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DEVELOPMENT AND EVALUATION OF A *PIPER BETLE* L. LEAF EXTRACT CLAY MASK WITH MOLECULAR DOCKING STUDIES FOR ANTI – ACNE ACTIVITY

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ABSTRACT: The present study was carried out to develop and evaluate a *Piper betle* L. leaf extract-based herbal clay mask for potential anti-acne application and to determine the binding affinity of selected phytoconstituents towards acne-related molecular targets using molecular docking studies. Fresh leaves were collected and authenticated, followed by pharmacognostical studies and ethanolic extraction by cold maceration. Preliminary phytochemical screening revealed alkaloids, phenolic compounds, flavonoids, tannins, triterpenoids and steroids. TLC analysis using toluene:ethyl acetate (7:3) provided a preliminary fingerprint profile with two distinct coloured spots. Molecular docking showed favourable predicted interactions of hydroxychavicol with 5α-reductase, TLR2, TLR4 and MMP-9, with the most favourable predicted binding of −7.2 kcal/mol with MMP-9. The clay mask showed smooth and uniform appearance, good homogeneity, pH 5.0–6.0, good spreadability, easy washability and satisfactory stability. Further experimental studies are required to confirm actual anti-acne efficacy, mechanism of action and safety.

KEYWORDS:*Piper betle* L.; Hydroxychavicol; Herbal clay mask; Anti-acne; Molecular docking; Thin Layer Chromatography

INTRODUCTION

Herbal cosmetics are products formulated using natural plant materials such as herbs, extracts, and essential oils for skin and personal care. They contain bioactive phytoconstituents such as flavonoids, phenolics, tannins, and essential oils, which may exhibit antioxidant, antimicrobial, and anti-inflammatory activities. Acne is a common skin condition associated with excess sebum production, follicular blockage, microbial activity, and inflammation, particularly in oily skin. Herbal clay masks containing natural clays such as kaolin and bentonite help absorb excess oil and remove surface impurities. *Piper betle* L., commonly known as betel leaf and belonging to the family Piperaceae, contains phytoconstituentSuch as hydroxychavicol,eugenol, chavicol, and chavibetol, which have been investigated for their antimicrobial, antioxidant, and anti-inflammatory properties. The

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present study aims to develop and evaluate a *Piper betle* L. leaf extract-based herbal clay mask for potential anti-acne application through pharmacognostic studies, ethanolic extraction, preliminary phytochemical screening, thin-layer chromatography (TLC), molecular docking, and evaluation of physicochemical and cosmetic parameters, including appearance, colour, odour, pH, spreadability, washability, and stability.

Fig no 1: *Piper betle* clay mask

MATERIALS AND METHODS

1. PHARMACOGNOSTIC STUDIES

Collection and Authentication of Plant Material

Fresh *Piper betle* L. leaves were collected from Marthandam, Kanyakumari district, Tamil Nadu, and authenticated by Dr. N. Maybel Starlin, Head, Department of Botany, Nesamony Memorial Christian College, Marthandam. The plant belongs to the family Piperaceae.

Morphological Studies

The leaves were examined for colour, odour, taste, size, and shape, and the observations were tabulated.

Microscopical Studies

Fresh leaf sections were prepared using a sharp blade, stained with phloroglucinol–HCl and safranin, and observed under a microscope.

Preparation of Powder

The collected leaves were washed, shade-dried, and mechanically pulverized into coarse powder. The powder was used for pharmacognostic and phytochemical studies, while fine powder was used for powder microscopy.

Powder Microscopy

Temporary powder preparations were mounted in glycerin and examined under a microscope to identify the anatomical characteristics of the powdered leaves.

Determination of Stomatal Number and Stomatal Index

Small leaf pieces (2 mm²) were cleared by boiling in chloral hydrate solution for 30 minutes. The epidermis was peeled, mounted in glycerin, and examined microscopically. Stomata and epidermal cells were counted within a defined area, and at least four readings were taken to calculate the average stomatal number and stomatal index.

2. EXTRACTION

Extraction of Phytochemicals from *Piper betle* Leaf

Materials Needed

Dried *Piper betle* leaf powder
90% ethanol
Beaker and glass rod
Whatman No. 1 filter paper
Funnel

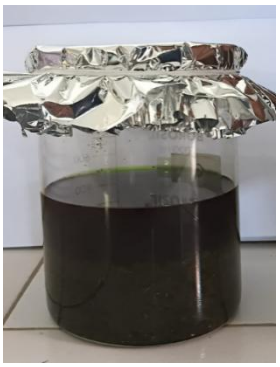
Procedure

Preparation of Plant Material: Fresh leaves were washed, shade-dried, and crushed into coarse powder.

Cold Maceration: About 50 g of dried leaf powder was mixed with 500 mL of 90% ethanol in a clean beaker. The mixture was covered with aluminium foil and kept at room temperature for 72 hours with occasional stirring.

Extraction Process: After 72 hours, the mixture was filtered through Whatman No. 1 filter paper to remove plant residues.

Extraction of Crude Extract: The filtrate was concentrated by evaporating the solvent on a water bath. The obtained extract was collected and stored for further phytochemical screening, TLC, and formulation studies.



Figno 2 : Cold maceration of powdered *Piper betle*for 72 hours

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3. PHYTOCHEMICAL STUDIES

Preliminary phytochemical screening was performed on the ethanolic extract to identify major constituents, including carbohydrates, proteins, phenolic compounds, flavonoids, tannins, glycosides, and alkaloids.

4. THIN LAYER CHROMATOGRAPHY (TLC)

Preparation of TLC Plates

Silica gel G slurry was prepared using distilled water and uniformly coated onto clean glass plates. The plates were air-dried and activated in a hot air oven at 110°C for 30 minutes.

Preparation of Sample Solution

The ethanolic extract of Piper betle leaves was used as the sample solution.

Application of Sample

A baseline was marked approximately 1 cm above the lower edge of the plate, and the extract was applied as a spot using a capillary tube.

Development of Chromatogram

The TLC plate was placed in a chamber saturated with toluene and ethyl acetate (7:3 v/v). After development, the plate was removed, air-dried, and sprayed with vanillin–sulphuric acid reagent to visualize the separated spots.

Calculation of Rf Value

The R_f was calculated using the formula:

R_f = Distance travelled by the sample spot / Distance travelled by the solvent front

5. MOLECULAR DOCKING STUDIES

Molecular docking is a computational technique used to predict ligand–protein interactions and estimate binding affinity. In this study, selected *Piper betle* phytoconstituents were evaluated against a suitable target protein, and their docking scores were compared.

Procedure

Phytoconstituents of *Piper betle* were identified using Dr. Duke's Phytochemical and Ethnobotanical Database. Their SMILES structures were retrieved from PubChem and submitted to SwissADME to assess physicochemical properties and drug-likeness. The compounds were also analysed using ProTox for in-silico toxicity prediction. Based on these results, suitable compounds were selected for docking studies.

Molecular docking was performed using the CB-Dock web server. Ligand structures were obtained from PubChem, and a suitable target protein was selected based on its PDB ID and biological source. The protein structure was downloaded and uploaded along with the selected ligands to CB-Dock. The resulting Vina scores and ligand–protein interaction images were recorded and saved for further analysis.

FORMULATION OF HERBAL CLAY MASK

SL.NO	INGREDIENTS	WEIGHING FORMULA (150 g)
1	*Piper betle* leaf ethanol extract	10% (15 g ≈ 15 mL)
2	Kaolin	25% (37.5 g)
3	Bentonite	1.5% (2.25 g)
4	Glycerin	8% (12 g ≈ 9.5 mL)
5	Propylene glycol	5% (7.5 g)
6	Methyl paraben	0.1% (0.15 g)
7	Rose water	qs

PROCEDURE

All ingredients were accurately weighed for a final batch size of 150 g.Methylparaben (0.15 g) was dissolved in a small quantity of propylene glycol (7.5 g) with gentle stirring.Glycerin (9.5 g) and the remaining propylene glycol were mixed thoroughly, followed by gradual addition of rose water to form a uniform liquid phase.Kaolin (37.5 g) and bentonite (2.25 g) were mixed uniformly using a clean, dry mortar and pestle.The clay mixture was gradually added to the liquid phase with continuous trituration to obtain a smooth, lump-free paste.*Piper betle* leaf ethanol extract (15 ml) was added gradually and mixed thoroughly for uniform distribution.Rose water was added if necessary to adjust the consistency while maintaining the final weight at 150 g.The prepared clay mask was transferred into a clean, dry, airtight container, labelled and stored appropriately for further evaluation.

EVALUATION OF HERBAL CLAY MASK

1. Organoleptic Evaluation

Colour, odour, appearance and texture were examined visually.

2. pH Determination

The pH was measured using a calibrated pH meter or universal pH indicator paper.

3. Homogeneity Test

The formulation was spread on a clean glass slide and examined for uniformity and lumps.

4. Spreadability Test

Approximately 1 g of formulation was placed between two glass slides. A 100 g

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weightwasapplied for 1 minute, and the spread diameter was measured.

5. Drying Time Test

A thin layer was applied to a clean glass plate, andthe time required for complete drying was recorded.

6. Washability Test

The dried clay mask was washed with distilled water, and its ease of removal was observed.

7. Grittiness Test

The formulation was gently rubbed between the fingers or on a glass slide to check for coarse particles.

8. Stability Study

The formulation was stored in an airtight container at room temperature and periodically checked for changes in colour, odour, pH, homogeneity, consistency and phase separation.

RESULT AND DISCUSSION

1.PHARMACOGNOSTICAL STUDIES

Morphological Evaluation of *Piper betle* leaf

Features	Description
Shape	Ovate to rounded ovate
Size	19cm × 9.2 cm
Texture	Membranous, glaborous
Colour	Green to dark green
Aroma	Aromatic and characteristic
Taste	Pungent, acrid and slightly bitter taste
Base	Cordate, usually Asymmetric/oblique
Apex	Narrowly and sharply acuminate

Microscopy and powder characteristics of *Piper betle*

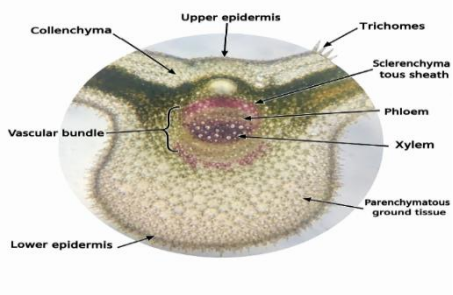

Fig no 3 : T.s of *Piper betle* L

Upper epidermis: Protects the leaf and reduces water loss.
Collenchyma: Provides flexible support.
Parenchymatous ground tissue: Stores food and water.
Sclerenchymatous sheath: Provides mechanical strength.
Phloem: Transports prepared food.
Xylem: Transports water and minerals.
Vascular bundle: Contains xylem and phloem.
Trichomes: Protect the surface.
Lower epidermis: Protects the lower leaf surface.

Powder characteristics of *Piper betle* leaf

Epidermal cells: Polygonal cells with distinct walls.
Stomata: Useful for leaf identification.
Trichomes: Simple covering hairs.
Parenchymatous cells: Mesophyll tissue fragments.
Vascular tissues: Xylem and phloem fragments.
Calcium oxalate crystals: Crystal fragments in leaf tissues.
Oil/secretory cells: Associated with the aromatic properties.
Spiral and annular vessels: Xylem vessel fragments.

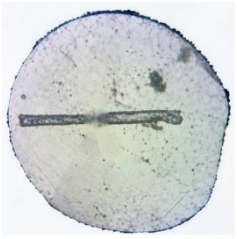

Fig no4 : Fiber

Figno 5 : Trichomes

Determination of stomatal number and stomatal index

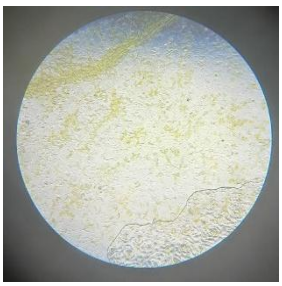

Fig no6:stomata

Lower epidermis: 38 stomata
Upper epidermis: 7 stomata

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REPORT

The stomatal index of the upper epidermis of the leaf was found to be 7.5, calculated from 7 stomata and 86 epidermal cells. The stomatal index of the lower epidermis was found to be 15.3, calculated from 38 stomata and 210 epidermal cells. Thus, the stomatal index was found to be higher in the lower epidermis (15.3) than in the upper epidermis (7.5), indicating that stomata are more abundant on the lower surface of the leaf. This observation is consistent with the typical distribution of stomata in many dicot leaves, where stomata are predominantly present on the lower epidermis.

PHYTOCHEMICAL STUDIES

S. No.	Phytochemical Group	Result
1	Carbohydrates	+ve
2	Proteins and Amino Acids	+ve
3	Alkaloids	+ve
4	Phenolic Compounds	+ve
5	Flavonoids	+ve
6	Glycosides	-ve
7	Tannins	+ve
8	Anthraquinone Glycosides	-ve
9	Saponins	-ve
10	Triterpenoids	+ve
11	Steroids	+ve

MOLECULAR DOCKING STUDIES

Molecular docking showed that hydroxychavicol from *Piper betle* exhibited favorable binding with 5α-reductase, TLR2, TLR4, and MMP-9, with the strongest affinity for MMP-9 (−7.2 kcal/mol). Kaempferol, apigenin, and β-sitosterol also showed favorable interactions. These findings suggest potential anti-acne activity, but further experimental studies are needed for confirmation.

THIN LAYER CHROMATOGRAPHY

TLC was performed on the ethanolic extract of *Piper betle* leaves using silica gel G as the stationary phase and toluene : ethyl acetate (7:3) as the mobile phase.
The plate was sprayed with vanillin–sulphuric acid reagent, revealing two distinct spots.
Spot 1 appeared orange with an Rf value of 0.78, while Spot 2 appeared violet with an Rf value of 0.63.
The results were compared with published TLC studies on *Piper betle* leaf extracts usingsilica gel plates and vanillin–sulphuric acid reagent.
The Rf value of Spot 2 was comparable to the published Rf value reported for hydroxychavicol under different chromatographic conditions.
These findings support preliminary phytochemical profiling; further analysis is required to confirm the identities of the compounds.

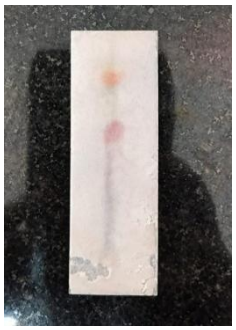

Fig no 7: visualized chromatogram

FORMULATION STUDIES

COMPOUNDS	5 α Reductase	TLR2	TLR4	MMP9
HYDROXYCHAVICOL	- 6.5	-6.9	-6.3	-7.2
KAEMPFEROL	-9.2	-8.3	-8	-9.2
APIGENIN	-9.3	-8.5	-8.2	-9.5
β- SITOSTEROL	-9.3	-10.6	-10.7	-7.2

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Formulation of *Piper betle* clay mask

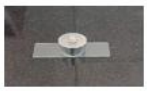
Fig 8: Dry powder phase

Fig 9: Aqueous phase

Fig 10: Mixing

Fig 11: Anti acne claymask

EVALUATION OF CLAY MASK

Sl.no	Test	Observation
1	Organoleptic evaluation	Colour: Pale greenish - grey to off-white Odour: Characteristic odour Appearance: Smooth, uniform paste Texture: Smooth and soft
2	PH determination	PH: 5.0 – 6.0
3	Homogeneity Test	Good homogeneity; uniform distribution of ingredients with no visible lumps.
4	Spreadability test	Easily spreadable on the glass slide. Diameter of spread: 4 cm 
5	Drying Time Test	The formulation dried uniformly after application. Drying time: 15 min.
6	Washability Test	Easily washable; formulation was removed without difficulty.
7	Grittiness Test	No coarse particles were observed; formulation felt smooth on gentle rubbing
8	Stability Study	No significant change in colour, odour, pH or consistency was observed. Homogeneity maintained; no phase separation observed.

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CONCLUSION AND FUTURE PERSPECTIVES

The present study was successfully carried out to develop and evaluate a *Piper betle L*. leaf extract-based herbal clay mask for potential anti-acne application. The leaves were collected, authenticated and subjected to ethanolic extraction by cold maceration, which produced an extract yield of 28% w/w. Preliminary phytochemical screening revealed the presence of important constituents such as alkaloids, phenolic compounds, flavonoids, tannins, triterpenoids and steroids. TLC analysis provided a preliminary fingerprint profile of the extract with distinct coloured spots. Molecular docking studies of selected phytoconstituents showed favourable predicted binding with the selected targets, and hydroxychavicol showed a favourable binding affinity of −7.2 kcal/mol with MMP-9. The formulated clay mask showed a smooth and uniform appearance, good homogeneity, characteristic odour, pH in the range of 5.0–6.0, good spreadability and easy washability. The formulation also showed satisfactory stability, with no significant changes in colour, odour, pH, consistency or homogeneity during the stability study. Overall, the findings indicate that *Piper betle L*. leaf extract has potential as a herbal ingredient for thedevelopment of an anti-acne clay mask. However, further experimental studies are required to confirm its actualanti-acneefficacy, mechanism of action and safety.

Future studies should evaluate the anti-acne activity of Piper betle L. leaf extract against Cutibacterium acnes and investigate its anti-inflammatory and antioxidant properties. Further research should identify active phytoconstituents and optimize the formulation for improved stability and skin compatibility. Safety, skin irritation and clinical studies are also required to establish its effectiveness and suitability for topical application. The extract may further be developed into gels, creams and other herbal cosmetic formulations.

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