–	–
Alkaloids	Mayer's test	+	+	+	+	_	_	+	+
	Dragondroff's test	+	+	+	+	_	_	+	+
Flavonoids		_	_	_	_	_	+	+	+
Coumarins		_	_	_	+	+	+	+	+
Saponins		+	+	+	+	+	+	+	+
Phenols		+	+	+	+	+	+	+	+

Emilia sonchifolia **(Asteraceae)**

Class of compounds		Hexane extract		Chloroform extract		Ethanol extract		Aqueous extract	
		S	L	S	L	S	L	S	L
Carbohydrates	Molisch's reagent	+	+	+	–	+	+	–	+
	Fehling's reagent	+	+	+	+	–	–	–	–
Alkaloids	Mayer's test	–	+	+	+	–	–	–	+
	Dragondroff's test	–	+	+	+	–	+	+	+
Flavonoids		–	–	–	–	–	–	–	–
Coumarins		–	+	+	+	+	–	–	–
Saponins		+	+	+	–	–	–	+	+
Phenols		+	+	–	+	–	–	–	–

Phytopharmacological Screening

Antioxidant Assay DPPH Radical Scavenging Method

Standard: Ascorbic acid

SL NO	Concentration (µg/ml)	Absorbance at 517 nm	% Activity
1	100	0.521	43.90
2	200	0.439	52.70
3	300	0.237	74.40
4	400	0.125	86.50
5	500	0.015	98.38

***Hemigraphis colorata* (Acanthaceae)**

SL NO	STEM			LEAF		
	Concentration (µg/ml)	Absorbance at 517 nm	% Activity	Concentration (µg/ml)	Absorbance at 517 nm	% Activity
1	100	0.729	21.52	100	0.739	20.45
2	200	0.531	42.84	200	0.627	32.50
3	300	0.304	67.27	300	0.437	52.96
4	400	0.167	82.02	400	0.328	64.69
5	500	0.103	88.91	500	0.191	79.44

***Emilia sonchifolia* (Asteraceae)**

SL NO	STEM			LEAF		
	Concentration (µg/ml)	Absorbance at 517 nm	% Activity	Concentration (µg/ml)	Absorbance at 517 nm	% Activity
1	100	0.734	20.99	100	0.639	31.21
2	200	0.602	35.19	200	0.528	43.16
3	300	0.404	56.51	300	0.482	48.11
4	400	0.222	76.10	400	0.238	74.38
5	500	0.102	89.02	500	0.082	91.17

CONCLUSION:

Hemigraphis colorata has a richer and more diverse phytochemical profile than Emilia sonchifolia, containing flavonoids, coumarins, saponins, and phenols in all extracts. This suggests it has broader therapeutic potential and stronger bioactive properties. In contrast, Emilia sonchifolia has fewer phytochemicals, mainly alkaloids and phenols, indicating narrower pharmacological applications.

Both plants exhibit good antioxidant activity that increases with concentration. In H. colorata, the stem extract shows stronger antioxidant activity than the leaf. In E. sonchifolia, the leaf extract demonstrates the highest activity (91.17% at 500 µg/ml), close to standard ascorbic acid (98.38%). This indicates that both plants contain compounds capable of neutralizing free radicals, with E. sonchifolia leaf being slightly more effective.

Overall, H. colorata is a better source of diverse bioactive compounds, while E. sonchifolia offers strong antioxidant activity, especially in its leaves. Both plants can be considered valuable natural sources of antioxidants.

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BIOACTIVE AND ECO-SAFE PHYTOCONSTITUENTS AS ALTERNATIVE PHOTOPROTECTANTS TO CONVENTIONAL SUNSCREENS: A REVIEW

Abstract

This review examines the mechanisms of skin damage induced by ultraviolet radiation (UVR) and explores the protective role of plant-derived phytomolecules. Utilizing a comprehensive review of UV classification, skin responses, and secondary plant metabolites, many research highlights how UVA and UVB rays generate reactive oxygen species and DNA damage, leading to photoaging, immunosuppression, and skin carcinogenesis. Plant-derived molecules with SPF activity, such as flavonoids (quercetin, rutin), phenolic acids (ferulic, caffeic), tannins, carotenoids, and coumarins, protect the skin primarily through dual mechanisms — UV absorption and antioxidant defence. Their conjugated aromatic structures enable direct absorption of harmful UVA and UVB radiation, while their potent antioxidant capacity neutralizes reactive oxygen species generated by UV exposure, preventing oxidative stress, inflammation, and DNA damage. Some also exhibit anti-inflammatory effects by downregulating COX-2 and cytokines, thereby enhancing cellular repair. In short, these phytoconstituents act as natural photoprotectants, offering eco-safe, multifunctional alternatives or enhancers to conventional sunscreens. This underscores the potential for developing herbal photoprotective formulations that leverage bioactive plant constituents to improve skin health and prevent UV-induced damage.

Keywords: Ultraviolet radiation, Reactive Oxygen Species, Natural Photoprotectants, Eco-safe

Introduction

Ultraviolet (UV) radiation, a component of solar electromagnetic energy, plays a dual role in human physiology—serving as both a vital and a potentially deleterious environmental factor. Spanning the wavelength range of 100–400 nm, UV radiation is classified into three major types: UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm). While the stratospheric ozone layer effectively absorbs nearly all UVC and most UVB radiation, UVA accounts for over 90% of solar UV energy reaching the Earth's surface. Despite its lower energy, UVA penetrates deeply into the dermal layers, contributing significantly to photoaging, immunosuppression, and oxidative stress. Conversely, UVB, though less prevalent, is highly energetic and primarily affects the epidermis, inducing erythema, sunburn, DNA damage, and carcinogenesis.[1][3][5]

The skin, functioning as the primary barrier against environmental insults, possesses intrinsic defence mechanisms—melanin synthesis, keratinocyte apoptosis, and antioxidant systems—to counteract UV-induced oxidative and inflammatory injury. Nevertheless,

prolonged or unprotected exposure leads to cumulative photodamage characterized by collagen degradation, elastin fiber disorganization, and mutagenic alterations in cutaneous cells. The pathophysiological mechanisms underlying UV-mediated skin damage involve the formation of reactive oxygen species (ROS), photodimerization of DNA bases, and lipid peroxidation, all of which compromise cellular integrity and accelerate dermal aging.[2][5]

Given the rising awareness of the adverse effects associated with synthetic UV filters as Gudsol and benzophenone-3 [14]—such as photoinstability, skin sensitization, and environmental bioaccumulation—the research focus has shifted toward plant-derived photoprotective compounds. Secondary plant metabolites (SPMs) such as flavonoids, phenolic acids, coumarins, carotenoids, terpenoids, and alkaloids have demonstrated substantial broad-spectrum UV-absorbing and antioxidant capabilities**.** These polyaromatic and conjugated molecules not only absorb UVA and UVB wavelengths efficiently but also quench free radicals and inhibit inflammation without eliciting adverse photochemical reactions.

Furthermore, several plant extracts—including *Aloe vera*, *Vitis vinifera*, *Curcuma longa*, and *Calendula officinalis*—have been incorporated into marketed sunscreen formulations, underscoring their translational potential in dermatocosmetic applications. These botanicals act synergistically to mitigate photodamage, promote collagen preservation, and enhance the skin's natural defence systems. Notably, established chemical classes of sun-protective phytomolecules such as flavonoids (quercetin, catechin), phenolic acids (ferulic acid, caffeic acid), carotenoids (β-carotene, lutein), and curcuminoids (curcumin) contribute to their UV-protective efficacy. Consequently, phytochemical-enriched sunscreens represent a sustainable, biocompatible, and efficacious alternative to conventional UV filters, aligning with contemporary trends in green chemistry and natural product-based therapeutics.

UV radiation and skin damage[1][3][4][5]

Ultraviolet radiation (UVR) ranges from 100 to 400 nm and is divided into UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm). The ozone layer blocks nearly all UVC and about 90% of UVB, making UVA account for over 90% of total daily UV exposure on earth.

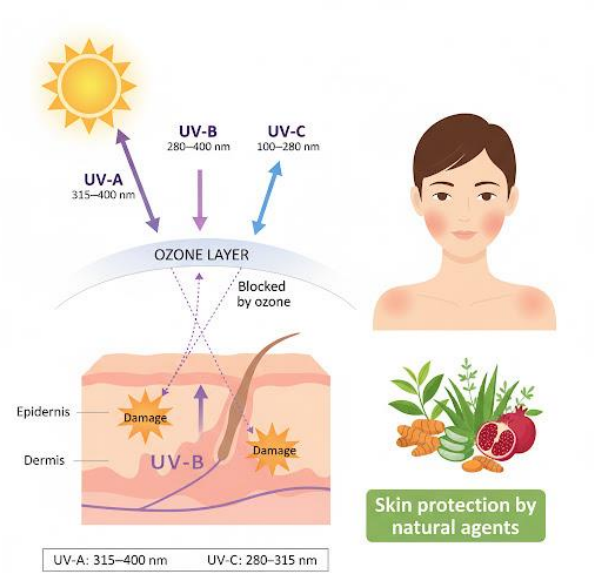


Figure 1: Different types of UV radiation on skin

Type of UVR	Characteristics	Acute Harmful Skin Effects	Chronic Harmful Skin Effects
Ultraviolet A radiation (UVA) 315 to 400 nm	Is not filtered by the stratospheric ozone layer in the atmosphere 90–99% reaches the earth's surface Can penetrate deeper into the skin	Immediate pigment darkening Tanning	Photoaging: skin elasticity reduction and increase wrinkling. Immunosuppression
Ultraviolet B radiation (UVB) 280 to 315 nm	Filtered by the stratospheric ozone layer in the atmosphere 1–10% reaches the earth´s surface Can penetrate the upper layers of the epidermis	Edema, erythema, darkening, sunburns. Thickening of the epidermis and dermis	Photoaging Immunosuppression Skin cancer
Ultraviolet C radiation (UVC) 100 to 280 nm	Completely filtered by the stratospheric ozone layer in the atmosphere Major artificial sources are germicidal lamps	Burn	Skin cancer

Table 1: Classification of UV radiation, characteristics and harmful effects

Mechanism of skin damage

UVA radiation penetrates deeply into the skin, causing immediate tanning, premature aging, and immunosuppression by generating reactive oxygen species (ROS) that damage DNA, proteins, and lipids, ultimately leading to collagen degradation and wrinkle formation.

In contrast, UVB radiation primarily affects the epidermal basal layer, being far more potent in inducing sunburn, inflammation, and skin cancer through inflammatory mediator release, collagen reduction, and cellular damage.[1][2]

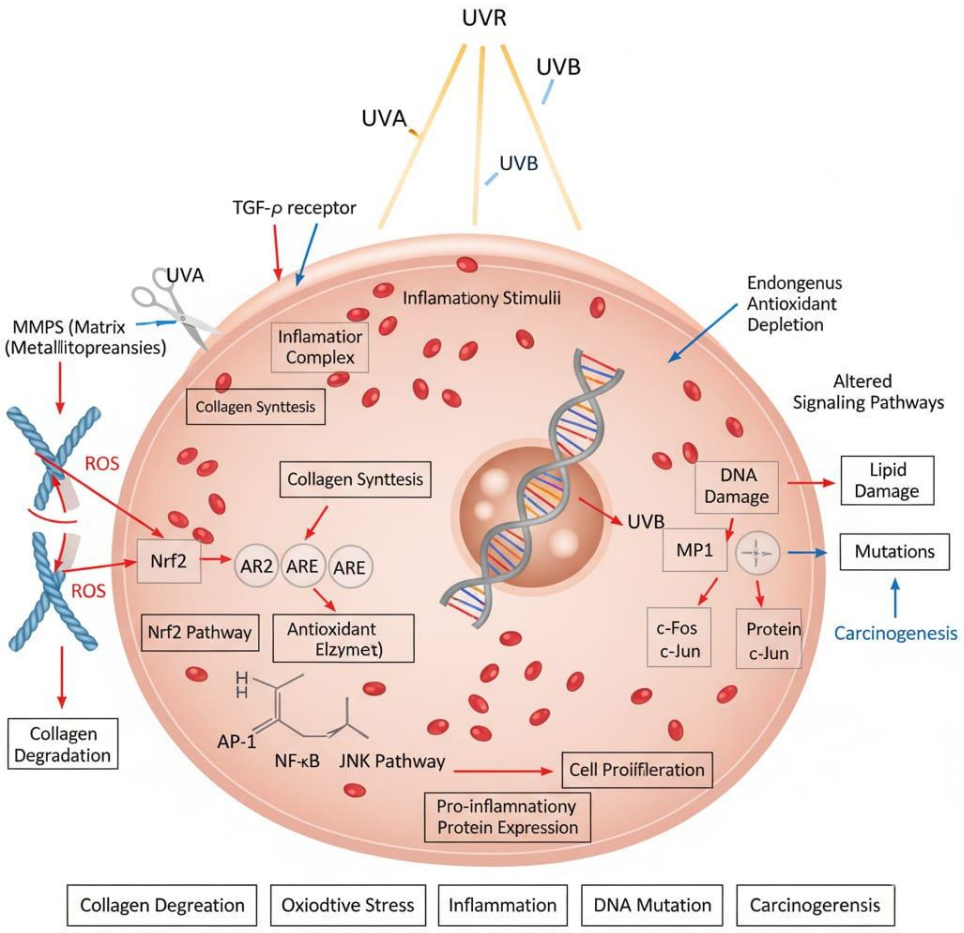


Figure 2: Mechanism of skin damage

Ultraviolet (UV) rays can exert both harmful and beneficial effects on human skin. UV-C is highly mutagenic due to its ionizing properties and can contribute to immune-mediated diseases and skin cancer, with certain genetic and hereditary factors increasing susceptibility. The skin defends itself through melanin, which absorbs and dissipates UV radiation, and through apoptosis of keratinocytes via cell-surface death receptors like Fas to prevent malignant transformation. UV radiation can cause DNA damage through photodimer formation and reactive oxygen species (ROS), with the melanocortin 1 receptor (MC1R) playing a key role in pigmentation, tanning, and skin cancer risk. On the beneficial side, UV-B stimulates pre-vitamin D3 formation and provides mild photoprotection through tanning and epidermal hyperplasia, highlighting the need to balance prevention of harmful effects while harnessing the positive ones. [2][5]

Synthetic sunscreens

Synthetic sunscreens are formulations that combine organic (chemical) and inorganic (physical) UV filters to provide broad-spectrum protection against harmful ultraviolet radiation. Chemical filters such as avobenzone, octocrylene, and oxybenzone absorb high-energy UV rays, while inorganic compounds like titanium dioxide (TiO_2) and zinc oxide (ZnO) reflect, scatter, and absorb UV radiation, enhancing overall protection. Advances in nanotechnology have enabled these metal oxides to be used as nanoparticles, improving texture

and transparency on the skin while maintaining high Sun Protection Factor (SPF). Despite ongoing studies on nanoparticle safety, their incorporation has made sunscreens more effective and cosmetically appealing. Popular marketed synthetic sunscreens include Neutrogena Ultra Sheer Dry-Touch SPF 100, La Roche-Posay Anthelios Melt-in Milk SPF 100, and Coppertone Sport SPF 50, which utilize a combination of these organic and inorganic filters for optimal UV defence.

Demerits of synthetic sunscreens

- Incompatible with all skin type
- Synthetic sunscreens can trigger the formation of reactive oxygen species (ROS) in human skin; however, natural antioxidants such as alpha-carotene, ascorbic acid, and flavones possess electron-donating abilities that help neutralize these free radicals and interrupt oxidative chain reactions.[15]
- Nanoscale Titanium dioxide (TiO_2) and Zinc oxide (ZnO) exhibit higher photocatalytic activity and potential skin penetration, posing risks of toxicity. These nanoparticles primarily block shorter UV wavelengths (UVB–UVAII) and may generate reactive oxygen species (ROS) upon UV exposure, leading to oxidative stress, photoallergic dermatitis, and premature skin aging.[16]
- Sunscreens containing Titanium dioxide (TiO_2) and Zinc oxide (ZnO) nanoparticles primarily absorb and scatter shorter UV wavelengths (UVB to UVA-II) rather than longer UVA or visible light. However, these nanoparticles can generate reactive oxygen species (ROS) upon UV exposure and may penetrate the stratum corneum, potentially causing photoallergic contact dermatitis and premature skin aging with prolonged use.[16]
- Synthetic Sunscreen causes bleaching of coral reefs

Sun Protection Factor

The Sun Protection Factor (SPF) is the quantitative metric used to gauge a sunscreen's effectiveness against Erythema-Inducing Radiation (EIR), the UV rays that cause sunburn For a sunscreen to be truly effective, it must demonstrate a broad range of UV absorbance between 290 and 400 nm. Historically, a product's SPF was primarily determined through engthy, variable, and ethically complex in vivo tests on human volunteers. Now, while in vivo testing remains the standard, in vitro SPF is increasingly valuable for preliminary screening during the product development phase. Critically, a sunscreen's protective efficacy should be understood by evaluating the percentage of EIR transmitted to the skin, not the percentage absorbed by the sunscreen itself. For instance, doubling the SPF from 30 to 60 effectively doubles the protection by halving the % EIR transmitted (from approximately 3.3% to 1.7%).

Phytochemical	Plant Source	Type/Class	SPF Range
Quercetin	Onion, Tea, Apple	Flavanol	10-30

Rutin	Buckwheat, Citrus	Flavonoid Glycoside	8-18
Ferulic acid	Rice bran, oats	Phenolic acid	10-37
Silymarin/Silibinin	Milk thistle	Flavonolignan	5-14
Green tea polyphenols	*Camellia sinensis*	Catechins/Polyphenols	10-13
Ellagic acid	Pomegranate, Berries	Polyphenols	7-12
Resveratrol	Grapes, Berries	Stilbene	10-13
Lignin	Wood, Flax, Corn husk	Phenolic polymer	Up to 40
Carotenoids	Tomato, Carrot	Terpenoid pigment	1-2

Table 2: Phytomolecules with desirable SPF values

Among these, flavonoids like quercetin and rutin are the most widely investigated. Quercetin exhibits strong absorption in the UVA and UVB regions (250–380 nm) and produces SPF values ranging from 10 to 30 depending on concentration and formulation type. Rutin, a glycosylated form of quercetin, shows SPF values of 8–18 and enhances photostability of other sunscreen agents [17]. Phenolic acids, particularly ferulic acid, contribute to both UV absorption and antioxidant stability, and when incorporated into formulations with vitamins C and E, increase SPF by up to 37% in vivo [24]. Silymarin, a flavonolignan complex from Silybum marianum, provides SPF values between 5 and 14 and offers protection against UV-induced lipid peroxidation and inflammation [29]. Similarly, green tea catechins (EGCG) and ellagic acid from pomegranate possess moderate SPF (≈10–13) and reduce ROS generation and DNA damage [30][21]. Recent studies have also highlighted lignin nanoparticles as potent natural UV filters with SPF values exceeding 30–40 in optimized formulations[31].

Overall, these phytomolecules provide multifunctional photoprotection through direct UV absorption, antioxidant action, enzyme inhibition, and DNA protection, supporting their application as safe and eco-friendly alternatives to synthetic UV filters in herbal sunscreens.

Marketed Product	Company Name	Formulation	Active pharmaceutical ingredient (API)	SPF value	Benefits
Protect and Moisture Sun Lotion	Nivea	Lotion	• Octocrylene • Butyl Methoxydibenzoylmethane	SPF 50	• Protection against UV-A and UV-B. • Sun and Collagen Protection. •Moisturising.
Rosy Tone Broad Spectrum Sunscreen	L'Oreal Paris	Cream	• Avobenzone-3% • Homosalate-2.7% • Octisalate-5% • Octocrylene-7%	SPF 30	• Anti-ageing • Keeps skin hydrated
Sheer Zinc Face Dry-Touch Sunscreen Broad Spectrum	Neutrogena	Lotion	Zinc oxide (21.6%)	SPF 50	• Protect against UVA and UV-B rays. • Water resistant. • Non-Comedogenic
Sunforgettable total Protection Brush-On Shield	Colorescience	Brush-on	• Zinc oxide (22.5%) • Titanium Dioxide (22.5%)	SPF 50	• Water resistant. • Protect against UV-A and UV-B rays.

Table 3: Some commercial Sunscreen formulations and their label claims on SPF and benefits.

Major Classes of Secondary Plant Metabolites (SPMs) useful as Sunscreens[1][2][6][7][8]

1. Phenylpropanoids (also called Ethylpropanoids) and their derivatives

These are aromatic compounds derived from phenylalanine or tyrosine. They contain carbon (C), hydrogen (H), and oxygen (O) atoms, and usually have multiple hydroxyl (–OH) groups.These compounds are found in families or plants as *Aloe vera* (Liliaceae), *Camellia sinensis* (Theaceae), *Curcuma longa* (Zingiberaceae), *Vitis vinifera* (Vitaceae), *Calendula officinalis* (Asteraceae), and *Ocimum sanctum* (Lamiaceae).

Examples and types:

- Simple polyphenolics: acids, alcohols, aldehydes (e.g., cinnamic acid, p-coumaric acid).
- Aromatic or poly-aromatic polyphenols:

    - Flavonoids (e.g., Quercetin) present in Tomato (*Solanum licopersicum*)
    - Stilbenes (e.g., Resveratrol) present in Grape (*Vitis vinifera*)
    - Curcuminoids (e.g., Curcumin) present in Turmeric (*Curcuma longa*)
    - Coumarins (e.g., Umbelliferone) present in Carrot *(Daucus carota*)
- Glycosides: phenolic compounds linked with sugar moieties such as rhamnose, mannose, rutinose, etc.

2. Terpenoids

- Built from isoprene (C_5H_8) units.
- Contain long carbon–hydrogen (C–H) chains, often unsaturated.
- Usually have a non-aromatic carbon ring (C–H cycle) as their nucleus.
- Responsible for essential oils, resins, and pigments in plants.

Examples: Monoterpenes (menthol), Sesquiterpenes, Diterpenes (phytol), Triterpenes (squalene), Carotenoids .

3. Nitrogen-containing Heterocycles

These compounds contain nitrogen (N) in their ring structure.
They include several biologically active molecules:

Examples:

- Purines and Pyrimidines (e.g., Adenine, Cytosine — components of nucleic acids)
- Porphyrins (e.g., heme structure)
- Chlorophylls (contain porphyrin ring with Mg^{2+})
- Flavins (e.g., Riboflavin — vitamin B_2)

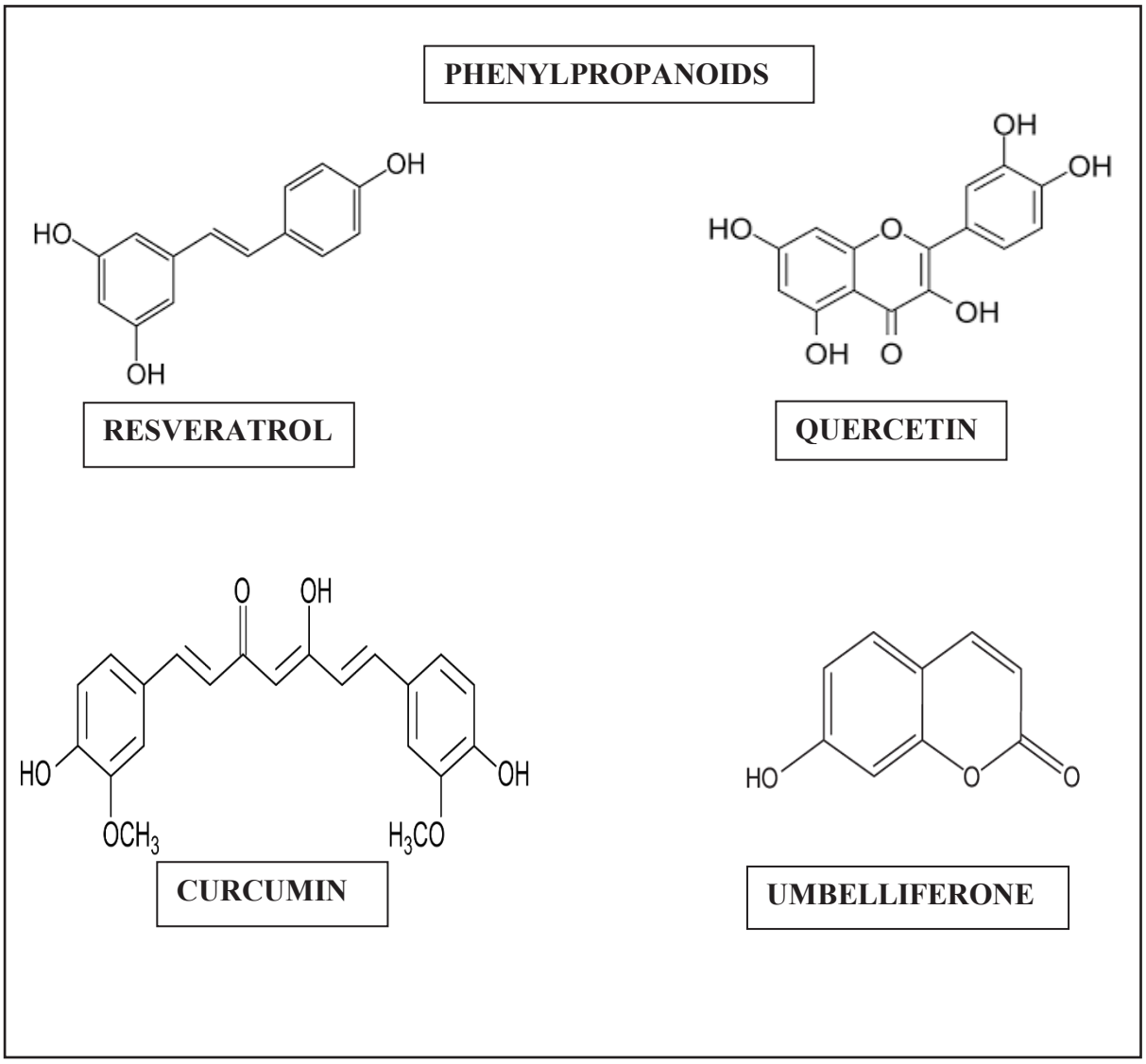
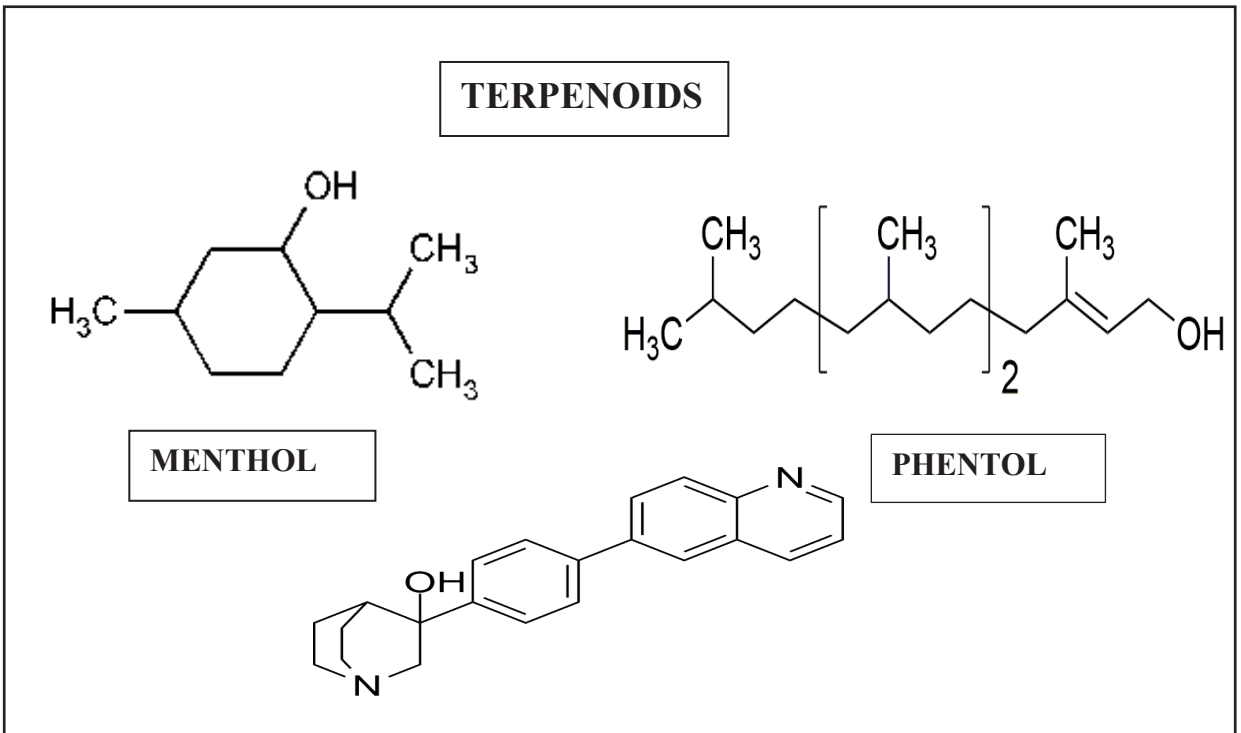


Figure 3: Examples of Phenylpropanoids



SQUALENE

Figure 4: Examples of Terpenoids

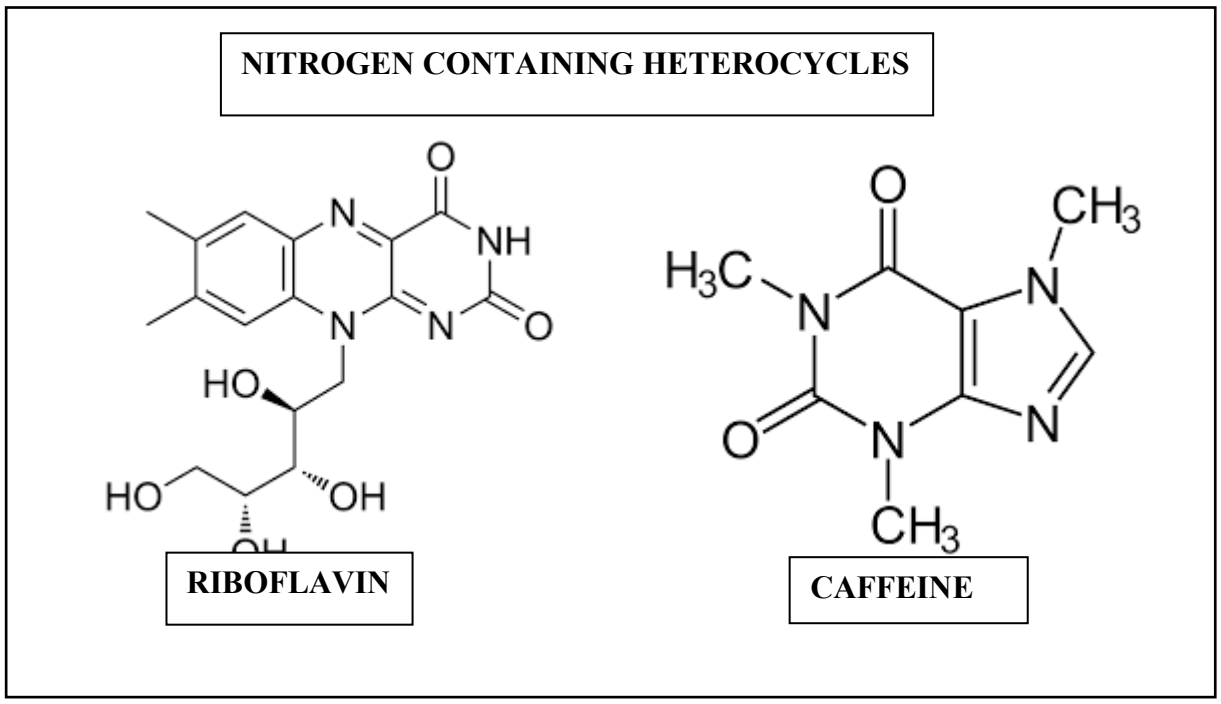


Figure 5: Examples of nitrogen containing heterocycles

Polyphenols, including phenylpropanoids, their glycosides, and bioflavonoids, are produced in plant cells through the phenylpropanoid pathway when exposed to UV light. These compounds have aromatic 5- and 6-carbon rings with multiple hydroxyl groups, allowing them to absorb UVA and UVB rays effectively, giving them natural sunscreen properties. Unlike synthetic sunscreens, they do not promote harmful photochemical reactions and are considered safer. Due to their highly conjugated aromatic structure, they require UV light to become excited, and the absorbed energy is safely dissipated within the molecule. The perpendicular arrangement of their rings helps trap electrons, enhancing stability. Additionally, when these compounds are glycosylated (combined with sugar molecules), their photo-stability increases, making them more resistant to UV damage. [8][9][12][3]

Plant name	Plant part(s) used	Extraction Method	Type of compounds	Activity
Amaranthus viridis	Fabrics	Methanolic and aqueous	Phenolics and flavonoids	Anti-inflammatory
Baccharis antioquensis	Aerial parts	Acidulated acetone	Total anthocyanins, total phenols	Photoprotective and anti-oxidant activity
Blackberry, raspberry	Fruits	Ethanol	Anthocyanins, flavonoids	Antioxidant,Anti-cancerous and antimicrobial
Calamagrostis effusa	Rhizome	Acidulated acetone	Total anthocyanins, total phenols	Antioxidant,Anti-cancerous and antimicrobial
Calea fruticosa	Aerial parts	Extracted successively with n-hexane, ethyl acetate, and ethanol	Flavonoids and terpenoids, sesquiterpenic lactone, flavonol, glucosylated coumarin	Photoprotection and anti aging
Coffea arabica	Coffee	80% acetone, or hydroalcoholic	Total phenolics	Antioxidant and photoprotective activity.Prevent the skin cancer
Disterigma alaternoides	Leaves	Acidulated acetone	Total phenolic; Total monomeric anthocyanin pigment, Total anthocyanins	Anti inflammatory
Drimys granadensis	Leaves	Acidulated acetone	Total phenolic; Total monomeric anthocyanin pigment, Total anthocyanins	photoprotective activity due to their UV-absorbing and antioxidant properties.
Dalbergia ecastaphyllum	Leaves	Hydroethanol	Carotenoids, chlorophylls, total phenolic and flavonoids	Photoprotective and antioxidative action
Elaeagnus angustifolia	Leaves	70%MeOH	Flavonoids, Phenols	photoprotection by directly absorbing UV radiation and by mitigating the downstream effects of UV exposure, such as oxidative stress,

				inflammation, and cellular damage
Ginkgo biloba	Purchased extract	Ethanol	flavonoid	photoprotective agent, mainly by defense systems against oxidative stress and inflammation caused by UV radiation
Hibiscus roseus	Leaves, flowers	Acidulated acetone	Phenolic compounds	Photoprotective and anti oxidative action
Helianthus annuus	Leaves	Methanolic extract	Hydroxycinnamic acids, cell wall-bound phenolics	against UV-induced damage and photoaging
Juglans regia	Male flower	Methanolic	Antioxidant fatty acids, flavonoids and other secondary metabolites	helps prevent UVB-induced apoptosis (programmed cell death) in skin cells
Nephelium lappaceum	Peel	Ethanolic	Tannins and flavonoids	Significant photoprotective and antiphotoaging action
Sophora japonica	Flower	Water	Polysaccharide	Decrease UVB-induced cytotoxicity (cell death) and apoptosis (programmed cell death)
Solanum nigrum	Fabrics	Methanolic and aqueous	Phenolics and flavonoids	Photoprotective activity
Vitis vinifera	Peel or fruits	Hydroalcoholic	Flavonoid enriched extract (FE)	1. Effects aginst skin cancer and skin tumorigenesis.
Daucus carota	Root	Ethanolic or hexane	Anthocyanins-cyanidin, peonidin, pelargonidin.	Anthocyanins is the antioxidant activities and fortification
Calendula officinalis	Flowers	Ethanolic	Apigenin	prevention of UVA/UVB-induced skin carcinogenesis.

Vitis vinifera	Seed extract	Ethanolic	Oligomeric proanthocyanidins, Catechin, epicatechin, and taxifolin	antioxidant activity and anti-aging action.
Melaleuca alternifolia	Leaves	methanolic or hexane	terpinen-4-ol, 1,8-cineole,, alpha-terpineol, and gamma-terpinen.	It is an effective antiseptic, fungicide, and germicide
Juglans regia	Fresh green shells	Acetone	myricetin and juglone	UV protection propery and antioxidant properties.
Emblica officinalis	Fruit extract	methanolic or ethanolic	1-O-Galloyl-β-D-glucose (β-glucogallin), β-Glucogallin	Photoprotection efficacy,antioxidant and anti-aging.
Citrus limon	Fruits and seed extract	Hexane or Ethanolic	Ascorbic acid (Vitamin C)	protection against acute UVB damage
Punica granatum	Fruits	Acetone	Ellagitannins and anthocyanins.	Photoprotection and antioxidant activity
Cucumis sativus	Fruits	Water or Ethanolic	ascorbic acid (vitamin C) and caffeic acid,	remove dead skin cells and tightens skin.
Aloe barbadensis	Leaves	Ethanolic or Methanolic	aloin, aloesin	effective moisturizer, and work as a healing agent for the skin
Glycyrrhiza glabra	Root extract	Ethanloic	Methyl and methyl anthranilate	Photoprotective activity
Curcuma longa	Rhizome	Methanolic	Curcumin (diferuloylmethane), polyphenolic compounds, curcuminoids, demethoxycurcumin	Curcumin possesses anti-inflammatory, antitumoral, and antioxidant properties.
Crocus sativus	Powder	Aqueous methanolic or ethanolic	Homosalate	Saffron can be used as a natural UV absorbing agent

Table 4: Source, isolation method and properties of phytoconstituents with sun screen potential.

Phytomolecules used in marketed sunscreens

Herbal sunscreens utilize phytomolecules —plant-derived bioactive compounds such as flavonoids, phenolic acids, coumarins, carotenoids and tannins—to provide natural UV protection and antioxidant support. These molecules absorb UVA and UVB radiation and neutralize reactive oxygen species, helping to prevent photo-oxidative skin damage . Popular herbal sunscreen brands in India that leverage these plant-based actives include Mamaearth, Soulflower and Biotique.Increased concerns around synthetic UV filters—such as their environmental impact or potential skin sensitization—have driven growth in these botanical alternatives as safer, more sustainable options. Consequently, Phytomolecules - enriched herbal sunscreens are emerging as promising choices for everyday broad-spectrum photoprotection with skincare benefits. [3][10][11][13][15]

Popular herbal sunscreen brands	Natural sunscrenns	Key ingredients
Mamaearth	Mamaearth Hydragel Indian Sunscreen (50 g)	Aloe Vera, Raspberry , Glycerine
Biotique	Bio Sandalwood Sunscreen Lotion (120 ml)	Squalene,Santalols (Sandalwood oil) Ashwagandha Arjuna, Honey,
Soulflower	Soulflower Broad Spectrum Sunscreen Spf 50+ Pa+++	2% Turmeric, 5% Aloe Vera, 4% Olive Oil & 0.5% Retinol (Vitamin A)
Greenberry Organics	SPF 40+ Sunscreen Spray Lotion PA+++ UVA/UVB Protection (120 ml)	Jojoba oil, Argan oil, Shea butter

Table 5: Popular herbal sunscreen brands with their Sun Protection Factor (SPF) and PA (Protection Grade of UV-A) label claims.

Several cosmetic and dermatological brands have developed herbal or phytochemical-based sunscreens that utilize bioactive plant compounds with proven UV-absorbing, antioxidant, and skin-protective properties. These formulations combine natural UV filters such as flavonoids, phenolic acids, and polyphenols with traditional herbal extracts to achieve broad-spectrum protection.

Plant name	**Marketed products**
Black carrot (*Daucus carota*)	BIOTIQUE Sun Shield Carrot Sunscreen Ultra Protective Lotion
Marigold (*Calendula officinalis*)	Mamaearth Rich Moisturizing Ultra Light Sunscreen-SPF 50 Baby Sunscreen-With Calendula & Zinc Oxide
Grape seed (*Vitis vinifera*)	Lilymin SPF 50 Sunscreen With Grape Seed Extract
Tea tree (*Melaleuca alternifolia*) oil	Naturali Acne Control Sunscreen With Tea Tree Oil, Hemp Oil & Salicylic Acid
Walnut (*Juglans regia*)	ASSENTIA Sunscreen with SPF 50 & PA+++ for UVA/UVB & Blue Light
Amla (*Emblica officinalis*)	NutriPro Vitamin-C Sunscreen Lotion SPF 50 PA+ + , UVA & UVB | Amla Fruit Extract & Orange Peel Extract| For Oily, Light & Non-Sticky Skin
Lemon (*Citrus limon*)	Lacto Calamine Sunscreen SPF 50 , PA +++ Sunscreen for Oily Skin | UVA – UVB Sunscreen ,With Kaolin Clay and Lemon Extracts
Tomato (*Solanum lycopersicum*)	Aqualogica Detan+ Dewy Sunscreen SPF 50+ With Cherry Tomato and Hyaluronic Acid
Pomegranate (*Punica granatum*)	PURE ELEMENTS Ultralight Sunscreen SPF 50 with Pomegranate & Green Tea
Cucumber (*Cucumis sativus*)	Aroma Magic Cucumber SPF 30 Sunscreen Lotion
Aloe Vera	Biotique Aloe Vera ,75+SPF .UVA/UVB Sunscreen Ultra Soothing Body Lotion
Avocado (*Persea Americana*)	Aqualogica 5 Barrier+ Repair Sunscreen with Avocado & 5 Essential Ceramides ,SPF 50 PA++++

Licorice (*Glycyrrhiza glabra*)	Kimirica Glow Sunscreen, SPF 50, PA+++ For UVA/B Protection With Liquorice And Carrot Root Extract
Turmeric (*Curcuma longa*)	Mamaearth- Ultra Light Indian Sunscreen with Carrot Seed, Turmeric, and SPF 50 PA+++
Jasmine (*Jasminum officinale*)	SVARASYA Nivr- Natural Sun Protection Sunscreen, SPF 21 (Jasmine, Clove & Cardamom)
Saffron (*Crocus sativus*)	NutriGlow Kashmiri Saffron Sunscreen, SPF50 PA+++

Table 6: Major marketed herbal sunscreens with Sun Protection Factor (SPF) and PA (Protection Grade of UV-A) label claims.

Modern herbal sunscreens incorporate phytochemicals such as flavonoids (rutin, quercetin), polyphenols (green tea catechins, resveratrol), carotenoids (β-carotene, lycopene), and phenolic acids (ferulic acid), which exhibit UV-absorbing, antioxidant, and anti-inflammatory properties. These bioactives either contribute directly to SPF value or enhance the performance and stability of conventional UV filters.

Herbal formulations like Mamaearth, and Himalaya represent a growing trend toward natural, multifunctional sunscreens that combine traditional plant-based remedies with scientific photoprotection. However, most of these rely on synergistic effects rather than phytochemicals acting as stand-alone UV filters.

In Vitro Methods for Evaluating Photoprotective Action of Herbal Phytochemicals

In vitro evaluation methods play a vital role in assessing the photoprotective potential of herbal phytochemicals before their use in sunscreen formulations. The most common technique is the spectrophotometric SPF determination (Mansur method), which estimates SPF by measuring UV absorbance in the 290–320 nm range. UV–Visible spectral analysis further identifies compounds with significant absorption in the UVA and UVB regions, indicating direct UV-filtering potential. To evaluate antioxidant-mediated protection, assays such as DPPH, ABTS, and FRAP are employed to measure free radical scavenging and reducing power, which contribute to prevention of UV-induced oxidative stress. Cell-based assays like the MTT test assess cytoprotective effects on skin cells (e.g., HaCaT keratinocytes), while ROS assays detect intracellular reactive oxygen species reduction following UV exposure. Together, these in vitro methods provide a comprehensive understanding of how phytochemicals—such as quercetin, rutin, ferulic acid, silymarin, and green tea catechins—protect skin through UV absorption, antioxidant activity, and cellular defense mechanisms, supporting their development as natural photoprotective agents in herbal sunscreen formulations

Method /assay	Purpose	Principle	Example of phytochemicals	Measured outcomes

Spectrophotometric SPF determination (in vitro SPF method)[22][23]	Estimate SPF value of extracts or formulations	UV absorbance of sample measured (290–320 nm) using spectrophotometer; SPF calculated via Mansur equation	Quercetin, Rutin, Ferulic acid, Green tea extract	SPF value *(in vitro)*
UV–Visible absorption spectrum analysis[24]	Assess UV absorption capacity. Measures absorbance profile (200–400 nm) to identify UVA/UVB-absorbing peaks. Silymarin, Ellagic acid, Pomegranate extract λmax and absorbance in UVB/UVA range	Measures absorbance profile (200–400 nm) to identify UVA/UVB-absorbing peaks	Silymarin, Ellagic acid, Pomegranate extract	λmax and absorbance in UVB/UVA range
DPPH radical scavenging assay[25]	Determine antioxidant potential contributing to photoprotection	DPPH (2,2-diphenyl-1-picrylhydrazyl) purple radical reduced to yellow; absorbance decrease = antioxidant activity	Green tea polyphenols, Curcumin, Resveratrol	% radical scavenging activity (IC_{50})
ABTS radical cation scavenging assay[26]	Evaluate total antioxidant capacity	$ABTS^{+}$ decolorization by antioxidant; absorbance at 734 nm	Rutin, Quercetin	TEAC (μmol Trolox equiv./g)
Cell viability (MTT or Alamar Blue) assay[28]	Assess cytoprotection of extracts against UV damage	HaCaT keratinocytes or fibroblasts exposed to UVB with/without treatment; viability quantified by metabolic dyes	EGCG, Curcumin, Silymarin	% cell viability, cytotoxicity
ROS (Reactive Oxygen Species) assay (DCF-DA)	Measure intracellular oxidative stress reduction	DCFH-DA dye oxidized to fluorescent DCF; fluorescence ↓ = less ROS	Resveratrol, Ellagic acid, Green tea extract	Relative fluorescence intensity (ROS level)

	after UV exposure			
FRAP(Ferric Reducing Antioxidant Power) assay[27]	Assess reducing power of extract	Reduction of Fe^{3+}–TPTZ complex to Fe^{2+}–TPTZ (blue) measured at 593 nm	Ferulic acid, Lignin, Polyphenol extracts	FRAP value (μmol Fe^{2+} equiv./g)
Lipid peroxidation assay (TBARS / MDA)	Evaluate inhibition of UV-induced lipid oxidation	Measures malondialdehyde (MDA) reacting with thiobarbituric acid; absorbance 532 nm	Green tea catechins, Curcumin	MDA concentration (nmol/mg protein)
Comet assay (Single-cell gel electrophoresis)	Assess DNA protection against UV-induced damage	DNA strand breaks visualized as "comet tails"; % tail DNA indicates damage	Silymarin, Ferulic acid	DNA damage % before & after UV
Collagenase / Elastase inhibition assays	Assess anti-photoaging activity	Enzymatic inhibition measured spectrophotometrically; lower enzyme activity = less collagen degradation	Rutin, Resveratrol, Luteolin	% inhibition of enzyme activity

Table 7: *In Vitro* Methods for Evaluating Photoprotective Action of Phytocomponents.

Conclusion

Ultraviolet (UV) radiation, though essential for certain physiological processes like vitamin D synthesis, poses serious risks to skin health through oxidative stress, DNA damage, photoaging, and carcinogenesis. Conventional chemical sunscreens, while effective, may cause allergic reactions and environmental concerns. Hence, natural plant-derived photoprotective agents have gained increasing importance. Many terrestrial and marine plants produce secondary metabolites—such as flavonoids, phenolic acids, terpenoids, alkaloids, and carotenoids—that effectively absorb UVA and UVB rays, scavenge reactive oxygen species, and enhance the skin's antioxidant defence. These phytoconstituents, including curcumin, resveratrol, silymarin, and lycopene, have been successfully incorporated into herbal sunscreen formulations that offer broad-spectrum protection and added skincare benefits. Therefore, Phytomolecule-enriched sunscreens represent a safe, effective, and eco-friendly alternative to synthetic filters, providing a sustainable approach to preventing UV-induced oxidative stress, premature aging, and skin disorders.

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RATIONAL DRUG DESIGN, QSAR AND DOCKING STUDIES BASED ON THE PHYTOCONSTITUENTS IN DIFFERENT FORMULATIONS OF *Withania somnifera*

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ABSTRACT

Withania somnifera is a remarkable source of therapeutic agents for different pharmacological activities. The root and stem bark of *Withania somnifera* was extracted with alcohol, methanol, ethanol, petroleum ether, benzene using various extraction methods. These extracts were analysed structurally and qualitatively to identify the phytoconstituents present responsible for the activity. The physicochemical properties and toxicity studies of these compounds were determined using softwares. The neuroprotective action of five phytoconstituents was screened using docking software AutoDock vina against target protein NADPH-diaphorase. It was found that ANAFERINE is the compound responsible for the neuroprotective action also having high number of amino acid interacting residues and BBB penetration.

KEY WORDS

Withania somnifera, Neuroprotective, Phytoconstituents, Rational drug design

INTRODUCTION

The medicinal plants contain a mass array of bioactive compounds which can be utilized for the development of potent therapeutic drugs. The beneficial medicinal effects of plant materials typically result from combinations of secondary products in them. *Withania somnifera* is commonly known as Ashwagandha, holds the most prominent place among the Ayurveda rasayana herbs with multiple pharmacological actions. More than 91 pharmaceutical products are produced from this plant. The biologically active chemical constituents of *Withania somnifera* consist of alkaloids, steroidal lactones and saponins. A wide range of activity comprising antistress, anticancer, antibacterial, anti-inflammatory, anticonvulsant, CNS depressant, hepatoprotective, immunomodulatory properties are reported. Several molecular docking studies have enlightened the compounds extracted from showing prominent pharmacological activity.

Synonym: Indian ginseng, Winter cherry

Biological Sources: It consists of the dried roots and stem bases of *Withania somnifera*, belonging to family Solanaceae. Shrub grows in India, Middle east and parts of Africa

Chemical composition in Ashwagandha: Laboratory analysis has revealed over 35 chemical constituents contained in the roots of *Withania somnifera*. The biologically active chemical constituents are alkaloids (isopellertierine, anferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanoloides with a glucose at carbon 27 (sitonidoside XI and X). Among the various alkaloids, withanine is the main constituent. The other alkaloids are somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a gloyloxytropane,

choline, cuscohygrine, isopelletierine, anaferineandanahydrine. Two acyl steryl glucoside viz. sitoindoside VII and sitoindoside VIII have been isolated from root. The leaves contain steroidal lactones, which are commonly called withanolides. Much of Ashwaganda's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D. Further chemical analysis has shown the presence of the following: Anaferine (Alkaloid), Anahygrine (Alkaloid), Beta-Sisterol, Chlorogenic acid (in leaf only), Cysteine (in fruit), Cuscohygrine (Alkaloid), Iron, Pseudotropine (Alkaloid), Scopoletin, Somniferinine (Alkaloid), Somniferiene (Alkaloid), Tropanol (Alkaloid), Withanine (Alkaloid), Withananine (Alkaloid) and Withanolides A, Y (Steroidal lactones).

Over these chemical constituents some are really beneficial to human health and using these constituents in marketed formulations are also available. From that a few marketed formulations are taken and observed the activity of each chemical constituent on the basis of their particular activity specified on the formulations.

General uses: Reduces stress and anxiety, Neuroprotective, Immunomodulator, Anti-inflammatory action, Anti- bacterial, Cancer treatment, Parkinson's disease, Huntington's disease, Alzheimer's disease Diabetes, arthritis, epilepsy, Skin disorders.

Neuroprotective Activity

Preclinical research and clinical trials support the use of *W. somnifera* for the treatment of neurological conditions such as anxiety, depression, cognitive disorders, senile dementia and neurodegenerative disorders. Earlier, it was reported that the neuroprotective activity of *W. somnifera* root extract could be because of presence of glycowithanolides and their ability to inhibit lipid peroxidation because of their antioxidant actions.

Marketed formulations: Capsules, Tablets, Powder, Ointment, Thailam etc.

REVIEW ON FEW MARKETED FORMULATION

ASHWAGANDHA TABLET FORMULATION- Each Tablet contains 250 mg of root extract of Ashwagandha helps in nourishing body tissues; helps strengthen the immune system and reduces stress. The people after using these tablets recommended by physicians gets better results. The major chemical constituents present in these formulations are ANAFERINE & WITHAFERINE A

ASWAGANDHA PAIN RELIEF BALM - Balm is prepared using the ingredients camphor, eucalyptus, menthol, and root extract of *Withania somnifera*. And it is used as a pain reliever and as an anti-inflammatory agent. Due to the presence of steroidal lactones like withanolides and withaferine it will show the anti-inflammatory action.

MATERIALS AND METHODS

Studies have demonstrated that a diverse range of chemical constituents are present in *Withania somnifera* and some are really beneficial to human health, these constituents in marketed formulations are also available. From that two marketed formulations are taken and observed the activity of each chemical constituent on the basis of their particular activity specified on the formulations and five chemical constituents were selected for this study, its structures were drawn using chemsketch.

The physicochemical properties of the chemical constituents were determined using Molinspiration software. It offers broad range of cheminformatics software tools supporting molecule manipulation and processing, including SMILES, normalization of molecules, etc.

Drug Likeness: Druglikeness may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs. The criteria most frequently used for drug-likeness is the Lipinski rule of five, The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME").However, the rule does not predict whether a compound is pharmacologically active or not.

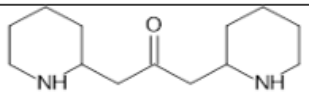
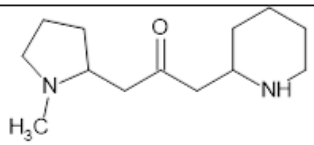
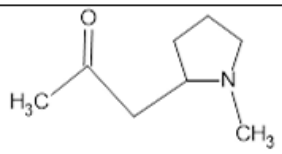
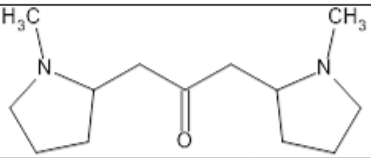
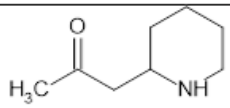
In-Silico Toxicity Studies: Toxicity is a measure of any undesirable or adverse effect of chemicals. Lazar toxicity predictions were the software that used for in-silico toxicity studies. This is free online software that gives toxicity data of drugs by drawing the chemical structure of drug in its dashboard or pasting the smiles of structure.

Molecular Docking Studies: Software's used

- AutoDock vina Tools- version 1.5.6
- Open Babel
- Pymol
- BIOVIA I Discovery Studio
- Chemsketch

RESULTS AND DISCUSSIONS

STRUCTURE CONSTRUCTION USING CHEMSKETCH

COMPOUND NO.	COMPOUND NAME	STRUCTURE	MOLECULAR FORMULA	MOLECULAR WEIGHT	IUPAC NAME
1	ANAFERINE		C13H24N2O	224.34g/mol	1,3-di(piperidin-2-yl)propan-2-one
2	ANAHYGRINE		C13H24N2O	224.34g/mol	1-(1-methylpyrrolidin-2-yl)-3-(piperidin-2-yl)propan-2-one
3	HYGRINE		C8H15NO	141.21 g/mol	1-(1-methylpyrrolidin-2-yl)propan-2-one
4	CUSCOHYGRINE		C13H24N2O	224.34 g/mol	1,3-bis(1-methylpyrrolidin-2-yl)propan-2-one
5	ISOPELLETIERENE		C13H24N2O	141.21g/mol	1-(piperidin-2-yl)propan-2-one

DRUGS PHYSIOCHEMICAL PROPERTIES OF DIFFERENT CHEMICAL CONSTITUENTS USING MOLINSPIRATION SOFTWARE

COMPOUND CODE	COMPOUND NAME	LogP	TPSA	natoms	nON	nOHNH	nviolation	nrotb	volume
1	ANAFERINE	1.38	41.12	16	3	2	0	4	236.41
2	ANAHYGRINE	1.12	32.34	16	3	1	0	4	236.55
3	HYGRINE	0.55	20.31	10	2	0	0	2	150.72
4	CUSCOHYGRINE	0.86	23.55	16	3	0	0	4	236.69
5	ISOPELLETIERENE	0.81	29.10	10	2	1	0	2	150.58

DRUGS BIOLOGICAL ACTIVITY

COMPOUND CODE	COMPOUND NAME	GPCR LIGAND	ION CHANNEL MODULATOR	KINASE INHIBITOR	NUCLEAR RECEPTOR LIGAND	PROTEASE INHIBITOR	ENZYME INHIBITOR
1	ANAFERINE	-0.08	0.17	-0.60	-0.58	-0.14	0.08
2	ANAHYGRINE	0.28	0.53	-0.39	-0.61	0.15	0.18
3	HYGRINE	-0.54	0.24	-1.50	-1.41	-0.78	-0.26
4	CUSCOHYGRINE	0.07	0.40	-0.42	-0.43	-0.09	0.11
5	ISOPELLETIERENE	-0.79	-0.15	-1.81	-1.64	-0.87	-0.32

In-silico TOXICITY STUDIES USING LAZAR SOFTWARE

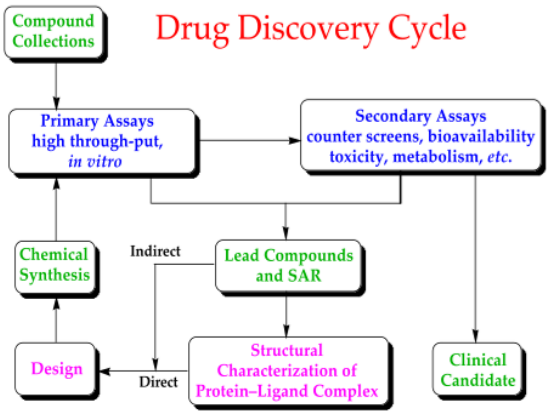
COMPOUND CODE	COMPOUND NAME	ACUTE TOXICITY (DAPHNIA MAGNA) (mmol/L)	BLOOD BRAIN BARRIER PENETRATION (HUMAN)	CARCINOGENICITY (RAT)	LOWEST OBSERVED ADVERSE EFFECT LEVEL(RAT)	MAXIMUM RECOMMENDED DAILY DOSE(HUMAN) (mmol/kg-bw/day)	MUTAGENICITY (Salmonella typhimurium)
1	ANAFERINE	0.00864 (mmol/L)	penetrating	non-carcinogenic	0.0733 (mmol/kg_bw/day)	0.0127 (mmol/kg-bw/day)	non-mutagenic
2	ANAHYGRINE	0.00948 (mmol/L)	penetrating	non-carcinogenic	0.0262 (mmol/kg_bw/day)	0.0133 (mmol/kg-bw/day)	non-mutagenic
3	HYGRINE	-	penetrating	-	0.00847 (mmol/kg_bw/day)	0.00324 (mmol/kg-bw/day)	non-mutagenic
4	CUSCOHYGRINE	-	-	-	0.00786 (mmol/kg_bw/day)	0.00684 (mmol/kg-bw/day)	non-mutagenic
5	ISOPELLETIERENE	0.00837 (mmo	penetrating	non-carcinogenic	0.00296 (mmol/kg_bw/day)	0.00679 (mmol/kg-bw/day)	non-mutagenic

ADME PRDICTION

COMPOUND CODE	COMPOUND NAME	GI ABSORPTION	BBB PERMEANT	P-GP SUBSTRATE	CYP1A2 INHIBITOR	CYP2C19 INHIBITOR	CYP2C9 INHIBITOR	CYP2D6 INHIBITOR	CYP3A4 INHIBITOR	LOG Kp(skin permeation) (cm/s)	BIOAVAILABILITY SCORE	SYNTHETIC ACCESSIBILITY	DRUGLIKENESS
1	ANAFERINE	HIGH	YES	YES	NO	NO	NO	NO	NO	-7.11	0.55	2.60	YES
2	ANAHYGRINE	HIGH	YES	NO	NO	NO	NO	NO	NO	-7.04	0.55	2.62	YES
3	HYGRINE	HIGH	NO	NO	NO	NO	NO	NO	NO	-6.83	0.55	1.80	YES
4	CUSCOHYGRIN	HIGH	YES		NO	NO	NO	NO	NO	-6.96	0.55	2.60	YES
5	ISOPELLETIERENE	HIGH	YES	NO	NO	NO	NO	NO	NO	-6.91	0.55	1.79	YES

DRUG DESIGN

Drug design, often referred to as rational drug design or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it. Drug design frequently but not necessarily relies on computer modelling techniques. This type of modelling is sometimes referred to as computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure-based drug design.



TARGET SELECTION

A biomolecular target (most commonly a protein or a nucleic acid) is a key molecule involved in a particular metabolic or signalling pathway that is associated with a specific disease condition or pathology or to the infectivity or survival of a microbial pathogen. Potential drug targets are not necessarily disease causing but must by definition be disease modifying. In some cases, small molecules will be designed to enhance or inhibit the target function in the specific disease modifying pathway.

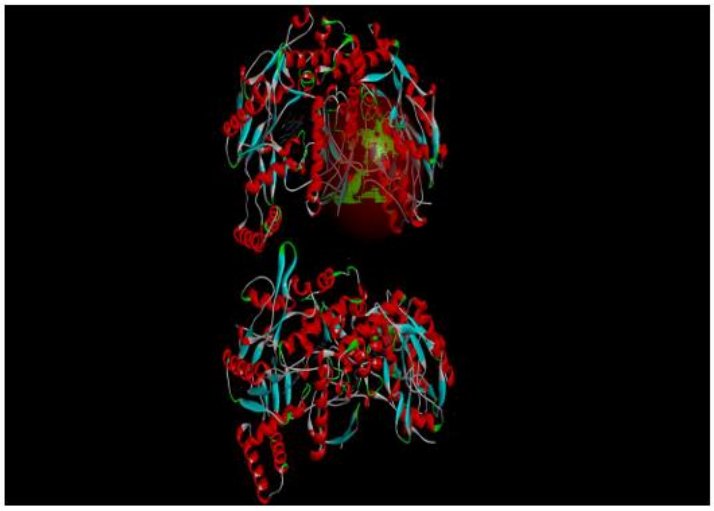
PDB ID : 4NOS

➢ Alternating name: NADPH-Diaphorase or NO Synthase

Based on studies;

- The enzyme which produces NO in brain, NITRIC OXIDE SYNTHASE (NOS) serves as an interphase between social interactions and anxiety, stress like disorders.
- NOS transforms arginine into NO and citrulline.
- Upon inhibition of NOS, it will eliminate the effect of anxiety like responses.

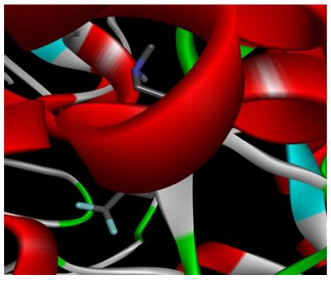
PROTEIN STRUCTURE



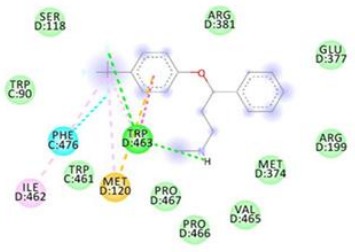
PDB ID :4NOS

FLUOXETINE AS STANDARD DRUG

Fluoxetine, sold under the brand names Prozac and Sarafem, among others, is a selective serotonin reuptake inhibitor (SSRI) class used in the treatment of anxiety and stress.

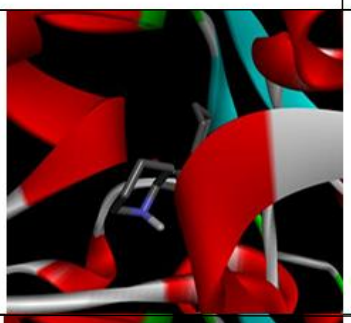
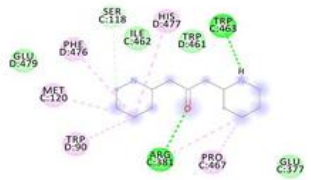
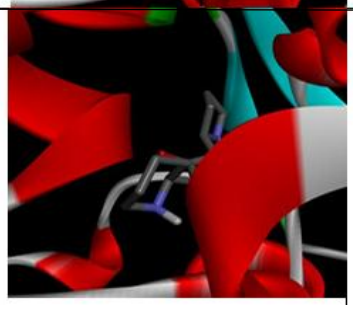
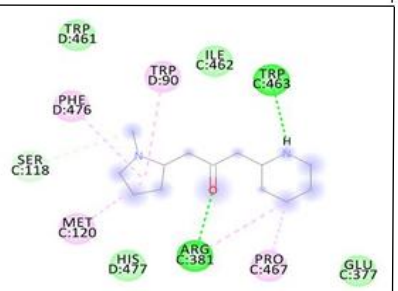
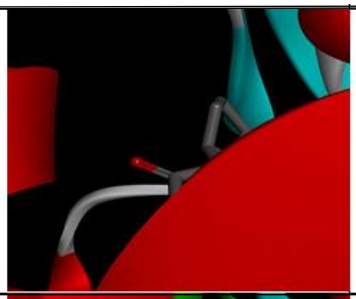
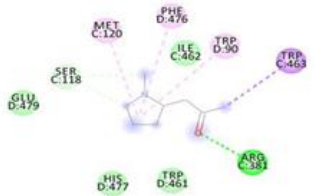
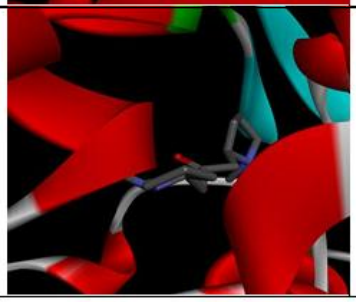
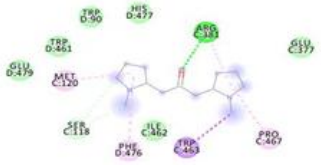
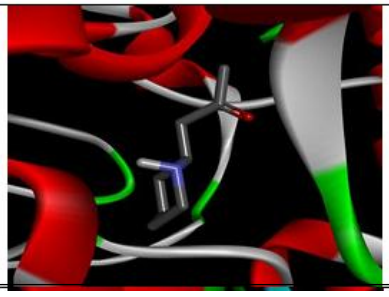
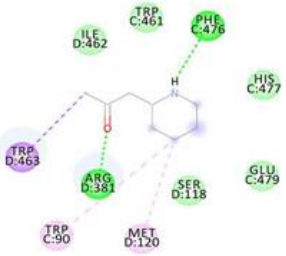


3D Structure of the compound



2D structure of the compound

DOCKING STUDIES

Sl NO.	COMPOUND NAME	3D STRUCTURE	2D STRUCTURE
1	ANAFERINE		
2	ANAHYGRINE		
3	HYGRINE		
4	CUSCOHYGRINE		
5	ISOPELLETIERENE		

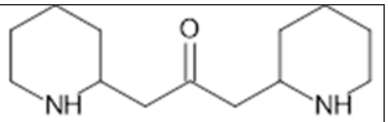
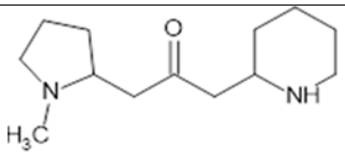
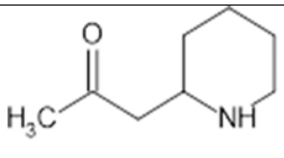
INTERPRETATION OF RESULTS

COMPOUND NAME	AFFINITY (kcal/mol)	NO.OF HYDROGEN BONDS
ANAFERINE	-7.6	2
ANAHYGRINE	-7.4	2
HYGRINE	-5.6	1
CUSCOHYGRINE	-6.9	1
ISOPELLETIERENE	-6.0	2
FLUOXETINE (STANDARD DRUG)	-8.1	2

CONCLUSION

The alcoholic, methanolic, ethanolic, petroleum ether extracts of *Withania somnifera* were extracted using various extraction methods and identified the chemical constituents using different identification tests. The phytoconstituents present in different formulations of *Withania somnifera* shows different pharmacological activities. Various *in-silico* studies were carried out to determine drug likeness property and pharmacological activity of these phytoconstituents. Not all the five compounds penetrate BBB; ANAFERINE, ANAHYGRINE, CUSCOHYGRINE, ISOPELLETIERENE only shows BBB penetration. Docking studies were conducted using 4NOS protein structure.

BBB PENETRATING COMPOUNDS HAVING PIPERIDINE RING

ANAFERINE	
ANAHYGRINE	
ISOPELLETIERENE	

The synthetic accessibility value of the steroidal lactones is very high; so, it is difficult to synthesise, also these compounds not penetrate BBB. The major phytoconstituent which is responsible for neuroprotective action in *Withania somnifera* is ANAFERINE. ANAFERINE has the highest number of interacting residues when compared to other five compounds. The studies on phytoconstituents in *Withania somnifera* continue to be an interesting novel approach in the future development of anxiety disorders with less adverse effects.

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Exploring Student Assessment Challenges in classroom

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Abstract

Classroom assessment is a dynamic and continuous process essential for effective teaching and student success. This article outlines the necessity of transitioning from assessment solely *of* learning (summative) to integrating assessments *for* and *as* learning (formative and self-regulatory). It details the core student evaluation criteria, structured using Bloom's Taxonomy to cover a cognitive hierarchy from foundational knowledge to advanced critical and creative thinking. This articles highlights a range of assessment methods, emphasizing the importance of alignment with learning objectives, and promoting student metacognition. Effective assessment practices transform the classroom into an adaptive, data-driven learning environment.

Introduction

Assessment in the classroom serves as the critical link between instruction and mastery. It moves beyond simply assigning grades to become a powerful mechanism for diagnosis, motivation, and instructional adjustment.[1] Effective assessment practices require a framework that defines what success looks like (criteria) and how that success will be measured (methods). The goal is to establish a continuous feedback loop that informs both the student's journey toward competence and the educator's teaching strategies. High-quality assessment must be valid (measuring what it intends to) and reliable (yielding consistent results).[2]

Student Evaluation Criteria

To ensure fairness and clarity, student evaluation criteria must be explicitly defined and shared. These criteria typically fall into three broad categories:

Criteria	Purpose	Focus
Knowledge and Comprehension[3]	The student's ability to recall, define, and explain core facts, concepts, theories, and terminology	Depth of understanding of subject matter content
Skills and Process[4]	The student's proficiency in practical tasks, procedures, critical thinking, problem-solving, communication, and collaboration	The student's proficiency in practicals
Application and Synthesis (Competence):	The student's ability to apply learned knowledge and skills to new or complex situations, evaluate information, create novel work, and justify decisions.	Transferring learning to real-world or novel contexts (e.g., solving a complex case study or designing a project).

Student Evaluation Criteria: A Bloom's Taxonomy Framework

To ensure assessments accurately target different levels of cognitive complexity, student evaluation criteria are often structured using the Revised Bloom's Taxonomy (2001). This hierarchical model classifies learning objectives into six progressively challenging levels, ensuring that students develop skills from simple recall to sophisticated judgment and creation.[5]

The three broad categories of criteria align directly with Bloom's Taxonomy as follows:

Cognitive Level	Bloom's Levels	Evaluation Criteria & Focus	Assessment Goal
1.Foundational Knowledge	**Remembering & Understanding**	**Lower-Order Thinking Skills (LOTS):** Recalling facts, defining terms, explaining core concepts, theories, and terminology.	**Basic comprehension** of subject matter content.
2. Skills and Application	**Applying & Analyzing**	**Mid-Level Thinking:** Proficiency in practical tasks, problem-solving, and demonstrating synthesized knowledge (breaking down information).	The ability to **use knowledge** to solve structured problems and dissect information.
3. Advanced Competence	**Evaluating & Creating**	**Higher-Order Thinking Skills (HOTS):** Critically appraising, justifying decisions, generating novel solutions or products.	**Transferring learning** to novel, complex contexts, demonstrating judgment and and innovation.

By using this framework, educators ensure that assessments are scaffolded, progressively challenging students to move from rote recall to true mastery and critical thinking.[6]

Assessment Methods and Purpose

Assessment methods should be varied to accurately capture the full range of criteria. They are often categorized by their function in the learning process:[7&8]

Assessment Type & Purpose	Aligned Bloom's Levels	Example Methods
Formative Assessment (*for* Learning)	**Remembering, Understanding, Applying**	Exit Tickets, brief Quizzes, Concept Maps, Thumbs-Up Checks.
Self/Peer Assessment (*as* Learning)	**Analyzing, Evaluating**	Peer Review of essays using a detailed rubric, Self-Correction, Reflection journals.
Summative Assessment (*of* Learning)	**All Levels (LOTS & HOTS)**	Traditional Exams, Case Study Analysis, Capstone Projects/Theses (measuring Creating).

Conclusion

Effective student assessment is the linchpin of an adaptive and responsive classroom. By clearly defining robust evaluation criteria leveraging Bloom's Taxonomy to ensure a balanced assessment covering the full cognitive spectrum from basic Remembering to advanced Creating and employing a balanced mix of formative and summative assessment methods, educators ensure that assessment serves its highest purpose: driving learning. This intentional, criterion-referenced approach moves the focus from simply passing judgment to providing actionable feedback, fostering deep understanding, and cultivating students who are capable, self-aware, and ready to apply their learning beyond the classroom. The strategic alignment of methods with the Bloom's hierarchy ultimately transforms evaluation into an essential component of the instructional process itself.

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Pharmacological Evidence and Safety Profiling of ASU&H Drugs: A Comprehensive Review as per Standard Guidelines

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Abstract

Ayurveda, Siddha, Unani, and Homeopathy (ASU&H) constitute the traditional medicine systems widely practiced in India. While their clinical use is longstanding, systematic pharmacological evaluation and safety profiling are essential to ensure efficacy, reproducibility, and patient safety. This review consolidates preclinical, clinical, and post-marketing evidence on ASU&H drugs, focusing on pharmacological mechanisms, therapeutic potential, toxicological studies, and compliance with standard regulatory guidelines such as AYUSH, WHO, and ICH protocols. Indian and global literature was reviewed to assess experimental models, pharmacokinetics, pharmacodynamics, adverse effects, herb–drug interactions, and post-market surveillance data. The review highlights the gaps in standardization, quality control, clinical trial reporting, and regulatory harmonization. Future directions include integration of modern pharmacological tools, molecular profiling, systematic safety evaluation, and development of evidence-based guidelines to enhance credibility and safe use of ASU&H medicines in India and globally.

Keywords: ASU&H, Ayurveda, Siddha, Unani, Homeopathy, pharmacology, safety profiling, standard guidelines, toxicology.

1. Introduction

Traditional systems of medicine have been integral to Indian healthcare for centuries. Ayurveda, Siddha, and Unani (collectively called ASU), along with Homeopathy (H), are widely used for preventive and therapeutic purposes across diverse populations [1]. Despite extensive historical use, systematic pharmacological validation and safety profiling remain limited, creating challenges for evidence-based integration into mainstream medicine.

Modern regulatory frameworks, including the Ministry of AYUSH, WHO Traditional Medicine Guidelines, and ICH Safety Guidelines, emphasize rigorous preclinical and clinical evaluation, pharmacovigilance, and quality control for ASU&H products [2,3]. Pharmacological studies provide insights into mechanisms of action, bioactive compounds, pharmacokinetics, and potential interactions, while toxicological profiling ensures safety, identifies dose limits, and monitors organ-specific effects [4].

This review aims to comprehensively summarize **pharmacological evidence and safety data for ASU&H drugs**, evaluate adherence to standard guidelines, identify knowledge gaps, and suggest future research directions to promote safe and effective use.

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2. Pharmacological Evidence of ASU&H Drugs

2.1 Ayurvedic Drugs

- **Preclinical Studies:**
  - Many Ayurvedic herbs have demonstrated anti-inflammatory, antioxidant, antidiabetic, hepatoprotective, and cardioprotective activities in animal models.
  - Example: *Withania somnifera* (Ashwagandha) shows anxiolytic, neuroprotective, and immunomodulatory properties in rodent models [5].
  - *Tinospora cordifolia* exhibits immunomodulatory and antidiabetic effects [6].
- **Mechanistic Insights:**
  - Modulation of inflammatory cytokines, oxidative stress pathways, insulin signaling, and neuroendocrine axes has been reported.
- **Clinical Evidence:**
  - Randomized controlled trials (RCTs) in India indicate efficacy in anxiety, arthritis, diabetes, and chronic fatigue [7].
  - Limitations: Small sample sizes, variable standardization of formulations, and inconsistent outcome measures.

2.2 Siddha Drugs

- **Preclinical Evidence:**
  - Siddha formulations such as *Nilavembu Kudineer* and *Kabasura Kudineer* show antiviral, anti-inflammatory, and immunomodulatory activities in animal models [8].
- **Clinical Evaluation:**

    - Siddha formulations have been studied for respiratory illnesses, fever, and viral infections, particularly during COVID-19, demonstrating symptomatic improvement and immunomodulation [9].
- **Mechanistic Insights:**
    - Bioactive compounds modulate inflammatory pathways, antiviral responses, and antioxidant mechanisms.

2.3 Unani Drugs

- **Preclinical Evidence:**
    - Unani herbs like *Gul-e-Banafsha* (Viola odorata) and *Terminalia chebula* show hepatoprotective, antidiabetic, and anti-inflammatory effects in rodents [10].
- **Clinical Evidence:**
    - Studies on Unani formulations for respiratory disorders, metabolic syndrome, and gastrointestinal ailments report safety and symptomatic improvement [11].
- **Mechanistic Insights:**
    - Antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory activities are commonly reported.

2.4 Homeopathic Drugs

- **Preclinical Evidence:**
    - Homeopathic preparations, particularly complex nosodes and microdoses, have shown immunomodulatory and anti-inflammatory effects in experimental models [12].
- **Clinical Evidence:**
    - Clinical trials demonstrate potential benefits in upper respiratory tract infections, allergy, and chronic pain [13].
    - Limitations: Dose-response relationships are poorly defined; reproducibility remains a concern.
- **Mechanistic Insights:**
    - Proposed mechanisms include modulation of cytokine profiles, gene expression, and immune cell activity.

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3. Safety Profiling of ASU&H Drugs

3.1 Toxicological Studies

- **Acute Toxicity:**

    - Most Ayurvedic and Siddha herbs show high LD50 values in rodents, indicating low acute toxicity [14].
- **Subchronic and Chronic Toxicity:**
    - Long-term administration studies report hepatoprotective and nephroprotective safety in multiple formulations.
    - Some herbs (*Glycyrrhiza glabra*, *Aristolochia spp.*) may induce organ toxicity; caution advised [15].
- **Genotoxicity and Carcinogenicity:**
    - Limited data; most modern studies show no genotoxic effects at therapeutic doses.

3.2 Clinical Safety and Adverse Events

- RCTs and observational studies report mild gastrointestinal complaints, headache, or fatigue in <5–10% of subjects [16].
- Severe adverse effects are rare but include hepatotoxicity and allergic reactions in specific populations.

3.3 Herb–Drug Interactions

- Potential interactions with anticoagulants, antidiabetics, antihypertensives, and immunosuppressants have been reported.
- Example: *Withania somnifera* may potentiate sedatives; *Guggulu* may interact with lipid-lowering agents [17].

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4. Standard Guidelines and Regulatory Framework

4.1 Ministry of AYUSH Guidelines

- Emphasizes pharmacopoeial standardization, Good Manufacturing Practices (GMP), and clinical trial documentation [18].

4.2 WHO Guidelines

- WHO traditional medicine strategy (2014–2023) advocates for quality, safety, efficacy, and integration into national health systems [19].

4.3 ICH Safety Guidelines

- Provides protocols for acute, subchronic, chronic toxicity, genotoxicity, and reproductive toxicity testing [20].

4.4 Gaps in Compliance

- Many traditional formulations lack comprehensive pharmacokinetic, pharmacodynamic, or standardized safety data.
- Limited multi-centric clinical trials and heterogeneity in outcome measures reduce evidence reliability.

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5. Tables Summarizing Pharmacological Evidence and Safety

Table 1: Selected Ayurvedic Drugs – Pharmacological Evidence and Safety

Drug/Herb	Pharmacological Activity	Preclinical Evidence	Clinical Evidence	Safety Profile
Withania somnifera	Anxiolytic, immunomodulatory	Rodent models: ↓cortisol, ↑immune cells	RCTs: ↓stress, fatigue	Mild GI symptoms, safe in recommended dose
Tinospora cordifolia	Antidiabetic, immunomodulatory	Rodent: ↓blood glucose, ↑antioxidants	RCTs: improved glycemic control	Generally safe; rare allergy
Curcuma longa	Anti-inflammatory, hepatoprotective	Rodent: ↓ALT, AST	Small clinical trials: ↓CRP, liver enzymes	Well tolerated; mild dyspepsia

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Table 2: Selected Siddha and Unani Drugs – Pharmacological Evidence

Drug/Formulation	System	Activity	Preclinical Evidence	Clinical Evidence	Safety Profile
Nilavembu Kudineer	Siddha	Antiviral, antipyretic	Rodent viral models	Symptomatic relief in dengue, COVID-19	Well tolerated; mild GI upset
Terminalia chebula	Unani	Antidiabetic, hepatoprotective	Rodent: ↓blood glucose, hepatoprotection	Observational studies: improved glycemic control	Mild diarrhea possible

Drug/Formulation	System	Activity	Preclinical Evidence	Clinical Evidence	Safety Profile
Viola odorata	Unani	Anti-inflammatory, respiratory	Rodent: ↓inflammation	Clinical trials: improved asthma symptoms	Safe at recommended doses

Table 3: Summary of Pharmacological Evidence and Safety Profiling

System	Key Drugs/Formulations	Preclinical Evidence	Clinical Evidence	Safety Considerations
Ayurveda	Ashwagandha, Guduchi, Curcuma	Anti-inflammatory, antioxidant, immunomodulatory	RCTs show efficacy in anxiety, diabetes, fatigue	Mild GI upset, allergic reactions rare
Siddha	Nilavembu Kudineer, Kabasura Kudineer	Antiviral, anti-inflammatory	Observational/clinical trials for respiratory ailments	Well tolerated; mild GI symptoms
Unani	Gul-e-Banafsha, Terminalia chebula	Hepatoprotective, antidiabetic	Observational trials for diabetes, respiratory disorders	Generally safe; rare diarrhea/allergic reactions
Homeopathy	Nosodes, complex remedies	Immunomodulatory, anti-inflammatory	Trials in respiratory and chronic pain	Safe at recommended dilution; reproducibility concerns

6. Challenges and Gaps in ASU&H Research

1. **Standardization Issues:** Batch-to-batch variability, unknown bioactive compounds.
2. **Limited High-Quality Clinical Trials:** Small sample sizes, lack of placebo controls.
3. **Inadequate Toxicological Data:** Few long-term studies; underreporting of adverse events.
4. **Herb–Drug Interaction Awareness:** Often under-recognized in polypharmacy.
5. **Regulatory Harmonization:** Need for uniform guidelines across ASU&H systems.

7. Future Directions

- **Systematic Pharmacological Evaluation:** Mechanistic studies using modern tools (molecular docking, omics technologies).
- **Standardization and Quality Control:** Authentication, marker-based standardization, GMP compliance.
- **Evidence-Based Clinical Trials:** Well-designed RCTs, multi-centric studies, long-term safety monitoring.
- **Pharmacovigilance Programs:** National database for adverse events.
- **Integration with Modern Medicine:** Combining ASU&H with conventional therapy under safety protocols.

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8. Conclusion

ASU&H drugs hold substantial therapeutic potential across diverse health conditions. Preclinical and limited clinical evidence supports their pharmacological efficacy, while overall safety is generally acceptable at recommended doses. However, comprehensive pharmacological validation, systematic safety profiling, and adherence to standard guidelines are necessary to ensure reproducibility, credibility, and safe integration into modern healthcare. Future research should focus on standardization, high-quality clinical trials, herb–drug interaction studies, and pharmacovigilance to optimize the use of ASU&H medicines in India and globally.

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Recent Trends in Indian Preclinical Research in Diabetes: Advances, Experimental Models, Phytochemicals, Technologies, and Future Directions

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Abstract

India has emerged as a global hub for preclinical diabetes research owing to its unique demographic, genetic, dietary, and cultural attributes. With over 100 million individuals currently living with diabetes, the country faces an urgent need for innovative preventive and therapeutic solutions tailored to South Asian populations. This review provides a comprehensive overview of recent trends in Indian preclinical diabetes research, including advancements in experimental animal and cellular models, mechanistic insights, traditional medicinal plants, phytochemicals, nanotechnology-derived formulations, omics-based tools, and stem cell approaches. Indian researchers employ a diverse spectrum of diabetes models ranging from classical streptozotocin (STZ) and alloxan-induced models to high-carbohydrate and cafeteria diet models that closely resemble Indian dietary habits. The emergence of zebrafish models, organoids, and gene-editing tools marks a significant methodological transition. Molecular investigations in India focus heavily on oxidative stress, inflammation, insulin resistance, mitochondrial dysfunction, microbiome–metabolism interactions, and pathways driving diabetic complications. India's vast ethnopharmacological repertoire continues to stimulate research on antidiabetic phytochemicals such as curcumin, berberine, gymnemic acids, charantin, and various flavonoids, with recent work emphasizing standardization and nanoformulation strategies. Technological innovations—including metabolomics, proteomics, bioinformatics, machine learning, nanomedicine, and stem-cell–based β-cell regeneration—are rapidly expanding the scope of Indian diabetes research. Despite these advances, challenges such as limited GLP facilities, inconsistencies in herbal standardization, and translational gaps persist. This review consolidates contemporary Indian research efforts and suggests future directions for developing cost-effective, mechanism-driven therapeutic candidates suited to India's epidemiological context.

Keywords: Diabetes mellitus, India, preclinical models, phytochemicals, nanomedicine, omics, stem cells, insulin resistance, oxidative stress.

1. Introduction

Diabetes mellitus represents one of India's most significant public health threats, with prevalence rising exponentially in both rural and urban settings. In 2023, India surpassed 100 million diabetic individuals, positioning it as the second most affected country worldwide [1]. Adult-onset type 2 diabetes mellitus (T2DM) constitutes over 90% of the national disease burden, and is driven by a constellation of factors including genetic predisposition, urbanization, dietary transitions, sedentary lifestyle, and rising obesity levels [2]. Notably, the "South Asian phenotype," characterized by abdominal adiposity, enhanced insulin resistance at lower BMI, and early β-cell dysfunction, uniquely shapes disease progression and necessitates population-specific therapeutic strategies [3].

Against this backdrop, Indian preclinical research in diabetes has grown extensively, driven by improved laboratory infrastructure, increasing research funding, and heightened interest in leveraging India's traditional medicinal systems for drug discovery. Indian scientists contribute significantly to global diabetes literature through in vivo rodent models, in vitro cellular assays, zebrafish models, and mechanistic studies exploring key metabolic pathways. Traditional Ayurvedic and ethnomedical plants, widely used across the Indian subcontinent, serve as abundant sources of novel antidiabetic compounds, reinforcing the nation's long-standing phytomedicinal heritage [4].

Simultaneously, India's integration of cutting-edge technologies—including metabolomics, transcriptomics, nanotechnology, stem-cell biology, and computational modeling—reflects a paradigm shift toward multidisciplinary diabetes research. These innovations address persistent gaps in understanding disease mechanisms, improving drug delivery, and identifying biomarkers for early detection. However, issues such as inadequate standardization of herbal extracts, limited GLP-compliant facilities, and insufficient translation from bench to bedside continue to slow progress [5].

This review synthesizes the landscape of recent Indian preclinical diabetes research, focusing on experimental models, mechanistic insights, phytomedicines, advanced technologies, and future possibilities. By consolidating current trends, the review aims to highlight India's unique contributions and identify paths toward more effective and translatable diabetes therapeutics.

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2. Epidemiological Context and Rationale for Indian Preclinical Research

2.1 Growing Diabetes Burden in India

India's rapid socio-economic transformation has dramatically altered the prevalence of metabolic disorders. Key contributors to rising diabetes incidence include:

- **Dietary transition:** Replacement of traditional fiber-rich diets with refined carbohydrates, sugary beverages, processed foods, and trans-fats [6].
- **Sedentary behavior:** Increased screen time, vehicular dependence, and reduced occupational physical activity [7].
- **Urbanization:** Exposure to obesogenic environments and chronic stress [8].
- **Genetic susceptibility:** Polymorphisms in *TCF7L2*, *PPARG*, and *FTO* genes are highly prevalent in Indians [9].
- **Early-onset insulin resistance:** Indians exhibit metabolic abnormalities at lower BMI compared to Caucasians [3].

These factors create an urgent need for diabetes therapies that consider population-specific metabolic traits.

2.2 Why India Requires Tailored Preclinical Models

Western high-fat diets (typically 45–60% fat) used in global diabetes research inadequately represent Indian dietary patterns dominated by:

- High carbohydrates (55–75% of caloric intake)
- Low protein
- Saturated fatty acids from ghee and vanaspati
- Refined grains (maida, white rice)

Hence, Indian laboratories increasingly employ **high-carbohydrate diets**, **fructose models**, and **cafeteria diets incorporating Indian snacks** (samosa, bhujia, sweets) to better simulate local nutritional habits [10].

2.3 Growth of Indian Research Infrastructure

Institutions like IISc, IITs, NIPERs, CSIR institutes, AIIMS, and ICMR-affiliated centers now house advanced facilities for molecular biology, proteomics, animal studies, nanotechnology, and computational analyses. Enhanced government funding through DBT, DST-SERB, and AYUSH has accelerated diabetes research.

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3. Experimental Models in Indian Preclinical Diabetes Research

Indian researchers use a variety of animal and cellular models tailored to different pathophysiological aspects of diabetes. These include chemical, dietary, genetic, and hybrid models.

3.1 Chemical Models

3.1.1 Streptozotocin (STZ)

STZ remains the most widely used diabetogenic agent across Indian institutions due to its reproducibility and affordability.

- **Single high-dose STZ (50–60 mg/kg):** Induces near-complete β-cell destruction resembling type 1 diabetes [11].
- **Multiple low-dose STZ:** Mimics autoimmune β-cell damage; increasingly used to model chronic hyperglycemia and complications [12].
- **Neonatal STZ model:** Produces early-life β-cell injury, leading to adult-onset glucose intolerance akin to T2DM [13].

3.1.2 Alloxan

Used less frequently due to instability, but remains common in smaller institutes. It generates oxidative stress–mediated β-cell necrosis [14].

3.2 Dietary Models

Indian laboratories extensively use diet-induced models to emulate nutritional transitions in the country.

3.2.1 High-Fat Diet (HFD)

Indian HFDs commonly contain:

- Ghee, coconut oil, palm oil
- Vanaspati (trans fats)
- Milk fat

These induce:

- Insulin resistance
- Hyperlipidemia
- Adipose inflammation [15]

3.2.2 High-Carbohydrate Diets

Reflecting Indian dietary patterns, these models include:

- Polished rice

- Refined wheat flour
- Sucrose and fructose
- Jaggery-sweetened foods

High-carbohydrate diets effectively induce hepatic steatosis and hyperinsulinemia [16].

3.2.3 Fructose Models

Widely used to mimic metabolic syndrome. 10–20% fructose in drinking water induces:

- Hypertriglyceridemia
- Hypertension
- Insulin resistance [17]

3.2.4 Cafeteria Diet Models

Unique to India, using:

- Samosas
- Namkeen
- Gulab jamun
- Chips
- High-sugar milk products

These induce obesity, oxidative stress, and hepatic pathology more rapidly than HFDs [18].

3.3 Genetic and Transgenic Models in India

While India still has limited access to large-scale transgenic breeding facilities, several models are widely used:

- **Zucker fatty rats**
- **db/db mice** (leptin receptor mutation)
- **ob/ob mice** (leptin mutation)

Indian researchers combine these with dietary triggers to model severe insulin resistance [19].

3.4 Zebrafish Models

Zebrafish (*Danio rerio*) research is expanding due to advantages such as transparency, rapid breeding, and genetic tractability.

Indian studies use zebrafish for:

- Pancreatic β-cell imaging
- Glucose-induced oxidative stress
- Screening of herbal compounds
- Nanoparticle toxicity in diabetes [20]

STZ and alloxan effectively induce β-cell damage in zebrafish larvae.

3.5 In Vitro Models

Commonly used Indian cell lines:

- **L6 myotubes:** GLUT4 translocation studies
- **3T3-L1 adipocytes:** Adipogenesis and insulin signaling
- **INS-1 and MIN6 cells:** β-cell toxicity and insulin secretion assays
- **HepG2 cells:** Glucose production and lipogenesis studies

Phytochemicals are often screened using these models before in vivo validation [21].

3.6 Hybrid and Combination Models

Recent Indian studies use multi-hit models combining:

- Low-dose STZ + HFD
- Fructose + HFD
- Neonatal STZ + High-carb diet

These simulate progressive T2DM more realistically [22].

Table 1. Commonly Used Diabetes Models in Indian Research

Model Type	Key Features	Common Use in India
STZ (single dose)	β-cell destruction; hyperglycemia	T1DM, complications
Low-dose STZ + HFD	Partial β-cell damage + insulin resistance	T2DM
Alloxan	ROS-mediated β-cell death	Entry-level labs
High-fat diet	Obesity, insulin resistance	Dietary diabetes

Model Type	Key Features	Common Use in India
High-carbohydrate diet	Hyperinsulinemia, steatosis	Indian dietary relevance
Fructose model	Metabolic syndrome	Cardiometabolic studies
Cafeteria diet	Severe obesity; oxidative stress	India-specific
Zebrafish	Rapid drug screening	Molecular imaging
L6/3T3-L1/INS-1	In vitro metabolic studies	Mechanistic assays

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4. Mechanistic Insights from Indian Preclinical Research

Indian studies explore multiple molecular and cellular pathways central to diabetes pathology.

4.1 Oxidative Stress

Hyperglycemia elevates reactive oxygen species (ROS) via:

- Mitochondrial electron transport chain impairment
- NADPH oxidase activation
- Protein glycation [23]

Indian researchers frequently measure:

- MDA
- SOD
- Catalase
- GSH
- GPx

Phytochemicals like curcumin and eugenol consistently improve antioxidant status [24].

4.2 Inflammatory Signaling

Chronic inflammation contributes to insulin resistance.

Markers studied in India include:

- TNF-α
- IL-6
- IL-1β
- MCP-1
- CRP
- NF-κB activation [25]

Compounds like *Tinospora cordifolia*, berberine, and neem suppress inflammatory pathways.

4.3 Insulin Resistance and Signaling Pathways

Major pathways investigated:

4.3.1 IRS-1/PI3K/Akt Pathway

Indians commonly exhibit impaired IRS-1 phosphorylation and reduced Akt activation [26].

4.3.2 GLUT4 Translocation

L6 myotube studies reveal defective GLUT4 trafficking in Indian models, restored by gymnemic acids and fenugreek extracts [27].

4.3.3 AMPK Pathway

Reduced AMPK activation contributes to dysregulated lipid metabolism [28].

4.4 β-Cell Dysfunction and ER Stress

Markers evaluated include:

- CHOP
- GRP78
- XBP1
- Caspase-3
- Bcl-2/Bax ratio [29]

Curcumin, ashwagandha, and green tea polyphenols show β-cell protection.

4.5 Mitochondrial Dysfunction

Indian studies document:

- Decreased ATP synthesis
- Reduced complex I/III activity
- UCP2 overexpression
- Increased mitochondrial ROS [30]

4.6 Gut Microbiome

Dysbiosis is evident in Indian diabetes models:

- ↓ Lactobacillus
- ↓ Bifidobacterium
- ↑ Firmicutes/Bacteroidetes ratio
- ↑ LPS levels [31]

4.7 Mechanisms of Diabetic Complications in India

Indian researchers intensely study:

4.7.1 Diabetic Neuropathy

- Axonal degeneration
- Na^+/K^+-ATPase inhibition
- Oxidative stress [32]

4.7.2 Nephropathy

- TGF-β1 elevation
- Collagen IV deposition
- Podocyte injury [33]

4.7.3 Cardiomyopathy

- Increased cardiac fibrosis
- Mitochondrial dysfunction [34]

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5. Phytochemicals and Herbal Research in India

India’s ethnomedicinal heritage drives extensive phytochemical research.

5.1 Major Indian Plants Studied for Antidiabetic Activity

5.1.1 *Gymnema sylvestre*

Contains gymnemic acids that:

- Regenerate β-cells
- Reduce intestinal glucose absorption
- Enhance insulin secretion [35]

5.1.2 *Momordica charantia* (Bitter gourd)

Contains charantin and polypeptide-p; improves GLUT4 activity [36].

5.1.3 *Curcuma longa* (Turmeric)

Curcumin reduces oxidative stress, inflammation, and ER stress [37].

5.1.4 *Tinospora cordifolia*

Immunomodulatory, antioxidant, and insulinotropic activity [38].

5.1.5 *Pterocarpus marsupium*

Epicatechin-rich heartwood rejuvenates β-cells [39].

5.2 Polyherbal Formulations

Ayurvedic combinations (e.g., **Nisha-Amalaki, Triphala, Chandraprabha vati**) show synergistic effects in rodent models.

5.3 Challenges in Herbal Research

- Lack of standardization
- Batch-to-batch variability
- Inadequate phytochemical quantification
- Differences in extraction methodologies [40]

Table 2. Key Indian Medicinal Plants and Their Mechanisms

Plant	Key Phytochemicals	Mechanisms
Gymnema sylvestre	Gymnemic acids	β-cell regeneration; insulin secretion
Momordica charantia	Charantin, vicine	GLUT4 translocation; insulin-mimetic
Curcuma longa	Curcumin	Anti-inflammatory; antioxidant
Tinospora cordifolia	Berberine-like alkaloids	Insulin sensitivity; immunomodulation
Pterocarpus marsupium	Epicatechin	β-cell protection

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6. Emerging Technologies in Indian Diabetes Research

6.1 Nanotechnology and Nanoformulations

Indian researchers develop:

- Curcumin nanoparticles
- Berberine-loaded liposomes
- Silver nanoparticles for glucose sensing
- PLGA-based insulin carriers [41]

These enhance stability, bioavailability, and targeted delivery.

6.2 Omics Approaches

6.2.1 Metabolomics

Used to profile:

- Amino acids
- Lipid species
- Organic acids
- Oxidative stress markers [42]

6.2.2 Transcriptomics and Proteomics

Identify:

- Upregulated inflammatory genes
- Downregulated insulin-signaling proteins [43]

6.2.3 Gut Microbiome Sequencing

16S rRNA sequencing reveals microbial shifts in Indian high-carbohydrate diets [44].

6.3 Stem Cells and Regenerative Medicine

Indian studies explore:

- Mesenchymal stem cells (MSCs) for β-cell regeneration
- Pancreatic progenitor cell differentiation
- Transplantation into diabetic rodents improving glucose tolerance [45]

6.4 Artificial Intelligence and In Silico Modeling

Applications include:

- Docking herbal compounds with insulin receptor
- Machine-learning prediction of drug toxicity
- Computational modeling of metabolic networks [46]

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7. Challenges in Indian Preclinical Diabetes Research

Despite progress, several limitations persist:

7.1 Lack of GLP-Compliant Facilities

Only a few Indian institutions have internationally accredited animal facilities.

7.2 Limited Transgenic Animal Access

Import restrictions and high costs limit advanced genetic tools.

7.3 Herbal Extract Standardization Issues

Variability in plant materials hampers reproducibility.

7.4 Translation Gap

Few preclinical discoveries progress to human trials.

7.5 Funding and Infrastructure Variability

Disparities between metropolitan and rural institutions.

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8. Future Directions

Key areas needing emphasis:

8.1 Development of Indian-Specific Animal Models

- Diets matching regional cuisines
- Genetic models reflecting Indian polymorphisms
- Microbiome-relevant models

8.2 Advanced Technologies

- CRISPR gene editing
- Single-cell sequencing
- Organoids
- Multi-omics integration

8.3 Standardization and Validation of Herbal Drugs

- Quantification of active constituents

- Pharmacokinetics and toxicity profiling
- Development of phytopharmaceuticals

8.4 Public–Private Partnerships

To accelerate drug development and translation.

8.5 Focus on Early-Stage Diabetes

Given early β-cell exhaustion in Indians.

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9. Conclusion

India has emerged as a powerful contributor to global preclinical diabetes research, leveraging diverse experimental models, mechanistic investigations, and a rich repository of medicinal plants. The integration of modern technologies such as nanomedicine, omics platforms, and stem-cell research has significantly advanced the field. However, persistent challenges in standardization, reproducibility, and translational research must be addressed to fully realize India's potential. By aligning indigenous knowledge with cutting-edge biomedical approaches, India can play a leading role in developing effective, accessible, and culturally relevant diabetes therapeutics.

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