PHYTOCHEMICAL SCREENING, FORMULATION AND EVALUATION OF ANTIBACTERIAL CREAM CONTAINING EPIPHYLLUM OXYPETALUM EXTRACT

Mansi Dadasaheb karande[1] , Varsha Digambar bherje[2]

[1]*Student, Raigad college of Pharmacy, Mohopre, Raigad , Maharashtra, India*
Email:mansikarande2003@gmail.com

[2]*Department of pharmacology, Raigad college of Pharmacy, mohopre ,Raigad,*

Maharashtra, India

ABSTRACT

Background: Epiphyllum oxypetalum (Queen of the Night) is a night-blooming cactus known for its medicinal properties, particularly antimicrobial activity.

Objective: This study aims to investigate the phytochemical constituents and antimicrobial efficacy of E. oxypetalum extract, and to formulate and evaluate an antibacterial herbal cream incorporating the extract.

Methods: Phytochemical screening was performed to identify bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and terpenoids. Ash value and acid-insoluble ash were determined to assess plant material quality. Advanced techniques including Thin Layer Chromatography (TLC), Paper Chromatography, and Column Chromatography were employed for phytochemical analysis, with further characterization via Gas Chromatography–Mass Spectrometry (GC-MS). An antibacterial cream was formulated using the extract and subjected to evaluations including PH, spreadability, viscosity, stability, and microbial efficacy. Antibacterial activity was assessed using the agar well diffusion method against Staphylococcus aureus and Escherichia coli.

Results: GC-MS identified several antimicrobial bioactives. The formulated cream exhibited significant antibacterial activity, indicating its potential use in therapeutic skincare.

Conclusion: The study supports the use of E. oxypetalum in herbal antibacterial formulations, highlighting the value of traditional medicinal plants in modern dermatological applications.

Keywords: *Epiphyllum oxypetalum, antibacterial cream, phytochemical screening, herbal formulation, GC-MS, antimicrobial activity*.

1.INTRODUCTION:

Epiphyllum oxypetalum, commonly known as "Queen of the Night" or "Brahma Kamal," is a cactus species renowned for its large, fragrant, white flowers that bloom exclusively at night. Native to tropical and subtropical regions, the plant rarely flowers, and when it does, the blossoms typically wither by the next morning. It features long, flat, green stems that resemble leaves, which may be wavy or lobed, measuring up to 16 inches in length and 8 inches in width.

Medicinal plants have played a significant role in traditional healthcare systems for centuries due to their rich content of bioactive compounds with therapeutic properties. Among these, Epiphyllum oxypetalum holds traditional value for its

potential anti-inflammatory, antimicrobial, and wound-healing activities. Despite its use in ethnomedicine, limited scientific literature is available on its phytochemical profile and pharmacological potential.

Phytochemical screening is essential in identifying secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids—compounds that often contribute to antimicrobial and healing effects. For quality assessment and authentication of plant materials, parameters such as ash value and acid-insoluble ash are determined. Additionally, chromatographic techniques including Thin Layer Chromatography (TLC), Paper Chromatography, and Column Chromatography aid in the separation and identification of phytoconstituents. Further characterization using Gas Chromatography–Mass Spectrometry (GC-MS) allows for the identification of volatile and semi-volatile compounds within the extract.

In this study, Epiphyllum oxypetalum extract was utilized in the formulation of an herbal antibacterial cream. The formulated cream was assessed for its physicochemical properties and antibacterial efficacy against common pathogenic bacteria. This research aims to validate the plant's potential as a natural therapeutic agent in topical applications for the management of skin infections.

Kingdom	**Plantae**
Phylum	Angiosperm
Class	Magnoliopsida
Order	Caryophyllales
Family	Cactaceae
Genus	Epiphyllum
Species	E.oxypeatlum
Binomial name	Epiphyllum oxypetalum

Table 1: Taxonomy of Epiphyllum oxypetalum[1]

Figure 1: Epiphyllum oxypetalum plant

1.2AIM AND OBJECTIVE:

- To prepare antimicrobial cream by using epiphyllum oxyepetalum.
- To determine the antimicrobial activity of brahma kamal.
- To provide a natural treatment for drug resistant bacteria by avoiding any adverse effect.
- To improve health benefit.
- Herbal creams are semi-solid formulations that are used topically to treat infected skin. The active ingredients in the formulations are used to create antimicrobial creams that protect the skin from microbial

2.Material and Methods

2.1Collection of the plant material:

Fresh leaves of Epiphyllum oxypetalum were collected from a nursery and surrounding local areas in Raigad district, Maharashtra, India. The plant material was taxonomically identified and authenticated by Department of Botany, Dr Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, affiliated with Shri Bhairavnath Nisarg Mandal Sanchit College of Agriculture, Achloli. The leaves were washed thoroughly with distilled water and shade-dried at ambient temperature for several days until a constant weight was achieved. Subsequently, 30–40 g of the dried leaves were blended into a coarse powder using a mechanical blender and stored in airtight containers for further use.[2]

2.2Extraction process:

Twenty grams of the powdered leaf material were subjected to Soxhlet extraction using 100 mL of ethanol as the solvent. The plant material was loaded into a thimble and placed in the main chamber of the Soxhlet extractor, which was connected to a 250 mL round-bottom flask containing ethanol. A condenser was attached to ensure continuous solvent recycling, with water circulating through the inlet and outlet pipes. The extraction was carried

out at 78 °C (boiling point of ethanol) using a heating mantle. The process was continued for approximately 12 hours, until the siphon tube of the Soxhlet apparatus became clear, indicating complete extraction. Upon completion, the ethanol extract was allowed to cool to room temperature and subsequently filtered. The filtrate was collected in a conical flask (iodine flask) and stored at 4 °C until further phytochemical and formulation analyses were performed.[3]

2.3Reagents and Chemicals:

chemicals and reagents used in the present study Ethanol, sulphuric acid, hydrochloric acid, Fehling solution A and B, ferric chloride and chemical reagents such as Wagner's reagent, Mayer's reagent, Dragendorff's reagent , Molisch's reagent, Benedict's reagent, Ninhydrin reagent, Barfoed's reagent used for different tests.

2.4Organoleptic evaluation:

Sensory parameters of the leaves such as colour, odour and taste were studied by organoleptic evaluation.[4]

SR.NO	PARAMETERS	OBSERVATION
1	Colour of the plant	Dark green
2	Colour of the leaf	Dark to light green
3	Colour of the powder	Yellowish brown
4	taste	Astringent
5	Odour	Characteristic odour
6	Texture	Glabrous

Table 2: Organoleptic evaluation of E.oxyepetalum leaf

2.5 Physicochemical Evaluation

2.5.1ASH VALUE:

Weigh 2 gm of the air-dried plant powder accurately. Pre-weigh a clean, dry crucible and note the weight. Transfer the powder into the crucible. Place the crucible in a muffle furnace at 500–600°C for 3–4 hours or until the sample turns white. Cool the crucible in a desiccator for 30 minutes. Weigh the crucible with ash. Calculate the total ash content as a percentage of the initial air-dried sample.

Ash value calculation:

weight of empty crucible: 18.50g

weight of powdered crude drug (brahma kamal leaves): 2g

weight of crucible with ash:19.25g

Ash value: weight of crucible with ash-weight of empty crucible

: 19.25-18.50 g

:0.75. g

Ash value of 2g of crude brahma kamal leaves is obtained 0.75g.

therefore ash value of 100g of crude brahma kamal leaves is 37.5g

2.5.2Acid insoluble ash value:

Boil the ash obtained from the total ash procedure with 10 mL 2N HCl for 5 minutes. Filter through an ashless filter paper. Wash the residue with hot distilled water until neutral. Place the filter paper (with residue) in the original crucible. Incinerate in a muffle furnace at 500–600°C until white or nearly white ash is obtained. Cool in desiccator and weigh.[5]

Acid insoluble ash value Calculation:

Weight of ash with crucible: 19.25g

Weight of ash after 1 hour of process: 19.54g

Acid insoluble ash value: Weight of ash after 1 hour of process - Weight of ash with crucible

: 19.54-19.25g

: 0.29g

Acid insoluble ash value is 0.29g.

2.5.3EXTRACTIVE VALUE:

Weigh 4.0 g of air-dried, coarsely powdered Brahma Kamal leaves and transfer it to a conical flask. Add 100 mL of distilled water to the flask. Stopper the flask and shake well. Macerate for 6 hours, stirring frequently (at least every hour), and then allow to stand for 18 hours at room temperature. After 24 hours, filter the mixture through Whatman No. 1 filter paper. Pipette 25 mL of the filtrate into a pre-weighed china dish. Evaporate the filtrate on a water bath to dryness. Dry the residue in a hot air oven at 105°C for 1 hour. Cool in a desiccator and weigh the dish with the dried extract. Subtract the tare weight of the china dish and calculate the extractive value.[6]

Extractive value calculation:

Weight of empty china dish: 69.35g

Weight of filtrate of brahma kamal leaves: 25ml

Weight of china dish after extractive value: 69.50g

Extractive value: Weight of china dish after extractive value - Weight of empty china dish

: 69.50-69.35g

: 0.15g

Extractive value of brahma kamal leaves extract is obtained after 24 hours is 0.15g.

3. Phytochemical Screening

3.1PRELIMINARY TEST:

Name	test	observation	inference
Molisch's test	1 ml of the extract and 2 ml of a-naphthol were mixed. 2 ml of concentrated H_2SO_4 is added along the sides of the test tube	The appearance of purple coloured ring at the interface of test solution	Presence of carbohydrates
Benedict's test	1 ml of the extract and 2 ml of Benedict's reagent were mixed	Formation of brick red precipitate	Presence of carbohydrates
Barfoed's test	2 ml of leaf extract and 2 ml of Barfoed's reagent were taken in a test tube. This test tube was immersed in a boiling water bath for 5 minutes	Brick red precipitates	Presence of carbohydrates

Fehling's test	Extracts were dissolved in 5 mL of distilled water and filtered. Fehling A and B solution was added into it. test tube boiling in water bath	Formation of red precipitate gives a positive result.	Presence of carbohydrates
Biuret test	3 ml extract is mixed with 1 mL NaOH and 0.5 mL CuSO4, solution.	blue colour in the test tube	Presence of proteins
Millon's test	To 2 ml extract in test tube, 1 mL Millon's reagent is added.	see the presence of brick red colour	Presence of proteins
Ninhydrin test	1 mL extract was mixed with 2 mL Ninhydrin reagent and the test tube is heated in a boiling water bath.	The appearance of a violet colour	Presence of proteins
Ferric chloride test	2 ml of the extract was mixed with 3 drops of $FeCl_3$ solution	appearance of bluish black or greenish black colour	Presence of tannins
Gelatine test	To 2 ml of the leaf extract, few drops of gelatin solution was added	Precipitation of gelatin directs the presence of tannins	Presence of tannins
Ferric chloride test	1 mL of extract is mixed with 3 drops of $FeCl_3$ solution	form bluish black precipitate	Presence of phenols
Wagner's test	1 ml of the extract and 3 drops of Wagner's reagent were mixed	development of brownish black colour	Presence of alkaloids
Mayer's test	1 ml of the extract and 3 drops of Mayer's reagent were mixed	appearance of white colour	Presence of alkaloids
Dragendroff's test	1 mL of the extract and 3 drops of Dragendorff's reagent were mixed	development of brownish red colour	Presence of alkaloids
Dragendroff's test	2 mL of the extract was mixed with 0.5 mL of $FeCl_3$ solution.	Development of intense green colour	Presence of flavonoids
Lead acetate test	the addition of lead acetate solution to the extract	no yellow precipitate	Absent or trace of

Table 3: preliminary test of plant extract

Group	Name of the test	Inference of the leaf extract in ethanol
Carbohydrates	Molisch's test	+
	Benedict's test	+
	Barfoed's test	+
	Fehling's test	+
Proteins	Biuret test	+
	Millon's test	+
	Ninhydrin test	+
Tannins	Ferric chloride test	+
	Gelatin test	+
Alkolides	Wagner's tesr	+
	Mayer's test	+
	Dragendroff's test	+
Flavonoids	Ferric chloride test	+
	Lead acetate test	-

Table 4: phytochemical analysis of plant extract using ethanol solvent

3.2 Chromatographic Techniques

3.2.1THIN LAYER CHROMATOGRAPHY:

Thin layer chromatography was employed for preliminary phytochemical analysis of the ethanolic extract. A TLC plate (silica gel 60 F254) was used, and a baseline was drawn with a pencil approximately 1 cm from the bottom edge. A small quantity of the extract was spotted on the baseline using a capillary tube and allowed to dry completely. The mobile phase was prepared using a solvent mixture of benzene: ethyl acetate: acetic acid in the ratio of 5:1.5:0.25 . The TLC plate was placed in a developing chamber saturated with the mobile phase, and the solvent front was allowed to migrate up the plate until approximately 3/4th of the plate length. The plate was then removed, and the solvent front was marked immediately. Spots were visualized, and retention factor (Rf) values were calculated using the formula:

Distance travelled by solvent=4.2

Distance travelled by yellow spot=2.3

Distance travelled by green spot=3.2

RF= Distance travelled by solute / Distance travelled by solvent

RF value of green spot=3.2/4.2=0.76

RF value of yellow spot=2.3/4.2=0.54

3.2.2PAPER CHROMATOGRAPHY:

Paper chromatography was performed to separate phytoconstituents in the ethanolic extract. A rectangular strip of Whatman filter paper No. 1 was used. A baseline was drawn 1 cm from the bottom, and a small spot of the extract was applied using a capillary tube and allowed to dry. The mobile phase consisted of benzene: ethyl acetate: acetic acid in a ratio of 5:1.5:0.25. The paper was suspended in a chromatography chamber containing the mobile phase, with the baseline just above the solvent level. The solvent was allowed to ascend the paper by capillary action.

After development, the paper was removed and the solvent front was marked. Spots were visualized and Rf values were calculated.

Distance travelled by solute=4.5

Distance travelled by solvent=4.6

RF=Distance travelled by solute/Distance travelled by solvent

RF value of green spot=4.5/4.6 =0.97

3.2.3 COLOUM CHROMATOGRAPHY:

Column chromatography was conducted for fractionation of the ethanolic extract. The mobile phase was composed of benzene: ethyl acetate: acetic acid (5:1.5:0.25). The stationary phase was prepared by mixing silica gel with the mobile phase to form a slurry. A glass column was packed with a small layer of cotton at the base, followed by the slurry to create a uniform column bed. Approximately 1–2 mL of the extract was loaded onto the column. The mobile phase was then added gradually to elute the compounds.

Different fractions were collected in separate beakers based on visible colour separation:

Green fraction – collected in one beaker

Yellow fraction – collected in another beaker

The separated fractions were concentrated and stored at low temperature (−20 °C) for further analysis. [7]

Summary Table:

Colour on column chromatography	Phytochemical Constituents
Yellow	Flavonoids, Coumarins, Phenolics
Green	Chlorophyll, possibly Terpenoids

Table 5: colour on column chromatography detection of phytochemical constituents

3.3GC-MS Analysis:

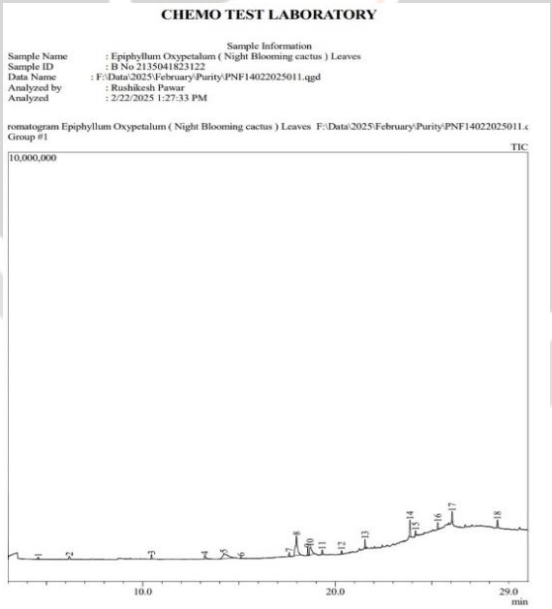

Figure 2: GC-MS chromatogram of alcohol extract of E. oxypetalum leaves dissolved in ethanol

Peak report TIC:

Peak#	Name	R.Time	Area	Area%
1	n-Hexane	4.576	171489	1.31
2	Pentanoic acid, 3-methyl-4-oxo-	6.187	303662	2.32
3	Toluene	10.460	277000	2.12
4	Urethane	13.223	448476	3.43
5	Dimethylsulfoxonium formylmethylide	14.212	2306646	17.62
6	Decane	15.102	90305	0.69
7	Cyclopentasiloxane, decamethyl-	17.612	266670	2.04
8	Phenol, 2-methoxy-	17.989	3553279	27.15
9	Dodecane	18.572	371287	2.84
10	Acetic acid	18.693	1447720	11.06
11	Benzenamine, 2,3-dimethyl-	19.322	309744	2.37
12	Cyclohexasiloxane, dodecamethyl-	20.324	264701	2.02
13	Tetradecane	21.544	414139	3.16
14	2,3-Epoxypropyl p-methoxyphenyl ether	23.875	943320	7.21
15	Heptadecane	24.162	265950	2.03
16	Megastigmatrienone	25.317	407668	3.11
17	Megastigmatrienone	26.068	561367	4.29
18	2-Pentadecanone, 6,10,14-trimethyl-	28.414	684015	5.23
			13087438	100.00

Table 6: Phytoconstituents identified in alcohol extract of E. oxypetalum leaves dissolved in ethanol by GC-MS analysis

GC-MS Analysis of Epiphyllum oxypetalum Leaf Extract:

The GC-MS (Gas Chromatography–Mass Spectrometry) analysis of Epiphyllum oxypetalum (Night Blooming Cactus) leaf extract revealed the presence of various bioactive compounds . The total ion chromatogram (TIC) showed multiple peaks indicating the complexity of the extract.

A total of 18 compounds were identified based on their retention times and peak area percentages . The major constituents include:

Phenol, 2-methoxy- (27.15%), Dimethylsulfoxonium formylmethylide (17.62%), Acetic acid (11.06%), 2,3-Epoxypropyl p-methoxyphenyl ether (7.21%), 2-Pentadecanone, 6,10,14-trimethyl- (5.23%) Other notable

compounds include toluene, decane, dodecane, cyclopentasiloxane derivatives, and various fatty acid esters and ketones.

Conclusion of GC-MS Analysis:

The results suggest that the leaf extract of Epiphyllum oxypetalum contains a wide range of bioactive compounds, primarily phenolic derivatives, sulfur-organic compounds, and aliphatic hydrocarbons. These constituents may contribute to the antibacterial and antioxidant potential of the plant, justifying its inclusion in herbal cream formulations.[8]

4. Formulation of Herbal Antibacterial Cream

4.1Material:

Stearic acid, cetylalcohol, almond oil, methyl paraben, propyl paraben, propylene glycol, triethanolamine, herbal extract.

4.2PROCEDURE OF HERBAL CREAM:

1.The cream consists of two phases; Oil and water. First mix accurately weighed Oil phase components mainly; Stearic acid, Cetyl alcohol and almond oil in a beaker and heat up to 75°C.

2.Then mix aqueous phase components such as; propylene glycol, Triethanolamine, methyl paraben, propyl parabens and herbal extract in water as prescribed in formula. Heat the mixture up to 75°C.

3.Mix the aqueous phase slowly into the oil phase with continuous stirring until a uniform cream is formed.

4.further transfer the cream in the container.[9]

Figure 3: formulation of cream

Material of Herbal Cream:

SR.NO	INGREDIENTS	QUANTITY TAKEN	PROPERTIES
1	HERBAL EXTRACT	2ML	ANTIBACTERIAL
2	STEARIC ACID	12GM	EMULSIFIER
3	CETYLALCOHOL	3GM	THICKENING AGENT,EMOLLIENT
4	ALMOND OIL	4ML	MOISTURISING
5	METHYL PARABEN	0.028GM	PRESERVATIEVS
6	PROPYL PARABENS	0.029GM	PRESERVATIVES
7	PROPYLENE GLYCOL	4ML	HUMECTANT, BASE
8	TRIETHANOLAMINE	4ML	EMULSIFIER
9	WATER	70ML	SOLVENT

Table 7: formulation of cream containing Epiphyllum oxypetalum extract

5.Evaluation of Formulated Cream:

To evaluate the prepared formulation various organoleptic and physicochemical test were performed such as colour, odour, texture, state, PH, spreadability , skin irritation test etc.

5.1Physical Appearance:

The formulation prepared was evaluated for the clarity, colour, odour and texture, state.

5.2PH:

The pH of the formulations was examined using a standardized pH paper at room temperature.

Figure 4: PH of cream

5.3Skin irritation test:

The skin irritancy test was performed by applying the formulations on the skin and observed for 30 minute.

Figure 5: Skin irritation test

5.4SPREADABILITY:

The spreadability of cream was determined by parallel plate method. In this method 2 glass slides were selected. About 1 gm of cream sample weighed and placed on one slide the other glass slides was placed on top of the cream. 1 gm of weight was placed on the slide so that cream was spread to form thin layer wait for some min. Weight was removed and the spread diameter was measured and note the time required to separate the glass slide.[10]

Figure 6: Spreadability test

5.5Viscosity:

Viscosity of formulated creams can be determined by using Brookfield Viscometer.

5.6Homogeneity:

The formulation was tested for the homogeneity by visual appearance and by touch.

5.7washability cream:

A portion of cream was applied over the skin of the hand and allowed to flow under the force of flowing tap water for 10 min. The time when the cream completely removed was noted.

Figure 7: Washability test

5.8Dilution test:

the dilution test was performed cream was taken and dissolved in distilled water cream was not completely dissolved in water.[11]

Figure 8: Dilution test

6.Antimicrobial test:

Prepare Nutrient Agar Mix nutrient agar powder with distilled water as per instructions. Sterilize by autoclaving. Pour into sterile Petri dishes and allow to solidify. Add E. coli culture into the molten agar before solidifying. Mix gently and pour into plates (or spread E. coli on pre-solidified agar). After solidification, use a sterile borer

to make four wells in the agar. Fill two well with standard antibiotic(amoxicilline). Fill the other well with herbal extract. Incubate the plates at 37°C for 24 hours. Measure the zone of inhibition around each well. Compare herbal extract zone vs. standard zone.[12]

Calculation:

Antimicrobial activity in percentage%:

Zone of sample/zone of standard x100

16mm/ 20mm x 100=80%

Figure 9: Antimicrobial test

Results :

SR.NO	PARAMETER	OBSERVATION
1	COLOUR	PALE GREEN
2	ODOUR	PLEASANT
3	TEXTURE	SMOOTH
4	STATE	SEMI-SOLID
5	PH	6
6	SKIN IRRITATION TEST	NO IRRITANT
7	SPREDABILITY TEST	SPREADABLE
8	WASHABILITY TEST	WASHABLE
9	DILUTION TEST	NOT DISSOLVE IN WATER

Table 8: Evaluation result for antibacterial cream containing Epiphyllum oxypetalum

8.Conclusion:

The comprehensive analysis of Epiphyllum oxypetalum extract confirmed the presence of several bioactive phytochemicals, including alkaloids, flavonoids, tannins, and saponins, which contribute to its medicinal properties. The ash value and acid-insoluble ash tests validated the purity and quality of the crude plant material, indicating minimal contamination and high authenticity. Chromatographic techniques-TLC, paper

chromatography, and column chromatography-successfully separated the individual constituents, while GC-MS analysis identified key phytocompounds with known antimicrobial activity.

The antibacterial cream formulated using Epiphyllum oxypetalum plant extract demonstrated effective antimicrobial activity against common pathogenic bacteria. The evaluation confirmed that the bioactive compounds present in the extract contribute to the inhibition of bacterial growth, making it a promising natural alternative for topical antibacterial applications. The formulation showed good stability, spreadability, and skin compatibility, suggesting its potential for use in dermatological preparations

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