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Development And Evaluation Of Herbal Ointment Formulation From The Plant *Calotropis Gigantea Linn*

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Abstract: This study focuses on the formulation and evaluation of a herbal wound healing ointment using the leaf extract of Calotropis gigantea, a medicinal plant widely used in traditional systems of medicine. The increasing interest in herbal therapeutics due to their affordability, accessibility, and minimal side effects has encouraged the development of plant-based formulations for wound management. The leaves of Calotropis gigantea were collected, authenticated, shade-dried, and powdered, followed by extraction using the cold maceration method with methanol as the solvent. The obtained extract, rich in bioactive compounds such as flavonoids, alkaloids, and glycosides, was incorporated into an ointment base prepared using ingredients like wool fat, cetostearyl alcohol, hard paraffin, and white soft paraffin through the trituration method. The pH (6.7) was found to be compatible with skin, and the formulation exhibited good spreadability and antimicrobial activity without any signs of microbial contamination. The findings suggest that the Calotropis gigantea herbal ointment possesses significant wound healing potential and meets the required evaluation standards. Therefore, it can be considered a safe, effective, and economical alternative for wound care. Further studies, including in vivo evaluations, are recommended to validate its therapeutic efficacy and support its large-scale application.

Keywords: Calotropis gigantea, Herbal ointment, Wound healing, Maceration, Antimicrobial activity, Traditional medicine

1. INTRODUCTION

Traditional medicine is “the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness” World Health Organization There are many different systems of traditional medicine, and the philosophy and practices of each are influenced by the prevailing conditions, environment, and geographic area within which it first evolved (WHO 2005), however, a common philosophy is a holistic approach to life, equilibrium of the mind, body, and the environment, and an emphasis on health rather than on disease. Generally, the focus is on the individual rather than on the particular ailment or disease from which the patient is suffering, and the use of herbs is a core part of all systems of traditional medicine. Herbal medicines are also very common in Europe, with Germany and France leading in over-the-counter sales among European

countries, and in most developed countries, one can find essential oils, herbal extracts, or herbal teas being sold in pharmacies with conventional drugs.

Herbs and plants can be processed and can be taken in different ways and forms, and they include the whole herb, teas, syrup, essential oils, ointments, salves, rubs, capsules, and tablets that contain a ground or powdered form of a raw herb or its dried extract. Plants and herbs extract vary in the solvent used for extraction, temperature, and extraction time, and include alcoholic extracts (tinctures), vinegars (acetic acid extracts), hot water extract (tisanes), long-term boiled extract, usually roots or bark (decoctions), and cold infusion of plants (macerates). There is no standardization, and components of an herbal extract or a product are likely to vary significantly between batches and producers.

2. METHODS OF EXTRACTION OF MEDICINAL PLANTS

2.1 Definition:

Extraction is a separation technique used to isolate active constituents or desired compounds from crude drugs or mixtures using a suitable solvent. "Extraction is the process of separating medicinally active portions of plant or animal tissues using selective solvents, through standard procedures." It is widely used in the pharmaceutical, herbal, and chemical industries to obtain alkaloids, glycosides, tannins, flavonoids, etc.

2.2 Classification of Extraction Methods:

Extraction techniques can be classified based on operating conditions, mechanism, or type of contact between the drug and the solvent.

I. Based on Temperature:

- Cold Extraction: Example: Maceration, Cold percolation.
- Hot Extraction: Example: Soxhlet extraction, Decoction, Infusion, Digestion.

II. Based on Process Type

- Batch Process:

  Example: Maceration, Cold percolation.
- Hot Extraction:

  Example: Maceration, Percolation
- Continuous Process:

  Example: Maceration, Cold percolation.
- Hot Extraction:

  Example: Counter-current extraction, Continuous percolation

III. Based on Equipment and Mechanism

- Simple Techniques:

  Example: Maceration, Infusion, Decoction
- Advanced Techniques:

  Example: Soxhlet extraction, Supercritical fluid extraction (SFE), Ultrasonic-assisted extraction, Microwave-assisted extraction.

3. METHODS OF EXTRACTION

MACERATION

Maceration is a simple and classical extraction method where the crude drug is soaked in a solvent for a prolonged time at room temperature to extract soluble constituents.

Procedure:

- Powdered drug is placed in a closed vessel.
- A sufficient quantity of solvent (e.g., alcohol, water) is added.
- The mixture is stirred occasionally and kept for 3-7 days.
- The extract is filtered and collected.

Uses:

- Preparation of tinctures, infusions.
- Suitable for heat-sensitive constituents.

Advantages:

- Simple, no heat required.
- Preserves thermolabile constituents.

Limitations:

- Slow process.
- Not suitable for hard or woody materials.

PERCOLATION:

Percolation is a continuous extraction method where solvent is passed through a column packed with powdered drug, extracting soluble compounds as it flows.

Procedure:

- Powdered drug is moistened and packed into a percolator.
- After macerating for 24 hours, fresh solvent is added from the top.
- The percolate (extract) is collected from the bottom.
- Continued until the drug is exhausted

Uses:

- Large-scale extraction.
- Used in fluid extracts and tinctures.

Advantages:

- More efficient than maceration.
- Continuous contact with fresh solvent.

Limitations:

- Requires control of flow rate and packing.
- Not suitable for very fine powders (can cause channelling).
- Not suitable for polar compounds without co-solvents.

ULTRASONIC-ASSISTED EXTRACTION (UAE)

Uses high-frequency ultrasonic waves to create cavitation in the solvent, disrupting cells and enhancing penetration.

Procedure:

- The crude drug is immersed in solvent.
- Ultrasonic waves are applied for a fixed time.

- Filtered to obtain the extract.

Advantages:

- Shorter time.
- Higher yield.

Limitations:

- Requires optimisation.
- Not suitable for industrial scale without high-end equipment.

FORMULATION OF OINTMENT

Definition of Ointment:

Ointments are soft semisolid preparation containing medication meant for external application to the skin or mucous membranes. Ointment are generally used for emollient, protective and therapeutic purposes.

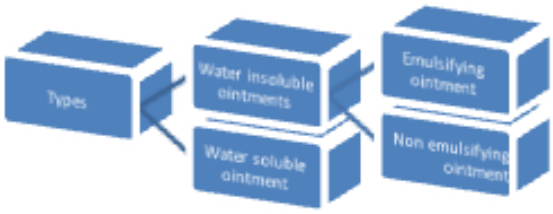

Fig.1

Classification of ointment:

1. According to penetration
   - Epidermic ointment
   - Endodermic ointment
   - Diadermic ointment
2. According to therapeutic
   - Antibiotic ointment
   - Antifungal ointment
   - Anti-inflammatory ointment
   - Astringent ointment
   - Counter irritant ointment

BASED ON PENETRATION OF OINTMENT

Epidermic ointments: They produce action on the surface of the skin. They are not absorbed and produce an only local effect. They are used as protective, antiseptic and local anti-infective.

Endodermic ointments: These are ointments that produce action on deeper layers of cutaneous tissues. They are partially absorbed. They are used as emollients and stimulants.

Diadermic ointments: These ointments are meant for deep penetration through the skin and release of the medicaments. They are absorbed and produce a systemic effect.

BASED ON THERAPEUTIC USES OF OINTMENT

1. Antiseptic / Antibiotic ointments: These ointments are used prevent kill micro-organisms. Example: Povidone-iodine, neomycin ointment

2. Anti-fungal ointments: These ointments are used to kill the fungi and used for fungal infection treatment. Example: Salicylic acid, benzoic acid, Nystatin ointment

3. Anti-inflammatory ointments: These ointments are used to relief the pain in the joints, arthritis and other inflammatory conditions muscle and joints of the bone. Example: Aceclofenac ointments,

4. Counter-irritant ointments: These ointments are used to get relief from muscle pain, headache and muscle-related pains. Example Methyl salicylate, Camphor, Menthol ointments

OINTMENT BASES

DEFINITION

Ointment base is a soft semisolid inert vehicle in which the medicament is incorporated or dispersed, or suspended

CHARACTERISTICS OF AN IDEAL OINTMENT BASE

⇒ It should be non-irritating to the skin

⇒ It should be non-greasy

⇒ It should be compatible with the medicaments

⇒ It should be physically and chemically stable

⇒ It should be easily removable from the skin

PREPARATION OF OINTMENT

- Fusion method
- Trituration method
- Chemical reaction method
- Emulsification method

AIM AND PLAN OF WORK

AIM:

The main aim of our project is to formulate and evaluate the herbal wound healing ointment using leaves extract of *Calotropis gigantea*.

Calotropis gigantea leaves wound healing activity which can be used to treat the infection.

PLANT PROFILE

Fig.2

TAXONOMICAL CLASSIFICATION

Species	:	Calotropis gigantea linn
Family	:	Asclepiadaceae
Genus	:	Calotropis
Kingdom	:	Plantae
Order	:	Gentianales
Vernacular name	:	Erukku

Table.1

Calotropis gigantea's Wound Healing Properties:

The leaves and aerial parts of C. gigantea exhibit various properties that contribute to enhancing wound healing. These parts contain abundant calotropin(Calotropagenin, β-Sitosterol, Amyrin Acetate, Ursolic Acid, Cardenolides), alkaloids, flavonoids, tannins, and saponins, each of which plays a distinct role in enhancing wound healing. According to Alafnan et al in 2021, several essential phytochemicals in C. gigantea, particularly flavonoids, have been reported for their wound healing activity. Secondary metabolite analysis has revealed the presence of flavonoids, such as tamarixin, which may contribute to the wound healing properties. In our study, flavonoids and terpenoids have been identified to possess astringent and antimicrobial properties, thereby promoting wound healing and epithelization. It is well-established that flavonoids also inhibit lipid peroxidation, not only by preventing or reducing cell necrosis but also by enhancing vascularity and improving the viability of collagen fibrils.[8] Additionally, cytoxin is also reported to be present in C. gigantea, playing an important role in wound healing.

PHYTOCHEMICAL	KEY ACTIVITIES	TEST	OBSERVATION	REPORT
Calotropagenin	Inhibits Na+/K+-ATPase, cancer apoptosis, anti-angiogenic, **anti-inflammatory**	Keller-Kiliani test	Reddish-brown ring (2-deoxy sugars) is produced	**PRESENT**
β-Sitosterol	Lowers LDL, BPH relief, **anti-inflammatory**, antioxidant, anti-cancer	Salkowski's test	Chloroform: bluish-red to violet; Sulphuric acid becomes : yellow - greenish glow visible	**PRESENT**
Amyrin Acetate	**Anti-inflammatory** (as potent as hydrocortisone)	Acetate ($FeCl_3$) test	Adding water to the and warming till formation of red precipitate.	**PRESENT**
Ursolic Acid	**Anti-inflammatory**, reduces ROS / oxidative stress	Salkowski's test	Chloroform: bluish-red to violet; Sulphuric acid becomes : yellow - greenish glow visible	**PRESENT**
Cardenolides	**Anti-inflammatory**	Baljet's (Picric acid + NaOH) reaction	Forming of Orange-red color (lactone ring)	**PRESENT**

Table.2

MATERIAL AND PROCEDURE

MATERIAL:

Plant – Calotropis Gigantea

Solvent – Methanol

APPARATUS:

Beaker, Glass rod, Funnel, Filter paper, Hotplate, Mortar and pestle, Aluminium tube Container

PROCEDURE:

Collection of the plant (leaves)

The leaf part of *Calotropis gigantea* was collected in the Madurai, Tamil Nadu then the plant (leaves) part was identified and authenticated by Dr. Stephen Ph.D. (Retd)., American college Madurai, Tamil Nadu.

Drying Of the Leaves

Leaf samples distributed evenly to facilitate homogenous drying. Shade drying is an important aspect to leave plant samples in shaded area or in a room with ambient temperature and adequate ventilation. The process of shade drying is important because it retains the secondary metabolites and prevents the loss of bioactivities of plant material. During shade drying, the regular monitoring is necessary to prevent molds growth and attack of insects and flies. Drying in direct sunlight should be avoided to minimize chemical reactions as the artifacts can be formed by the induction of ultraviolet rays. The plant material rich in high moisture content is difficult to dry in shaded area as this moist condition facilitates the growth of molds to contaminate the plant material. If the environmental condition is full of moisture or during rainy season, the chance of fungal contamination may increase. Therefore, dry conditions are very essential in shaded area or in room to prevent microbial fermentation and subsequent degradation of metabolites of plant materials.

Grinding

The aim of grinding is to fragmentation of the Leaves into smaller particles and improves the subsequent extraction by rendering the sample more homogenous, increasing the surface area, and facilitating the penetration of solvent into the cells. Mechanical grinder should be employed to shred the plant tissues to various particle sizes.

Extraction of Calotropis gigantea.

Method of Extraction:

Cold Maceration:

Cold maceration is an extraction process in which a coarsely powdered crude drug is soaked in a suitable solvent of Methanol at room temperature for 3-7 days with occasional stirring to dissolve the active constituents. calotropis gigantea leaf Methanolic extract produced a denaturation inhibition percentage of 85.71%.

Procedure:

Dry and powder the crude drug of calotropis gigantea. Place the powder in a stoppered container (maceration jar). Add the menstruum (water, alcohol, or Methanol solvent) until the drug is fully covered. Close the container tightly to prevent solvent loss. Allow to stand for 3–7 days at room temperature. Shake or stir occasionally to improve extraction. Filter the liquid by using filter paper after completion of maceration. Press the marc to recover absorbed solvent. Combine filtrate and washings to obtain the extract

Fig.3

FORMULATION OF CALOTROPIS GIGANTEA OINTMENT

Requirements:

S.no	Name of ingredients		Quantity to taken (10 gm)
1	Wool fat	:	0.5 gm
2	Cetostearyl alcohol	:	0.5 gm
3	Hard paraffin	:	0.4 gm
4	White soft paraffin	:	8.0 gm
5	Calotropis gigantea extract	:	0.5 gm
6	Methyl paraben	:	0.05 gm
7	Propyl paraben	:	0.05 gm

Table.3

Procedure for preparation of calotropis gigantea ointment

Simple ointment:

Melt all the ingredients in the china dish using water bath and stir well until room temperature is attained to form uniform consistency white soft paraffin is used as a base.

Ointment procedure:

Triturate the Methanolic extract powder with a portion of the simple ointment until smooth; gradually add the remainder of the simple ointment base and mixed thoroughly. Finally the ointment is prepared by trituration method.

Fig.4

EVALUATION

PHYSICAL APPERANCE:

The consistency, color transparency and appearance of the formulated ointment having evaluated

Consistency – smooth

Color – light green

Transparency – non transparent.

Appearance – semi-solid in nature

pH Test:

A 1gram sample of the ointment was weighed and dissolved or dispersed in 10 ml of distilled water, or the appropriate solvent based on the ointment type, the mixture was then stirred thoroughly until a homogenous suspension was formed, using a magnetic stirrer if necessary. The pH meter was calibrated using standard buffer solutions of pH 4.0,7.0,9.0 according to the manufacture's instruction. The electrode was rinsed with distilled water, blotted dry with tissue paper, and then immersed into the prepared ointment suspension, waiting for the pH reading to stabilize the pH value displayed on the meter was recorded.

Fig.5

SOLUBILITYTEST:

A one gram of the Ointment sample was weighed for each test solvent. Solvents of varying polarities were selected, including water for polar solubility, Methanol for semi-polar solubility, and oils for nonpolar solubility. The solvent 10ml was added to the ointment in a test tube or beaker and stirred manually using a glass rod or placed on a magnetic stirrer.

The mixture was observed for dissolution, partial dissolution, or insolubility and the appearance was noted, whether it was a clear solution, turbid solution, or phase separation. The solubility results for each solvent were recorded as soluble, partially soluble, or insoluble.

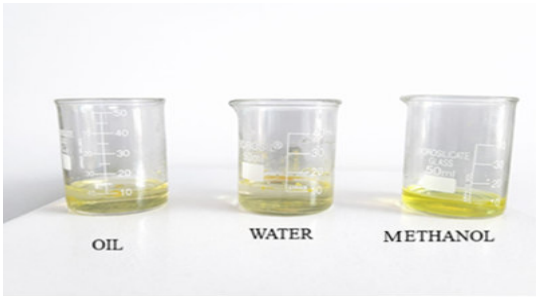

Fig.6

ANTI-MICROBIAL TEST:

Sterilized nutrient agar was poured into sterile petri dishes and allowed to solidify. The surface of the solidified agar was then swabbed evenly with the microbial suspension. A corkborer was used to create wells in the agar, which were filled with a fixed volume (50-100µL) of the ointment sample. Alternatively, sterile filter paper discs impregnated with the ointment were applied. Positive and negative controls were also set up, With the positive control being an antibiotic disc or standard antimicrobial agent, (Escherichia coli, Candida albicans, Bacillus subtilis) and the negative control being the solvent or base ointment without active ingredients. The plates were then incubated at 35˚C-

2˚C for 24-48 hours for bacteria and 48-72 hours for fungi. After incubation, the diameter of the clear zones (zone of inhibition) around the wells or discs was measured in millimeters. The zone of inhibition for each sample and control was recorded.

Fig.7

SPREADABILITY TEST:

Spread-ability of the ointment was done by using two sets of glass slides of standard dimensions. The herbal ointment formulation was placed over one of the slides, the other slide was placed on the top of the ointment, such that the ointment was sandwiched between the two slides. Weight was placed upon the upper slides so that the ointment between the two slides was pressed uniformly to form a thin layer. The weight was removed and excess of formulation adhering to the slides was scrapped off. The upper slide allowed slipping off freely by the force of weight tied to it. The time taken for the upper slide was noted & calculate by using the formula.

Spread-ability = m x1/t

- M= weight tied to the upper slide
- L= length of glass slide
- T= time taken in seconds

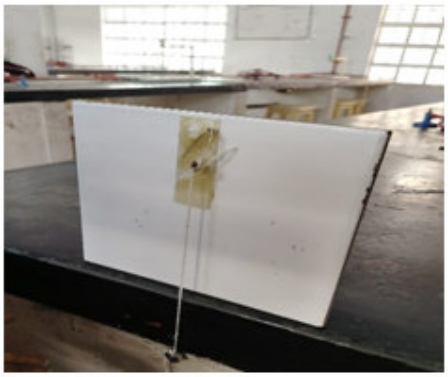

Fig.8

SKIN IRRITANCY TEST:

Label an area on the dorsal surface of the Right hand (1 sq.cm) for the defined area, the ointment was applied and time was noted. Irritants, erythema, edema were tested and reported at regular intervals of up to 24 hours. It was determined by applying the ointment on the surface of the skin of a person's volunteer (self, no ethical permission need because it is non-toxic, natural and safe components which makes it exceptional).

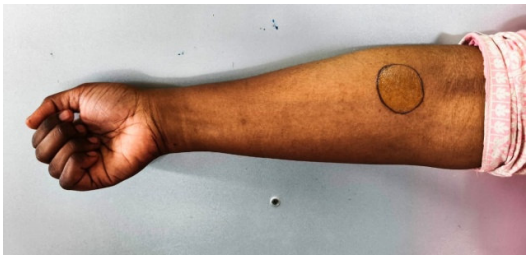

Fig.9

Accelerated stability test:

The ointment was kept at room temperature for a period of 30 days we did not notice any organoleptic change, pH and it is confirmed by taking this smear of the ointment.

Homogeneity

The formulation was tested for homogeneity by texture and by visual appearance.

CONTAINER TEST

EXTRUDABLE TEST

Principle -A standard load is allowed to fall on the empty tube and the resistance offered to this load is measured.

Apparatus

The apparatus used shall be as detailed in tube of diameter 13.5 mm, the auxiliary base plate as shown in the figure shall be inserted in the apparatus.

Procedure

Place the tube on the groove, or in the case of tube of diameter 13.5 mm on top of the auxiliary base plate, on the base of the apparatus with the open end touching the stopper. Release the shutter and allow to fall upon the tube. Read on the scale the position of the top of the shutter.

Fig.10

LEAKAGE TEST (TEST FOR CLOSURE EFFECTIVENESS)

Principle

The tube is tightly closed and placed in an inverted position. Water absorbed by the filter paper placed around the base of the nozzle will indicate leakage.

Procedure

Fill the tightly closed tube with water. Wipe the external surface of the tube thoroughly to make it dry. Place the tube in an inverted position with a filter paper fixed at the base of the cap. Allow the tube to stand for one hour.

LACQUER TEST:

Principle

The Lacquer is brought in contact with the product to be Packed and maintained at a fixed temperature and time. The lacquer is Visually examined for any damage. With this test, a suitable control testin a stoppered neutral glass test tube may be carried out simultaneously.

Procedure

Take Ten tubes for testing. Fill the product in tightly Stoppered tubes. Crimp the opened of the tubes properly. Subject these Tubes to 45°C in an oven for a period of 72 hours. After this period the Tubes are allowed to cool to room temperature and then are carefully cut Open lengthwise.

Product Compatibility

The contents of the tube should not show Any discolouration, change in odour, gas formation or signs of decomposition compared to control sample kept in the sealed neutral glass test tube and subjected to identical conditions at the same time.

Lacquer Compatibility

The contents are washed from the spread open tube body with water at about 45°C. The lacquer coat is dried with cotton wool avoiding rubbing and scratching.

Fig.11

PARTICLE SIZE

Select a tube, clean them, fill with ointment base, seal and allow to cool overnight. Express the base from each tube through a heated bacteriological filter with filter paper under suction. Wash the filter paper with chloroform, dry it and mount between glass for examination. Examine under the microscope count metal particles of different sizes.

(a) the number of all metal particles 1 mm in length and longer,

(b) the number in the range 0.5 mm to less than 1 mm and

(c) the number in the range 0.2 mm to less than 0.5 mm

Fig.12

RESULTS AND DISUCSSION

Preparation of the calotropis gigantea ointment

The ointment was prepared by trituration method, about 30gm of ointment was prepared and stored in an airtight container.

S.NO	EVALUATION PARAMETER	OBSERVATION	RESULT
1	Physical appearance	Light green	COMPILES
2	PH test	6.7	COMPILES
3	Solubility test	Soluble in Methanol	COMPILES
4	Total viable count	$80x10^{1}$ CFU/g	COMPILES
5	Spreadability test	30 mg.cm/sec	COMPILES
6	Skin irritancy test	Absence	COMPILES
7	Stability studies	Stable	COMPILES
8	Homogeneity test	Homogeneous	COMPILES
9	Extrudable test	Min 7 max 10	COMPILES
10	Leakage test	No absorption of water	COMPILES
11	Laquer test	No visible sign of softening	COMPILES

Table.4

Result:

a) Organoleptic test

S.NO	SPECIFICATION	OBSERVATION
1	Colour	Light colour
2	Consistency	Smooth
3	Transparency	Non transparent
4	Appearance	Semi solid in nature

Table.5

b) pH determination

Skin pH in normally acidic ranging of 4-6, while the body's internal environment a near neutral pH 7-9. Normal ointment pH range 4-6.9. the pH of the formulated ointment was range 6.7, which lies in the normal pH range of the skin.

c) Solubility

The formulated ointment was soluble in Methanol it shows no precipitation.

d) Spreadability

The spreadability of this ointment value is 30mg.cm/sec in Uniformly.

e) Skin irritancy test

The formulated ointment shows no redness, edema, inflammation and irritation during irritancy test.

f) Accelerated stability test

test for the 20 days at three different temperatures,40˚C, 30˚C, 25˚C temperature and observe. No colour change or physical change, detoriation occurs. Show the formulated calotropis gigantea ointment was stable and safe to use.

g) Homogeneity

It was found that the ointment was homogeneous and smooth and consistency in nature.

h) Anti-microbial test

1	Total viable count	80x10 ¹ CFU/g
2	Gram negative pathogen	Absent

Table.6

CONTAINER TEST

Extrudable test

When collapsible tube is tested in this manner. The minimum and maximum limit of collapsibility readings is min 7 and max 10 for 13.5mm diameter of tube.

Leakage test:

The filter paper shall not show any absorption of water at any time during the onehourtest period.

Lacquer test:

These shall be no visible signs of softening with scratching, lifting, peeling of lacquer when the surface is examined in each tube.

Particle size:

Examine under microscope and count metal particles of different sizes.

PARTICLE SIZE	**SCORE**
Particles 1 mm and above	50
Particles 0.5 mm but less than 1 mm	10
Particles 0.2 mm but less than 0.5 mm	2
Particles less than 0.2 mm	Nil

Table.7

CONCLUSION

The present work is formulation and evaluation of herbal ointment from the extract of calotropis gigantea. calotropis gigantea is herbal plant which has the capability to serve wound healing, analgesic, anti-microbial, skin ulcer, leprosy, digestive disorders, asthma and anti-inflammatory medicine. As the calotropis gigantea has more medicinal values it was aimed to formulate the herbal ointment using the extract of the selected plant. The prepared formulation was evaluated (table) and it was found that the herbal ointment in terms of texture and smoothness. the formulations produced any skin irritation. The formulation showed better stability in all aspects The formulation doesn't show any microbial contamination upon storage. Hence, from present investigation it was concluded that the herbal ointment was formulated and it complies with all the evaluation test being performed.

We have been done with the container test in further if we are permitted to proceed with animal

Studies we are prone to get approval under the criteria of the regulatory affairs.

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