

Formulation and Evaluation of Antimicrobial Herbal Ointment Containing Leaves Extract of *Calotropis gigantea*

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ABSTRACT

Herbal medication has very importance in medicinal as well as in economical field. The usage of herbal medication is increase rapidly in the recent years because of their good pharmacological action and their low adverse effects. The goal of ongoing research work has been formulate and evaluation of ointment from the leaves extract of *Calotropis gigantea*. Since the plant's leaves extract cannot be applied directly on the skin due to its anti-bacterial and anti-fungal qualities, formulations that are acceptable for application have been developed using the leaf extract. The leaves of *calotropis gigantea* has anti-bacterial and anti-fungal properties and leaves extract of this plant cannot be used directly on skin, so some suitable formulation has been develop by using leaves extract for application. According to research methodology, first collect the leaves extract of *Calotropis gigantea* by using ethanol through soxhlet extraction method and also performed the phytochemical screening and anti-microbial activity of leaves extract. The ointment formulation was designed by using hard paraffin, cetostearyl alcohol, yellow soft paraffin, wool fat and also determine the physicochemical parameters of formulation such as spreadability, pH, extrudability, solubility, consistency, diffusion study, washability as well antimicrobial activity. As a result, this ointment serves as a vehicle for effectively using the therapeutic benefits of *Calotropis gigantea*.

Keywords: *Calotropis gigantea*, Phytochemical Screening, Antimicrobial Activity, Antioxidant Activity, Agar Well Diffusion Method.

I. INTRODUCTION:-

Calotropis gigantea plant is distributed throughout India. It is popularly known as 'arka' in hindi and 'rui' in marathi. India being a tropical country is blessed with the best natural resources and ancient knowledge for its judicious utilization. *Calotropis gigantea* grows up to 8-13 feet (2.5-

4m), with sessile leaves and white to purple unscented flowers *Calotropis gigantea*, family Apocynaceae has been reported to possess antimicrobial activity.



Fig.1 *Calotropis gigantea* leaves

Medicinal plants have no doubt remained the major sources of traditional medicine worldwide. Today, many of the available drugs were derived from the medicinal plants which still till now the major source of drug. Most plants have medicinal potency, recently their extracts have been developed and proposed for use in food as natural antimicrobials. Alkaloids, glycoside, saponin, fats and oil, tannin and phenolic, flavonoids are bioactive which contained in leaves of *calotropis gigantea*.

Along with different dosage forms herbal drugs also available in the form of ointment which is semisolid preparation which applied locally for several purposes e.g. as antiseptics, emollients, antipuritics, astringents, etc.

Table 1: Systemic position of the selected plant

Kingdom	Plantae
Order	Gentianales
Family	Apocynaceae
Sub-family	Asclepiadoideae
Genus	<i>Calotropis</i>

II. MATERIAL AND METHODS:-

Plant Collection and Authentication-

The roots of *Calotropis gigantea* will be

collected from Gondia district, Maharashtra, India. The herbarium sheet of plant specimen will be authenticated by department of botany, D.B.Science Gondia and notated as identification voucher no. HS-ACC/11.09.2022/5222. The *Calotropis gigantea* leaves were cleaned from its debris dried and coarsely powder. It was stored in well-stopper container for the further work.

Extraction Of leaves of *Calotropis gigantea*-

Soxhlet extraction method was employed for the extraction process of crude plant. 500ml of methanol solvents were used to extract 100g of root powder that had been steadily squeezed into a container. After the extraction, solvents were evaporated using rotatory evaporator equipment, after that it also recorded the yield percentage. For use in subsequent research studies, dried extracts are transported from desiccators into airtight containers are stored there.

Phytochemical Screening of Plants Extract-

For the purpose of identifying the phytochemical components present in the hydroalcoholic extracts, various qualitative tests were carried out. For the presence of carbohydrates, tannins, flavonoids, steroids, glycosides, alkaloids, saponins, and other substances, several tests were carried out.

Evaluation of Antimicrobial Activity of Plant Extract-

Antimicrobial activity was evaluated using disc diffusion method with a standard antibiotic, 10 units per disc of Amikacin. The microorganisms used are *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*). Nutrient agar medium was used as culture media.

To examine the antimicrobial activity, the leaves of *calotropis gigantea* were extracted and diluted in distilled water at a concentration of 100mg/ml. For bacterial screening, the test organisms are seeded into sterile nutrient agar medium (20ml) in a clean test tube. 20µl of sterile melted nutritional agar are mixed uniformly with 1 µl of inoculum and cooled to 40-50°C and then put into sterile petri dishes. When the agar solidified, cellulose disk papers prepared by suitable disc cutter placed on the surface of agar medium which are pre-impregnated in the extract solutions. 10mg/ml dose of the reference standard streptomycin was used in sterile water. The widths of the zone of inhibition were measured on the

bacterial plated after a 24 hour incubation period at 30°C as a measure of activity.

Preparation of Herbal Ointment-

- Initially ointment get formulate by weighing precisely grated hard paraffin, which was then put in an evaporating dish on water bath at 65°C. After the hard paraffin was melted, remaining ingredients were added and vigorously stirred to help melting and homogeneous mixing. The ointment base was then allowed to cool.
- Herbal ointment was formulate by using a levigation method for mixing of accurately weighed *Calotropis gigantea* leaves extract with the ointment base to prepare a smooth paste that was 2 or 3 times the base's weight. More base was then gradually added until the ointment was homogenous, and finally transferred into an appropriate container.

Table2. Composition of Ointment base

S.No.	Name of the Ingredients	Quantity to be taken
1.	Wool fat	0.5g
2.	Cetostearyl alcohol	0.5g
3.	Hard paraffin	0.5g
4.	Yellow soft paraffin	8.5g

Table3. Formulation of Herbal Ointment

S.No.	Name of the ingredients	Quantity to be taken
1.	Leaves extract of <i>Calotropis gigantea</i>	0.06gm
2.	Ointment base	10gm

Evaluation of ointment-

Colour and odour- physical parameters like colour and odour were examined by visual examination.

Consistency- smoothness and consistency of prepared ointment is observed.

pH- pH of prepared herbal ointment was measured by using digital pH meter. The ointment was prepared by using 50ml of distilled water in 100ml of dry beaker and heat it for a few minutes and for cooling set aside for 2hrs 10 pH was determined by using pH meter. pH was determined in triplicate for the solution and average value was calculated.

Spreadability- when two slides are placed between them and a specific force is applied, spreadability is measured in terms of the number of seconds it

takes for the slides to separate from the ointment. The extra sample was sandwiched between the two glass slides, and a specific amount of weight was added to compress the uniformly thick glass slides. The time needed to separate the two slides was recorded. Spreadability was calculated by using the formula below-

$$S = M \cdot L / T$$

Where, S= spreadability, M= weight tide to the upper slide, L= length of glass slide, T= time taken to separate the slides.

Extruability- The formulation was placed into a container with a collapsible tube. Determine the consistency of the preparation. Extrudability can be expressed as the force necessary to extrude material out of the tube.

The following formula was used to determine the extrudability.

$$\text{Extrudability} = \frac{\text{applied weigh to extrude ointment from tube (gm)}}{\text{Area (cm}^2\text{)}}$$

Diffusion study- Nutrient agar medium was prepared for the diffusion research. Ointment was placed in a medium with a hole in the center of it. After the 60 minutes, it was noted the knot that the ointment had helped to diffuse.

Solubility- Insoluble in water, miscible with ethanol, chloroform, ether, and slightly soluble in distilled water.

Washability- formulation was applied on the skin and then ease and extent of washing with water and checked.

Stability study- Physical stability test of the herbal ointment was carried out for the four weeks at

various temperature conditions like 2⁰c, 25⁰c, 30⁰c. the herbal ointment was found to be physically a different temperature i.e. 2⁰c, 25⁰c, 30⁰c.

Antimicrobial Activity of Formulated Ointment-

Dimethyl sulfoxide (DMSO) was used to dissolve 10mg of ointments (0.5%, 1%, and 2%w/w), which were then weighed and used for activity experiments. The nutrient agar medium's component were all measured, weighed, and then dissolved. Agar was then added and dissolved. The medium's pH was then sterilized in an autoclave for 20 minutes at 15 pounds per square inch. Glassware such as pipettes, test tubes, and petri plates were sterilized by dry heat in an oven at 160⁰c for 1 hour.

The bacterial culture was spread on the culture medium and a well was bored in the middle of the agar. Then different samples and standard solutions of 0.05ml was spread on the medium and a well was bored in the middle of the agar. Then diffusion samples and standard solution of 0.05ml was poured inside these wells and plates were incubated at 37⁰c overnight for observation. The presence of inhibition zone was observed and noted.

III. RESULT AND DISCUSSION:-

Antimicrobial Activity of Leaves Extract-

Calotropis gigantean leaf extracts were tested for their ability to inhibit the growth of their bacterial species: Escherichia coli (E.coli), Staphylococcus aureus (S. aureus), and Pseudomonas aeruginosa (P. aeruginosa). Inhibition was at its greatest level against Staphylococcus aureus (14mm), Pseudomonas aeruginosa is inhibited with a (13mm) zone of inhibition, while E.coli is inhibited with a (12mm) zone of inhibition.

Table 4: Antimicrobial activity of leaves extract

Bacterial Species	Zone of Inhibition	
	Streptomycin	Leaves extract of Calotropis gigantea
E. coli	18mm	12mm
Staphylococcus aureus	23mm	14mm
Pseudomonas aeruginosa	21mm	13mm

Note: 1= Sample, 2= Streptomycin, 3= Distilled water



P. aeruginosa

E.coli

S.aureus

Phytochemical Screening of Calotropis gigantea-

Table 5- Phytochemical Screening of Calotropis gigantean-

Sr.no.	Class of Compounds	Test performed	Result
1.	Alkaloid	Drangendroff's test, Mayer's test	+
2.	Carbohydrates	Molish test, Fehling test	+
3.	Glycosides	Keller killiani test	+
4.	Flavonoids	Ammonia test	+
5.	Saponins	With water with Na ₂ CO ₃	+
6.	Terpenoids	Salkowski test	+
7.	Steroids	Libermann-Burchard test	+
8.	Phenolic compounds	Ferric Chloride test	+
9.	Polyuronoids	Haemotoxylin test	-
10.	Peroxides	Potassium Iodide test	-

The phytochemical analysis of the leaves extract of Calotropis gigantea revealed the presence of phenolic, steroids, glycosides, flavonoids, saponins, and alkaloids. Peroxides and polyuronoids were not present.

Antimicrobial Activity of Formulated Herbal Ointment-

Calotropis gigantea leaf extracts were tested for their ability to inhibit the growth of three

bacterial species: Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), and pseudomonas aeruginosa (P. aeruginosa). Inhibition was at its greatest level against staphylococcus aureus (12mm), pseudomonas aeruginosa is inhibited with a (10mm) zone of inhibition, while E. coli is inhibited with a (9mm) zone of inhibition.

Table 6: Antimicrobial activity of leaves extract

Bacterial species	Zone of inhibition	
	Streptomycin	Formulated herbal ointment
Staphylococcus aureus	23	12
Pseudomonas aeruginosa	21	10
E. coli	18	9



S. aureus

P.aeruginosa

s E.coli

Phytochemical Evaluation of Herbal Ointment-

Table 7- Physicochemical Evaluation of Herbal Ointment-

Physicochemical parameters	Observation
Colour	Green
Odour	Characteristics
Consistency	Smooth
Ph	7.1
Solubility	Soluble in ether, alcohol and chloroform
Washability	Good
Non irritancy	Non irritant
Stability studies	Stable at 2 ⁰ c, 25 ⁰ c, and 30 ⁰ c
Spreadability (sec.)	6.5 seconds

IV. CONCLUSION:-

The goal of this research project was to create a novel formulation of topical herbal ointment. The therapeutic characteristics of *Calotropis gigantea*, including their antibacterial, antifungal, anti-inflammatory, and anti-microbial effects, have been utilized for a very long time. As a result, this ointment may be used as a simple dose form to make effective use of these medicinal characteristics.

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