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Development of Blumea lacera Gel Targeting Skin Disease

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Tribal people utilize Blumea lacera leaves for the treatment of skin injury. This plant has antibacterial and therapeutic qualities, according to ayurveda. Present study was aimed to formulate and evaluate an antimicrobial gel using Blumea lacera leaf extract for the treatment of skin diseases. Chemical identification tests and phytochemical screening were carried out to ascertain the presence of bio-active compounds. The extract efficacy was initially assessed through the agar plate method and diffusion test. The chemical identification tests revealed presence of alkaloids, flavonoids, tannins, and terpenoids in the leaf extract. It also revealed inhibitory effects against various microbial strains. Molecular docking studies matched with the antimicrobial compounds in docking to prove its activity. The docking scores of the nominated phytoconstituents (PubChem ID – 1548943, 6989) showed a higher interaction score. Polymer carbopol 940 in 1.12% exhibited good gelling property to the formulated gel of leaf extract powder. It has shown sustained effect for 8 hours. This comprehensive analysis enhances our understanding for the antimicrobial potential of the formulated gel, paving the way for future developments in plant-based antimicrobial agents targeting skin diseases.

Keywords: Antimicrobial; Blumea lacera; Docking; Gel; Skin; Docking.

Medicinal plants have been used for millennia as an alternative source of medication to address human ailments.[1] The genus *Blumea* belongs to the *Asteraceae* family of flowering plants. These are little golden flowers on these plants. *Blumea lacera* (*B. lacera*) is an annual herb that grows from southeast asia to the tropical and subtropical areas of asia. The plant has a high stem, a corymbose-patterned pattern, and a camphor-like fragrance. The primary and secondary metabolite classes from this plant that were investigated were alkaloids, amino acids, sugars, tannins, phenolic compounds, reducing sugar, flavonoids, saponins, coumarin, and terpenoids.[2]

It has been reported in the literature that the phytochemical analysis showed trace amounts of flavonoids, triterpenoid, acetylenic compounds, thiophene derivative, diester, prenylated phenol glycosides and monoterpene glycosides. The Indian system of conventional medicine known as ayurveda describes its use as bitter, acrid, astringent, thermogenic, errhine, styptic, anti-inflammatory, digestive, ophthalmic, liver tonic, anthelmintic, febrifuge, expectorant, diuretic,

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antipyretic, stimulant, and deobstruent.[3] The studies for *Blumea lacera* reported its antifungal, antibacterial, cytotoxic, antipyretic, antiviral, antileukemic and antidiarrheal properties. The primary phytoconstituents utilized in the treatment are the leaves of the plant. This plant is mostly used to treat fever, burning sensations, and bronchitis.[4-6]

There is no marketed product of *Blumea lacera*. Market survey indicated presence of a tablet named Pilex forte which contains a species belonging to *Blumea* family. Pilex forte is used in the treatment of piles and helps to correct chronic constipation related to this condition.[7]

Tribal people utilize *Blumea lacera* to treat skin injuries. This plant has antibacterial and therapeutic qualities, according to ayurveda. It can be used to treat wounds. Research indicated that this plant's high tannins and flavonoid content is what gives it its activity.[8] The plant's crushed leaves exhibit strong activity, as seen in tribal areas. The survey carried out in the plant collected areas (multiple locations across Maharashtra, encompassing Khopoli (Raigad), Mulshi, and Chiplun) where tribal people indicated that they apply the leaves directly on the skin injuries. Negative effects of synthetic active pharmaceutical ingredients (APIs) include enhanced bacterial resistance, a higher risk of skin cancer, skin redness, rashes, dryness, as well as skin irritation from long term use.[9-10]

Aim of present research work was to formulate topical gel from the leaves which can be applied for its antimicrobial and anti-inflammatory property on skin diseases. By forming the herbal formulation into a gel, the potency can be sustained by targeted distribution over an extended period of time.

MATERIALS AND METHODS

Materials

Blumea lacera is widely spread around Indian subcontinent. The foliage used in this study was sourced from Khopoli (Raigad), Mulshi, and Chiplun. The climatic conditions were ideal for flowering of Blumea lacera as it is an annual herb. The collection period was one month at beginning and then as per need again collection was done. The botanical verification of the plant materials was carried out by experts affiliated with the Baburaoji Gholap College, Pune district education association.

Methods

Drying and grinding process

Leaves were meticulously cleansed with running tap water and subsequently subjected to the gentle process of shade-drying. The dried leaves were then meticulously ground into a fine powder using traditional mortar and pestle technique. The choice of shade-drying was made due to its capacity to retain essential oils. This technique employs lower temperatures compared to other drying methods. Mechanical method involving a mortar and pestle was employed to crush the dried leaves until a fine, uniform powder was achieved.

Extraction process

Two methods for extraction were used for *Blumea lacera* leaves as decoction and soxhlet extraction method.[11]

Decoction method

The extraction from the plant material was executed through a decoction method, which is suitable for compounds that exhibit thermal stability and solubility in water. The unprocessed plant material was boiled in water until it was reduced to one-fourth of the initial volume. This process was reiterated to ensure concentrated extraction. The resulting extract was filtrated, collected in a petri dish, and then heated to 60°C to promote evaporation. The dried, adhesive extract was subsequently collected and ground into a fine powder using mortar and pestle technique.

Soxhlet extraction method

Initially, the dried leaves were ground into a coarse powder. This powdered material was then packed into a porous thimble, which was inserted into the soxhlet extractor. Ethanol was used as the solvent, which was heated in a round-bottom flask. As the solvent vaporized and condensed in the thimble, it extracted various compounds from the leaves during several hours of extraction. The collected solvent was subsequently separated and subjected to evaporation to obtain a concentrated extract suitable for analysis or various applications.

Percentage yield

The yield was calculated by dividing the weight of leaves by the percentage of extract produced.

Organoleptic analysis

The extract was then characterized for colour, taste and odor.

Preliminary analysis

For ethanolic extract and aqueous extract test were carried out for to determine presence of steroidal constituents, flavonoids , tannins ,saponins and alkaloids.[11]

Docking

Molecular docking studies

Molecular docking was used to determine the binding capacity and strategy of the selected phytoconstituents and small molecules (ligands). The reference ligand was docked first, followed by the test ligands, for flawless confirmation of the docking research. These studies aimed to check the possibility of constituents to have antibacterial property.[12-13]

The software used were Schrodinger-Maestro 13.3 for protein and ligand preparation, docking and analysis of docking results. The databases was RCSB.PDB (www.rcsb.org) for protein and pub-chem database (pubchem.ncbi.nlm.nih.gov) for ligand retrieval.

Protein preparation

Protein 3D structures were retrieved from the RCSB.PDB database (https://www.rcsb.org) (Berman 2000). The chosen proteins (PDB ID-1QTN, 4ZZZ, 7XJ0) was energy minimized with Schrodinger-Maestro 13.3 and constructed with the same programme by removing water molecules, adding polar hydrogen atoms, and adding partial charges. The produced proteins were used in docking research.

Ligand preparation

The chosen ligands were obtained in SDF format from the pubchem database (https://pubchem.ncbi.nlm.nih.gov), and Schrodinger-Maestro13.3 was used for ligand production and stabilization.

Zone of Inhibition of Test Sample

The standard compound was Methicillin 5mcg (MET)& Norfloxacin 10mcg (NX).

Organisms used were *Staphylococcus aureus (S. aureus)* and *Pseudomonas aeruginosa* (*P. aeruginosa)*.[14-15]

The study involved cup plate diffusion method. Pure bacterial culture (0.1 ml) with optical density (OD) adjusted to 1 McFarland standard. It was pipetted onto the Mueller-Hinton agar plate using a sterile cotton swab and was spread evenly all over agar plate. After the plate was dried, it is divided into four equal quadrants. A hole is bored at the center of each quadrant on the agar surface using a sterile cork borer. Each test solution 0.1ml (aqueous extract powder) was added to the respective well. Using a sterile forcep, a standard reference antibiotic disc is placed on the media plate. Without inverting, the petri plates, they were kept for incubation at 37°C for 24 – 72 hours. After the incubation period, zone of inhibition for test and standard was observed and measured in mm.

Gel formulation

To formulate gel carbopol 940 was used as polymer. It was tried from 0.5 to 4 % concentration. After the formulation and homogenization process, carbopol 940 gel was combined with the aqueous plant extract powder. The addition of triethanolamine provided an optimal buffer to maintain pH neutrality. Final batch was formulated with *Blumea lacera* leaf extract powder (1%), carbopol 940 (1.12%), triethanolamine (1%), propyl paraben (0.5%) and propylene glycol (5%).[16] Gel was evaluated for its physical properties, spreading efficiency and % release through cellophane membrane. pH was measured by pH meter. Spreading potency was estimated using spreadability apparatus. The mass on the pulley was 120 gm. 1gm of gel was inserted on the fixed slide. The length travelled by the slide was 24 cm. Time taken for slide to move this distance was about 2 seconds. In primary dermal irritation index the gel was applied on open skin with spreading. The irritation on skin was measured at the time span of 4 minutes, 20 minutes, 30 minutes, 1 hour and 4 hours.

RESULTS

The odor of extract was strong camphor like. The color was brown to dark brown and taste was bitter.[17-19] Preliminary analysis is as shown in Table 1. The preliminary analysis showed presence of tannins, flavonoids, steroids, carbohydrates, saponins and alkaloids in etanolic as well as aqueous extract (Table 1)

Molecular docking

The selected (2) phytoconstituents were docked against the target CASPASE-8, PARP1 and TRPV3(PDB ID 1QTN, 4ZZZ and 7XJ0

respectively).[20] The docking details with all selected phytoconstituents with 1QTN, 4ZZZ and 7XJ0 can be visualized in Table 2 and Table 3

The docking scores of the nominated phytoconstituents (PubChem ID – 1548943, 6989) showed a higher interaction score (table 3), with multiple hydrophilic contacts with the chosen receptor. Capsaicin interacts with 4ZZZ. It showed good rest with a high score of -7.136. It may give a better result than Andrographolide.

Antimicrobial activity

The antimicrobial test indicated activity against *S. aureus* (table 4) and *P. aeruginosa* (table 5). This suggests that the extract exhibit antibacterial activity. This indicated that the formulation can be useful for the skin infections.[21]

UV spectral analysis

UV-Vis analysis gave maximum absorbance observed at 220 nm. The coefficient of regression was found to be 0.8385.This absorbance was considered as a reference point for further calculation of release study from gel. This study need to be carried out in detail after separation and characterization of individual constituent.

Evaluation of gel

The color was brown to dark brown. The

Table 1. Preliminary Analysis for ethanolic extract and aqueous extract

Method of Analysis	Observation	Results
Detection of sugars : Benedict's test – 3ml extract + equal amount of Benedict's reagent. Heating in water bath.	Green colour solution was observed.	Carbohydrates present.
Test for steroidal presence : Salkowski test	No blue colour seen	Steroids present
Test for flavonoids : Sulphuric acid test – extract + 80% sulphuric acid.	Deep yellow colour	Flavones and flavonols present
Test for tannins :		
• Lead acetate solution + extract	White precipitate	Tannins present
• Dilute HNO3 + extract	Reddish yellow colour was seen	Tannins present
Test For Saponins: Extract + water was shaken vigorously.	Persistent foam was observed	Saponins present
Test For alkaloids	Formation of orange or brown precipitate	Alkaloids present

Table 2. Docking Analysis

Pubmed ID of ligand	Binding score in Kcal/Mol	Hydrophilic bonds with distance in Å	Hydrophobic bonds with distance in Å
1QTN - CASPASE-8			
Capsaicin (1548943)	-4.06	GLU290(A) - H-Bond (1.92)	GLU290(A) - Aromatic Bond (2.62)
Thymol (6989)	-3.107	GLU290(A) - H-Bond (1.77)	-
4ZZZ - PARP1			
Capsaicin (1548943)	-7.136	ASP770-H-Bond (1.70)	ARG878 - Aromatic Bond (2.47)
Thymol (6989)	-6.009	GLY863 - H-Bond (1.88)	ILE879 - Aromatic Bond (2.65)
7XJ0 - TRPV3			
Capsaicin (1548943)	-4.022	GLU546(B) - H-Bond (1.85)	-

Table 3. Docking Score

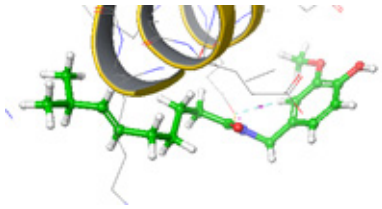
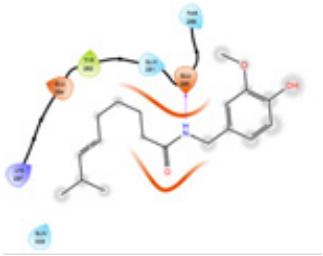
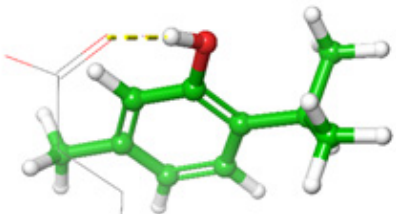
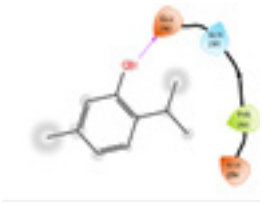
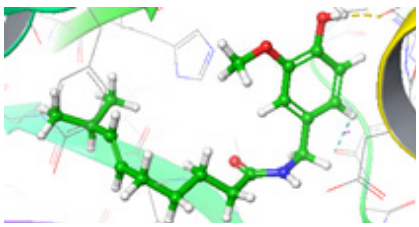
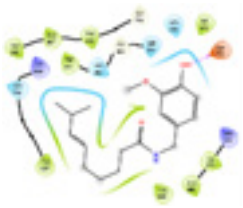
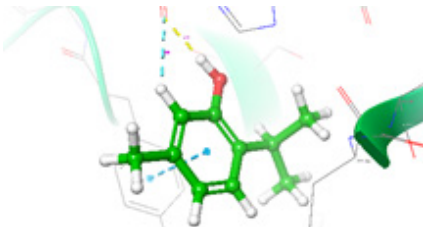
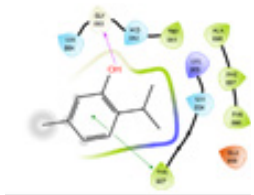
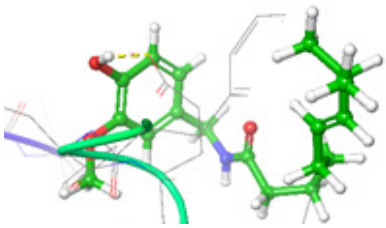
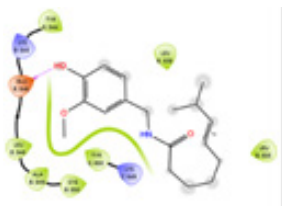
Pubmed Id of Ligand	3D Structure	2D Structure
1QTN - CASPASE-8		
Capsaicin (1548943)		
Thymol (6989)		
4ZZZ - PARP1		
Capsaicin (1548943)		
Thymol (6989)		
7XJ0 - TRPV3		
Capsaicin (1548943)		

Table 4. Zone of Inhibition for *S. aureus*

Dilution of test sample	Concentration of compound (mg/ml)	Zone of inhibition
Undiluted	100	15mm
1	50	12mm
2	25	8mm
3	12.5	7mm
4	6.25	5mm
5	3.625	2mm
Blank (sterile water)	-	0mm
Methicillin 5mcg	5mcg	24mm

Table 5. Zone of Inhibition for *P. aeruginosa*

Dilution of test sample	Concentration of compound (mg/ml)	Zone of inhibition
Undiluted	100	15mm
1	50	11mm
2	25	7mm
3	12.5	5mm
4	6.25	2mm
5	3.625	0mm
Blank (sterile water)	-	0mm
Norfloxacin	10mcg	33mm

gel gave a smooth texture with a cooling effect. The gel had dark brown in colour with a nice scent. The pH was in range of 6.5 to 7. The gel had a very smooth texture and a high spreadability. The gel was easy to wash and remove after use. Appropriate surface absorption was observed. The gel showed no irritation or sign of itching or stickiness. As tannins are present in the extract which work well as astringents, the area remained dry after application. With the use of organoleptic analysis, it was simple to describe the extract's strong aroma. The gel was uniform in nature and has been thoroughly blended. It is quite rare to find coarse particles. Spreadability was found to be 1440 gm.cm/sec.

Gel base and the extract worked together beautifully. The gel showed sustained release pattern in *in-vitro* release studies via cellophane membrane giving 80% cumulative release after 8 hours. The goal of creating a gel from an herb like *Blumea lacera* was to introduce the herb's undiscovered virtues and create a pharmaceutical dose form with minimal negative effects. When applied topically, this *Blumea lacera* leaf extract-based gel will show antimicrobial properties and can be used for skin infections.

CONCLUSION

Ayurveda as a secondary system of medicine, it has been the most well-known treatment. *Blumea lacera* is said to have anti-fungal, anti-septic, and anti-microbial antioxidant and antipyretic qualities. The docking details with all selected phytoconstituents with 1QTN, 4ZZZ and 7XJ0 were visualized and interpreted. Tannins, flavonoids, steroids, carbohydrates, saponins and alkaloids was confirmed in etanolic as well as aqueous extract. The extract had shown antimicrobial activity. *Blumea lacera* leaf extract powder was successfully converted into gel. The gel was created with a smooth texture and a cooling effect. The gel had a nice scent gave release in controlled manner. Further detailed evaluation in toxicological studies needed to be done to have deeper insights of the topic and to make it successful formulation in the market .

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Author Contributions

Arya Jayant Gijare: experimental studies and manuscript preparation. Kunal Jitendra Pardeshi : concept, literature search, experimental studies. Aryan Mangesh Mandot: experimental study. Swati Changdeo Jagdale: design, data analysis and manuscript editing.

Data Availability Statement

Not applicable.

Ethics Approval Statement

Not Applicable.

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