

Extraction, Isolation, Identification Tests For Poly Herbal Gel For Anti-Bacterial Using Artificial Intelligence

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Abstract

The increasing prevalence of antimicrobial resistance has created an urgent need for safe, effective, and plant-based alternatives to conventional antibacterial therapies. The present study aims to develop and evaluate a polyherbal antibacterial gel formulated from Punarnava (*Boerhavia diffusa*), Uttareni (*Achyranthes aspera*), Bilwa (*Aegle marmelos*), and Nela Usiri (*Phyllanthus niruri*). Plant materials will be subjected to Soxhlet extraction using suitable solvents, followed by phytochemical screening, isolation, and identification of bioactive constituents. Artificial Intelligence (AI)-assisted tools will be employed for literature mining, prediction of phytochemical–target interactions, and prioritization of potential antibacterial compounds. Molecular docking and enzyme–substrate interaction studies will be performed to investigate the binding affinity of selected phytoconstituents against bacterial target proteins. The optimized extracts will be incorporated into a topical gel formulation, which will be evaluated for physicochemical properties, spreadability, viscosity, pH, homogeneity, stability, antimicrobial activity, and in vitro drug release using diffusion studies. The findings are expected to provide scientific evidence supporting the development of a stable, effective, and AI-assisted polyherbal antibacterial gel with potential applications in wound care and topical infection management.

Keywords : Anti-bacterial activity, Punarava, Uttareni, Bilwa, Nela usiri, Gel formulation.

INTRODUCTION:

Medicinal plants have long been used in traditional systems such as Ayurveda, Siddha, and Unani for the treatment of various diseases due to their rich phytochemical content, including alkaloids, flavonoids, tannins, glycosides, and saponins. With the increasing problem of antimicrobial resistance and the limitations of conventional antibiotics, there is growing interest in plant-based antimicrobial agents. This study focuses on the antibacterial potential of Punarnava (*Boerhaavia diffusa*), Nela Usiri (*Phyllanthus niruri*), Uttareni (*Achyranthes aspera*), and Bilwa (*Aegle marmelos*), which are traditionally used to treat infections and inflammatory disorders. Molecular docking will be employed to predict the interactions of phytoconstituents with bacterial target proteins, supporting the identification of potential antibacterial compounds. The study aims to evaluate the phytochemical profile and antibacterial activity of these medicinal plants and explore their potential for developing effective herbal antimicrobial formulations.

DOCKING:

Docking in chemistry, its basically a way to look at how two molecules might connect. Like, theres this method that can be done on computers or even in labs, to predict stuff about their interaction. Usually its a small molecule, the ligand I guess, and then a bigger one, like a biomolecule thats the receptor [1].

The point is to understand how well they bind, you know, the affinity part. And also the orientation, how they sort of position themselves. It seems kind of important for figuring out that stuff, but im not totally sure on all the details yet. Sometimes it feels like the computer side is more common, though experiments help check it out.

MOLECULAR DOCKING:

Molecular docking is a computer-based technique used to predict how a small molecule (ligand) binds to a target protein or enzyme (receptor) [2]. It estimates the binding orientation, interaction pattern, and binding affinity, helping identify stable ligand–protein complexes. Lower binding energy indicates stronger and more stable interactions. Docking also analyzes interactions such as hydrogen bonding, hydrophobic, and electrostatic forces. It is widely used in drug discovery for virtual screening of potential drug candidates, understanding enzyme–substrate interactions, and rational drug design. Molecular docking can be performed using rigid, flexible, or high-throughput approaches depending on the complexity and purpose of the study.

Uses of Molecular Docking

Molecular docking is an important computational technique widely used in chemistry and pharmaceutical research to predict and analyze interactions between molecules. It plays a major role in drug discovery, enzyme studies, virtual screening, and computational chemistry applications.

Drug Discovery and Drug Design

Molecular docking is extensively used in rational drug design to predict how small molecules (ligands) bind with target proteins or enzymes. It helps researchers estimate binding affinity, orientation, and interaction patterns between ligands and receptors. Docking reduces the need for expensive laboratory experiments by allowing virtual testing of compounds before synthesis. It also assists in identifying potential inhibitors and optimizing drug molecules for better specificity, potency, and therapeutic effectiveness.

Enzyme–Substrate Interaction Studies

Molecular docking is used to understand enzyme–substrate and receptor–ligand interactions by identifying key amino acid residues involved in binding and explaining enzyme mechanisms. It plays an important role in designing enzyme inhibitors and studying biochemical pathways. Additionally, molecular docking enables virtual screening of large compound libraries to identify promising drug candidates and supports lead optimization by improving binding affinity, selectivity, and pharmacokinetic properties while reducing experimental time, cost, and toxicity.

Structure-Based and Computational Chemistry Applications

Docking is a major component of structure-based drug design (SBDD) and is often combined with computational methods such as molecular dynamics simulations and quantum chemical calculations. It helps study molecular recognition, supramolecular interactions, host–guest chemistry, and prediction of off-target effects or toxicity of compounds.

Materials and Supramolecular Chemistry

Apart from medicinal chemistry, molecular docking is also applied in materials science and supramolecular chemistry. It assists in designing synthetic receptors, nanomaterials, catalysts, sensors, and molecular devices by predicting molecular orientation, binding sites, and interaction strengths.

Summary of Uses

Thus, molecular docking is a versatile computational approach used for:

- Predicting molecular interactions
- Drug discovery and development
- Studying enzyme mechanisms

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- Virtual screening of compounds
 - Lead optimization
 - Designing functional materials and supramolecular systems

Its predictive ability saves time, reduces research cost, and supports the rational design of pharmaceutical and chemical systems.

Purpose of Molecular Docking

The primary purpose of molecular docking is to predict the most favorable binding orientation of a ligand with a target protein or receptor at a specific binding site. It helps determine the strength and stability of ligand–receptor interactions, which are essential for understanding biological activity and drug action.

Molecular docking is widely used in structure-based drug design and virtual screening to identify new drug candidates and improve existing drugs. By simulating molecular interactions computationally, docking provides valuable insights into binding mechanisms, molecular recognition, and biological processes. This technique accelerates drug discovery, minimizes experimental workload, and contributes significantly to modern pharmaceutical and computational chemistry research.

Advantages of Molecular Docking

1. Economical Approach
2. Faster Screening Process
3. Accurate Prediction of Molecular Interactions
4. Assists in Drug Repurposing
5. Supports Structure-Based Drug Design
6. Efficient Virtual Screening.

Disadvantages of Molecular Docking

1. Absence of a Universal Synergistic Model
2. Limited Availability of Quality Datasets
3. Lack of Standardized Methodology
4. Limitations of Scoring Functions
5. Complexity in Interpretation
6. Difficulties with Multi-Domain Proteins
7. Challenges in Evaluating Multi-Drug Interactions.

Gel Formulations

Herbal gel formulations are prepared by incorporating selected plant extracts such as Hibiscus and Cymbopogon into an appropriate gel base like Carbopol 934 or Carbopol 940 along with suitable excipients [3]. Common additives include propylene glycol as a penetration enhancer and triethanolamine for pH adjustment. The aim is to develop a stable and effective topical formulation with good therapeutic activity. After preparation, the gel is evaluated for parameters such as pH, viscosity, spreadability, homogeneity, drug release, and stability according to ICH guidelines. These evaluations help determine the product's texture, skin compatibility, stability, and efficiency in delivering the active constituents for applications such as wound healing and antimicrobial therapy.

Steps Involved in Gel Formulation

1. Preparation of Herbal Extract

Plant materials such as *Cymbopogon citratus* or other medicinal herbs are collected, dried, and extracted using suitable solvents like methanol or ethanol to obtain the active constituents.

2. Preparation of Gel Base

A gelling polymer such as Carbopol 934 or Carbopol 940 is dispersed in distilled water and kept undisturbed for sufficient time to allow proper swelling and hydration.

3. Addition of Excipients

Various excipients including preservatives like methyl paraben and propyl paraben, penetration enhancers such as propylene glycol, and other solubilizing agents are incorporated into the gel base to improve stability and performance.

4. Incorporation of Herbal Extract

The prepared herbal extract is gradually added to the gel base with continuous stirring to ensure uniform distribution throughout the formulation.

5. Adjustment of pH

Triethanolamine is added slowly to the formulation to adjust the pH close to the natural skin pH range (approximately 6.8–7.0). Proper pH adjustment improves stability, compatibility, and patient acceptability of the gel formulation.

Evaluation Parameters of Herbal Gel Formulations

1. Physical Appearance
2. pH Determination
3. Viscosity Measurement
4. Spreadability
5. Extrudability Test
6. Stability Studies.
7. Drug Release and Diffusion Study
8. Antimicrobial Activity
9. Skin Irritation Test.

Important Ingredients Used in Herbal Gel Formulations

1. Gelling Agents
2. Penetration Enhancers
3. Preservatives
4. pH Adjusting Agent.

PUNARNAVA:

Punarnava (Boerhaavia diffusa L.) Family: Nyctaginaceae

Punarnava is an important Ayurvedic medicinal herb widely used for treating inflammation, edema, liver disorders, and urinary diseases. It is valued for its anti-inflammatory, antioxidant, diuretic, and rejuvenating properties.

Botanical Features: A perennial creeping herb with reddish stems, fleshy roots, succulent leaves, and small pink flowers. The roots are the main medicinal part.

Common Names: Punarnava (Sanskrit), Hogweed (English), Attamamide (Telugu), Sant (Hindi).

Distribution: Commonly found in tropical and subtropical regions, especially throughout India.

Major Phytochemicals: Alkaloids (punarnavine), flavonoids, lignans, rotenoids, xanthones, phytosterols, and phenolic compounds.

Ayurvedic Properties: Balances **Kapha** and **Vata**; used for inflammation, edema, anemia, digestive disorders, and liver ailments [4].

Pharmacological Activities: Antibacterial, anti-inflammatory, antioxidant, hepatoprotective, antidiabetic, antiviral, immunomodulatory, analgesic, and anti-asthmatic activities.

Harvesting & Storage: Roots are harvested, shade-dried at **40–45°C**, and stored in airtight containers in a cool, dry place to preserve phytochemicals.

Chemical structure of Punarnavine:

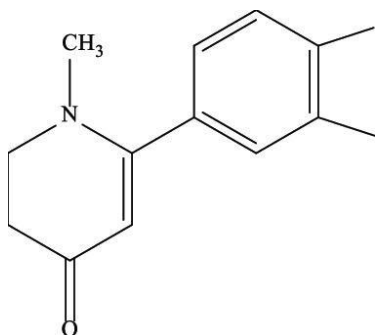


FIG 1.1 CHEMICAL STRUCTURE OF PUNARNAVINE NELA USIRI:

Nela Usiri (Phyllanthus niruri)

Family: Euphorbiaceae

Common Names: Nela Usiri, Bhumi Amla, Keezhanelli

Description:

Nela Usiri is a small annual medicinal herb (30–60 cm tall) widely used in Ayurveda for its antimicrobial, hepatoprotective, nephroprotective, anti-inflammatory, and antioxidant properties [5].

Major

icals:

Phyllanthin, Hypophyllanthin, Quercetin, Rutin, Geraniin, Corilagin, flavonoids, tannins, alkaloids, terpenoids, and saponins.

Medicinal Uses:

Phytochem

- Antibacterial, antiviral, and antifungal activity
- Liver protection
- Kidney stone management ("Stone Breaker")
- Anti-inflammatory and antioxidant effects
- Antidiabetic and digestive health support

Extraction:

The dried whole plant is commonly extracted using the **Soxhlet extraction** method with suitable solvents.

Advantages:

Natural antimicrobial, hepatoprotective, nephroprotective, antioxidant, and wound-healing properties.

Limitations:

May cause hypoglycemia, low blood pressure, mild gastrointestinal discomfort, and dehydration if consumed excessively.

Chemical structure of Phyllanthin:

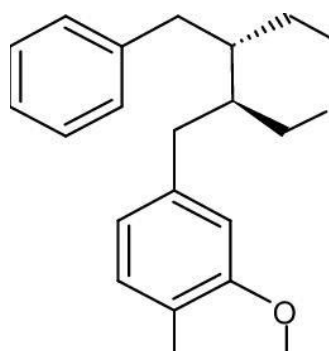


FIG 2.3 CHEMICAL STRUCTURE OF PHYLLANTHIN (NELA USIRI)

Methods of Use: Fresh juice, powder, decoction, capsules/tablets, and leaf paste for topical application.

Advantages: Hepatoprotective, supports kidney stone management, diuretic, antioxidant, anti-inflammatory, improves digestion, enhances immunity, helps regulate blood sugar and blood pressure, and promotes skin and hair health.

Disadvantages: May cause hypoglycemia, low blood pressure, mild gastrointestinal discomfort, drowsiness, and dehydration with excessive use.

UTTARENI SEEDS:

Uttareni (*Achyranthes aspera*)

- **Family:** Amaranthaceae
- **Common Name:** Uttareni (Prickly Chaff Flower)

Medicinal Importance:

- Traditionally used to treat infections, asthma, cough, skin diseases, liver disorders, wounds, and inflammation [6].

- Exhibits antibacterial, antioxidant, anti-inflammatory, and wound-healing properties.

Phytochemicals:

- Rich in alkaloids, flavonoids, saponins, tannins, phenolics, oleanolic acid, β -sitosterol, quercetin, and achyranthine.

Antibacterial Activity:

- Effective against bacteria such as *Staphylococcus aureus* and *Escherichia coli* due to its bioactive phytochemicals.

Plant Description:

- A perennial herb (1–2 m tall) with hairy stems, ovate leaves, greenish-white flowers, and brown seeds.
- Widely distributed across India and other tropical regions.

Cultivation:

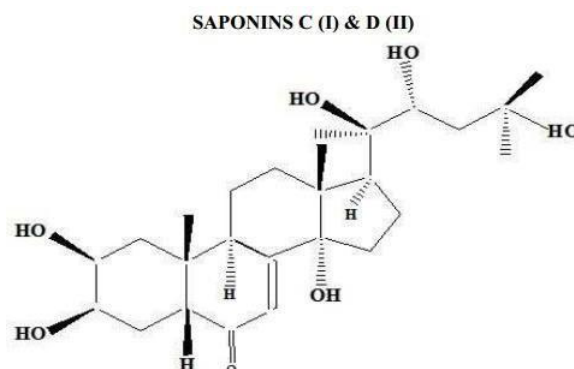
- Grows well in sunny locations with well-drained sandy loam soil.
- Propagated by seeds and requires moderate watering.

Seed Collection:

- Seeds are harvested from mature flowering spikes, shade-dried, and stored in airtight containers under cool, dry conditions.

Chemical structure of *Achyranthes aspera*:

FIG 3.1 CHEMICAL STRUCTURE OF ACHYRANTHES ASPERA



Description: Small, reddish-brown seeds traditionally used in Ayurveda.

Major Phytochemicals: Saponins, alkaloids (achyranthine, betaine), ecdysteroids, flavonoids, tannins, proteins, and carbohydrates.

Medicinal Uses: Possess antibacterial, antioxidant, anti-inflammatory, diuretic, digestive, expectorant, and wound-healing properties. Traditionally used for respiratory disorders, urinary infections, digestive problems, piles, and obesity management.

Methods of Use: Powder, decoction, and herbal formulations.

Advantages: Supports digestive, respiratory, urinary, and cardiovascular health; exhibits antioxidant and anti-inflammatory activities.

Side Effects: Excessive use may cause nausea, vomiting, skin irritation, and may affect male fertility with

prolonged use.

BILWA:

Bilwa (*Aegle marmelos*) Introduction:

Bilwa (*Aegle marmelos*), also known as bael or wood apple, is a medicinal plant widely used in Ayurveda for treating digestive disorders, infections, diabetes, and inflammation [7].

Description:

It is a hardy tree with a hard-shelled, aromatic fruit containing sweet, orange pulp rich in bioactive compounds.

Cultivation:

Bilwa grows well in tropical climates and well-drained soils. It is propagated by seeds or grafting and fruits are harvested after full maturity.

Major Phytochemicals:

Coumarins (marmelosin), alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, and essential oils.

Medicinal Uses:

- Antibacterial and antifungal
- Anti-inflammatory and antioxidant
- Anti-diarrhoeal and anti-ulcer
- Antidiabetic
- Supports digestive and cardiovascular health

Methods of Use:

Consumed as fresh fruit, juice, decoction, powder, or herbal formulations.

Advantages:

Natural antimicrobial, antioxidant, digestive aid, mild laxative, and rich in vitamins and minerals.

Disadvantages:

Excess consumption may cause diarrhoea, bloating, or indigestion due to its high fibre content.

Chemical structure of Bilwa fruit constituents:

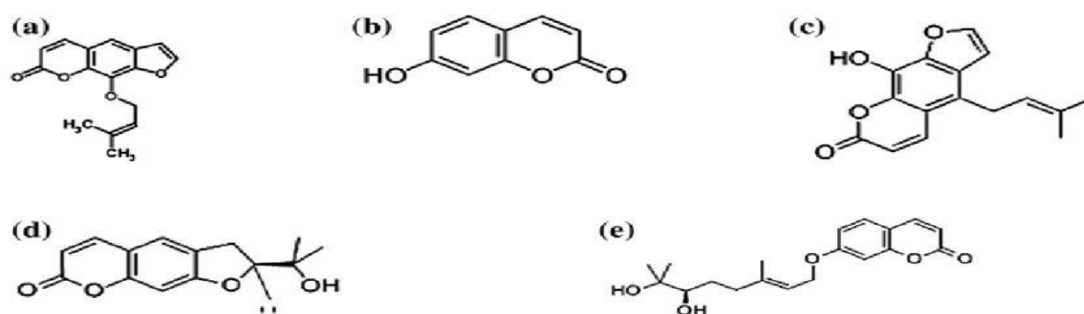


FIG 4.2 BILWA FRUIT CONSTITUENTS

2 Structure of some common coumarins. a Marmelosin, b Umbelliferone, c Alloimperatorin, d Marmesin, e

Marmin**SOXHLETATION PROCESS:**

Soxhlet extraction is a continuous extraction technique used to isolate bioactive compounds from plant materials [8]. The dried and powdered plant sample is placed in a Soxhlet apparatus, and a suitable solvent is heated to reflux. The solvent vapors condense, pass through the plant material, dissolve the phytochemicals, and siphon back into the flask. This cycle repeats until efficient extraction is achieved. The extract is then concentrated by evaporating the solvent and stored for further analysis and formulation.

Materials and Methods:**Materials**

- **Plants:** Punarnava (*Boerhaavia diffusa*), Nela Usiri (*Phyllanthus niruri*), Uttareni (*Achyranthes aspera*), Bilwa (*Aegle marmelos*)
- **Solvents:** Methanol, Ethanol, Distilled water
- **Gel ingredients:** Carbopol, Propylene glycol, Triethanolamine (TEA), Methyl paraben, Propyl paraben
- **Equipment:** Soxhlet apparatus, Hot air oven, Incubator, Autoclave, Laminar airflow, Electronic balance.

Method

The dried plant materials were powdered and extracted using **Soxhlet extraction** with methanol. The extracts were concentrated, dried, and subjected to phytochemical screening. Herbal gels were prepared by dispersing Carbopol in water, incorporating the plant extract with propylene glycol and preservatives, followed by neutralization with TEA to obtain a homogeneous gel [9]. The formulations were adjusted to **pH 6–7**, packed in airtight containers, and evaluated for antibacterial activity and physicochemical properties.

Method for Gel Formulation**1. Preparation of Uttareni Herbal Gel (*Achyranthes aspera*)**

Carbopol was dispersed in distilled water and allowed to swell. The ***Achyranthes aspera*** extract was dissolved in propylene glycol with preservatives and added to the Carbopol dispersion. Triethanolamine was added to form the gel and adjust the pH (6–7). The gel was mixed thoroughly, transferred to airtight containers, and stored in a cool, dry place.

2. Preparation of Punarnava Herbal Gel

The Punarnava extract was incorporated into a hydrated Carbopol gel base containing propylene glycol and preservatives. Triethanolamine was added to form the gel and adjust the pH (6–7).

The final volume was made up with distilled water, mixed thoroughly, packed in airtight containers, and stored under cool, dry conditions for further evaluation.

3. Preparation of Phyllanthus niruri Herbal Gel

Carbopol was dispersed in distilled water and allowed to swell. The herbal extract was dissolved in propylene glycol containing preservatives and then mixed with the Carbopol dispersion. Triethanolamine was added to form the gel, the pH was adjusted to 6–7, and the final volume was made up with distilled water. The gel was mixed thoroughly, packed in airtight containers, and stored for further evaluation.

4. Preparation of Bilwa Fruit Herbal Gel

The Bilwa fruit extract was incorporated into a Carbopol gel base prepared in distilled water. Preservatives and propylene glycol were added, followed by neutralization with triethanolamine (TEA) to obtain a clear gel. The

pH was adjusted to 6–7, mixed thoroughly to achieve a homogeneous formulation, and packed in airtight containers for storage.

RESULTS AND DISCUSSION:

Extraction of various herbal drugs and we isolated the **Aegeline**, Achyranthine, Allonorsecurinine, amoxicillin alkaloids from each plant their structures are drawn and subjected to the molecular docking studied the docking reports are as follows:

S.No	Derivative	C- Docker energy	C- Docker Interaction energy
1	Aegeline	-16.2655	-16.2655
2	Achyranthine	-6.21051	-24.6028
3	Allonorsecurinine	74.0794	-11.2795
4	Amoxycilin	65.2932	4.2632

TABLE -1 ALKALODS

Basing on the molecular docking studies one of the alkaloid from the plant Bilwa having highest negative docking score (Bilwa fruit and Aegeline) and remaining are also showed good negative dock scores hence the gel is formulated .

S.No	Ingredient	Quantity	Function
1	Herbal extract (Punarnava / Nela Usiri / Uttareni / Bilwa)	2 g	Active ingredient
2	Carbopol 934 / 940	0.5 g	Gelling agent
3	Propylene glycol	10 g	Humectant & penetration enhancer
4	Methyl paraben	0.2 g	Preservative
5	Propyl paraben	0.02 g	Preservative
6	Triethanolamine (TEA)	q.s.	pH adjuster & gel stabilizer
7	Distilled water	q.s. to 100	Vehicle

Uses

Punarnava (Boerhaavia diffusa): Treats skin infections, promotes wound healing, reduces inflammation, and helps manage edema-related skin disorders.

Nela Usiri (Phyllanthus niruri): Useful for acne, skin infections, wound healing, and relieving skin irritation and inflammation.

Uttareni (Achyranthes aspera): Acts as an antibacterial and anti-inflammatory gel for skin infections, wounds, ulcers, warts, and insect bites.

Bilwa (Aegle marmelos): Helps manage infected wounds, skin ulcers, bacterial and fungal infections, and protects damaged skin by promoting healing [10].

EVALUATION TESTS:

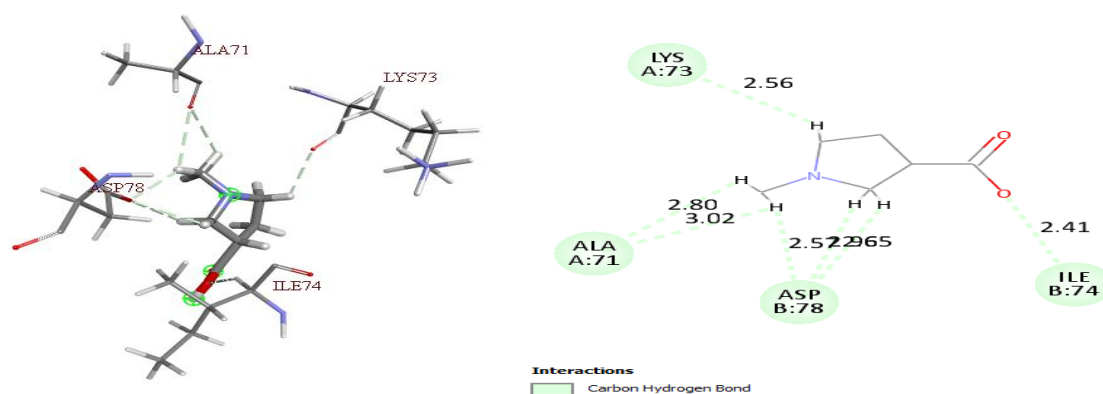
- **pH:** Measured using a digital pH meter to ensure skin compatibility.
- **Drug Content:** Determined by UV spectrophotometry to ensure uniform drug distribution.
- **Viscosity:** Evaluated using a Brookfield viscometer to assess gel consistency.
- **Spreadability:** Measured to determine the ease of application on the skin.
- **Extrudability:** Assessed by measuring the force required to extrude the gel from the tube.

- **Skin Irritation:** Evaluated on guinea pigs to determine the safety and irritation potential of the formulation.

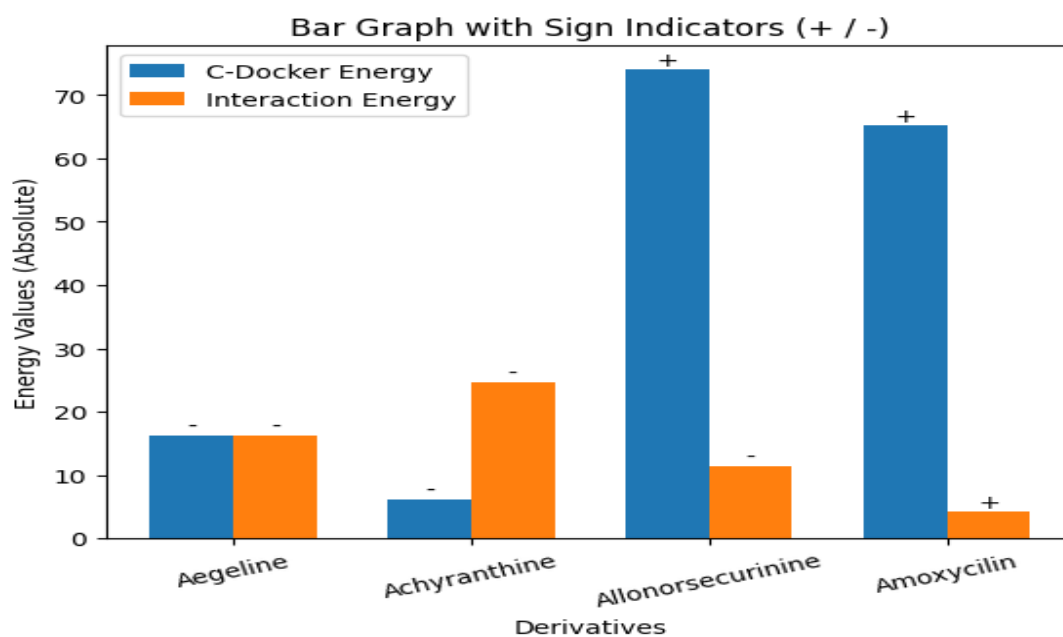
S. No.	Evaluation Test	Standard / Limit / Note	Observed Value
1	pH	~5.5 – 7 (skin compatible)	6.9
2	Drug Content	95% – 105%	95%
3	Viscosity	Should be consistent (Brookfield readings)	450cP
4	Spreadability	Less time → better spreading	1.00g/sec
5	Extrudability	Easily extrudable with moderate force	500g/cm ²
6	Skin Irritation	No irritation (Score: 0 preferred)	0

TABLE NO- 3 GEL EVALUATION PARAMETERS

Docking result



DOCKING RESULT FOR ACHYRANTHINE



DISCUSSION

This study evaluates the antibacterial potential of **Punarnava, Nela Usiri, Uttareni, and Bilwa**, which are rich in bioactive phytochemicals with therapeutic properties. Owing to increasing antibiotic resistance, these medicinal plants are being explored as safer alternatives to synthetic drugs. Molecular docking is used to predict phytochemical–protein interactions and identify potential antibacterial compounds. The findings highlight the potential of combining traditional herbal

medicine with computational techniques for developing effective antimicrobial agents

The present study focuses on the extraction and isolation of alkaloids from polyherbal drugs and the evaluation of their wound-healing potential with the support of artificial intelligence–based approaches. Medicinal plants containing bioactive alkaloids are considered important sources of natural therapeutic agents due to their antimicrobial and tissue-repairing properties.

Herbal gel formulations prepared from *Boerhaavia diffusa* (Punarnava), *Phyllanthus niruri* (Nela Usiri), *Achyranthes aspera* (Uttareni), and *Aegle marmelos* (Bilwa) are primarily intended for topical therapeutic applications. These formulations are widely used in the management of:

- Skin infections
- Wound healing
- Acne and pimples
- Inflammatory skin disorders
- Ulcers and other microbial skin conditions

The herbal gels provide localized antibacterial activity at the site of application and enhance the absorption of active constituents through the skin. In comparison with synthetic formulations, these herbal preparations are considered safer and are associated with reduced side effects, making them promising alternatives in topical antimicrobial and wound-healing therapy. Conflicts of interest: Authors does not have any conflicts of interest; all authors are equally shared the work. The research work was done with support of management and DST fist with central found.

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