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Calotropis Gigantea plant medicinal uses, properties and phytochemical test

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Calotropis Gigantea plant medicinal uses, properties and phytochemical test

Diksha Arya[1], Anubha Rawat[2], Rashmi Prabha Mishra [3]
Department of Biotechnology, Rapture Biotech Dehradun[1]
Corresponding mail: dikshaarya371@gmail.com

Abstract

Calotropis gigantea Linn., known as milkweed or giant milkweed, is a well-recognized medicinal plant that has played a vital role in traditional healing systems such as Unani, Ayurveda, and Siddha. Native to India, China, and Malaysia, it now grows widely across tropical and subtropical regions around the world. This hardy shrub, often called swallowwort, has long been valued for its remarkable therapeutic properties. Every part of the plant—the roots, leaves, flowers, bark, and latex used in traditional remedies and Unani formulations. Historical records describe its wide range of effects, including anthelmintic, tonic, expectorant, anti-inflammatory, sedative, and wound-healing actions. It has been traditionally prescribed for conditions such as asthma, Stomach-ache, cholera, amenorrhea, leprosy, and toothache, and used externally to treat joint pain and various skin ailments.

Keywords: Calotropis gigantea, Phytochemistry, Pharmacological activity, Traditional medicinal plant, serial dilution, spread plate, striking, biochemical test, Minimum Inhibitory Concentration (MIC),

1. Introduction

Calotropis gigantea Linn., commonly referred to as milkweed or giant milkweed, is one of the most remarkable medicinal plants known in traditional herbal medicine. A member of Asclepiadoideae family, this hardy shrub has long held an important role in healing practices across Asia and Africa. The Asclepiadaceae is a large family comprising of 175-180 genera and 2200 species distributed mainly in the tropical and subtropical region of the world [1]. Ethnos medical literature contains many plants including, Calotropis gigantea that can be used against diseases, like diabetes, atherosclerosis, ischaemic heart disease, disorders induced by free radicals and other reactive oxygen species. India is very rich in natural resources and the knowledge of traditional medicine and the use of plants as source of new drugs is an innate and very important component drug discovery [2] Reaching up to four meters in height, the plant is easily identified by its thick, pale green leaves, milky latex, and sturdy, branching structure. The latex, a distinguishing feature of the plant, has been a vital component in traditional medicine for centuries due to its complex mix of bioactive compounds.[3] Traditional medicines based upon broad knowledge of herbal plants offer promising capacity to cope with multidrug resistance [4]

Figure.1 Calotropis Gigantea plant

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemically active substances that produce a definite physiological action on human body.[5] Its parts roots, leaves, bark, flowers, and latex are known to contain compounds such as glycosides, flavonoids, tannins, and terpenes, each contributing to its therapeutic effects. Historically, it has been used to treat conditions like asthma, skin disorders, digestive issues, and even infections, making it a cornerstone species in traditional medicine systems.

1.1 Origin and Botanical Background-

Calotropis gigantea belongs to the same family as *Calotropis procera* and shares many similar traits with this close relative. he parts of the plant used in Ayurvedic medicine are leaves (fresh or dried), the roots, root bark and the flowers. The powered leaves are used for the fast healing of wounds, as a purgative and to treat indigestion. [6]. The plant produces milky white latex that exhibits analgesic activity and wound healing properties in various animal models [7].

1.2 Medicinal and Cultural Significance-

Throughout history, Calotropis gigantea has been praised for its wide range of healing properties. In Unani medicine, it is described as a tonic, expectorant, and anti-inflammatory agent. The most important property of these bioactive constituents of plants is that they are more effective with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. [8] Ayurveda mentions it as an anthelmintic, purgative, and stimulant. The plant's flowers are used to relieve coughs and asthma, while its roots and bark serve as digestive aids and emetics.

1.4 Role in Modern Medicine-

In recent decades, the rise of drug-resistant pathogens has presented a significant challenge to global health, prompting scientists to revisit natural remedies. Remedies based on traditional systems of medicine such as Ayurveda are often imported into Western countries, and their use remains popular without any evidence base.[9] Plants like Calotropis gigantea have become important subjects of study because of their potential to yield new antimicrobial and anti-inflammatory agents. Herbal medications exhibit a diverse array of biological actions, making them effective in the management of various diseases. [10]

2.Methods and material

2.1. Laboratory equipment-

1. Glass ware
2. Conical flask
3. Test tube
4. Beakers
5. Petri dishes

2.2. Collection and Preparation of plant extract-

C. gigantea mature plant fresh leaves were collected from local area of Dehradun. C. gigantea mature plant fresh leaves were collected from local area of Dehradun. Fresh leaves were washed with distilled water, then cut into small pieces and allowed to dry at room temperature. Ten grams of leaves were boiled in 100 mL distilled water for 20 min and filtered through Whatman No. 1 filter paper [11]. Crush the leaves using a mortar and pestle or blender to break the cell walls and release bioactive compounds. Place the crushed material in a clean glass beaker or flask. Measure a specific amount of the crushed plant material (for example, 10 grams) and soak it in an appropriate volume of distilled water (such as 100–200 mL) at room temperature for about 24 hours with occasional stirring.

2.3. Preparation of Microbial Culture Media-

Liquid broth (LB) medium was prepared by dissolving 13 g of LB powder (Sisco Research Laboratories Pt. Ltd., India) in 1 L of distilled water. The solution was sterilized by autoclaving at 121 °C and 15 psi for 25 minutes. After sterilization, the medium was allowed to cool to approximately 40–50 °C before being aseptically dispensed into sterilized 15 mL Falcon tubes, with each tube containing 5 mL of medium. The prepared LB medium was then used to inoculate bacterial seed cultures, and the cultures were incubated at suitable growth conditions for 24 hours.[12]

2.4. MHA preparation

The culture media were used mainly to cultivate fungus and bacteria. The list of media used Nutrient Agar (NA), Nutrient Broth (NB) and Mueller-Hinton-Agar MHA) for cultivation of bacteria. Calotropis gigantea extract is prepared by collecting fresh leaves, washing, drying them in shade, and grinding into powder. This powder is soaked in water or ethanol to extract the active compounds, then filtered and concentrated by gentle heating. The concentrated extract is added to sterile culture media like Potato Dextrose Agar before sterilization through autoclaving, allowing the plant's bioactive compounds to be studied in fungal or bacterial growth.

2.4.1 Preparation of MHA:

	Ingredients	**Gms/Litre**
I.	Beef, infusion from -	300.00
II.	Casein acid hydrolysate -	17.50
III.	Starch -	1.50
IV.	Agar -	17.00
V.	D.D water -	1000 ml

2.4.2 Preparation of NA and NB:

	Ingredients	**Gms /Litre**
I.	Peptone -	5gmI
II.	Beef extract -	- 1.5 gm/l-
III.	Yeast extract -	- 1.5 g/I
IV.	Nacl -	- 5g/l
V.	Agar -	- 20g/l
VI.	D.D water -	1000ml

(* NB is prepared without adding agar)

2.4.3 Serial dilution

To prepare a serial dilution of a plant extract, first make a concentrated stock solution by dissolving a known amount of plant extract (e.g., 100 mg) in a small volume of solvent (e.g., 1 mL) to get 100 mg/ml.

Then, label several tubes for dilutions. Add a set volume of diluent (like 9 mL of water or broth) to each tube except the first. Transfer 1 mL of the stock solution into the first tube, mix well to get a 1:10 dilution (10 mg/mL). Next, take 1 mL from the first tube, add it to the second tube, mix to get 1:100 dilution (1 mg/mL) Antimicrobial activity of the crude extracts was determined by the agar well diffusion method 10. All test organisms were inoculated in Mueller Hinton broth (pH 7.4.) for 8 hours [13]

2.4.4 Spread plate

Firstly, prepare serially diluted Calotropis extract solutions to create different concentrations for testing. Take a sterile petri dish containing nutrient agar and add a small drop (about 0.1 mL) of one diluted extract to the centre. Using a sterile glass rod or glass beads, gently spread the liquid evenly across the surface. Let the plate rest for about five minutes to allow absorption, then remove any beads if used. Finally, invert the plate and incubate it at around 37°C for 24 to 48 hours. After incubation, observe the plate to check for microbial growth or clear zones where the Calotropis extract inhibited growth.

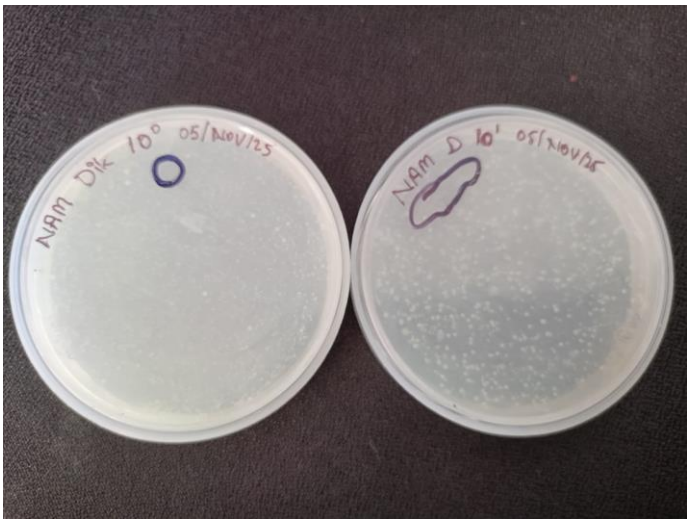

Figure 2: Spreading NAM plate for microbial growth

2.4.5 Streaking process-

First, bacterial cultures are prepared and spread evenly on nutrient agar plates to create a lawn of microbial growth. Small wells are then punched into the agar, into which measured amounts of Calotropis gigantea extract are added. The plates are chilled briefly to allow the extract to diffuse into the agar, then incubated at 37°C for 24 to 48 hours to let the microbes grow. After incubation, clear zones around the wells where bacterial growth is inhibited indicate the extract's antimicrobial effect. The size of these inhibition zones shows how effective the Calotropis extract is at stopping microbial growth, with larger zones meaning stronger activity.

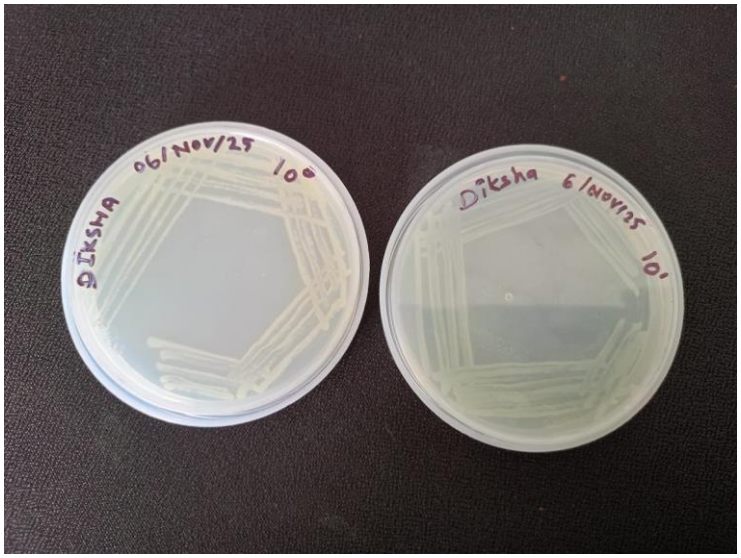

Figure 3: Streaking NAM plate for bacterial growth

2.4.6 Gram Staining-

The Gram staining process for bacteria isolated from Calotropis, start by gently collecting bacteria from the plant with a sterile swab and spreading it on a clean slide. Let it air dry, then quickly pass the slide through a flame to fix the sample. Next, stain the slide with crystal violet for a minute, rinse with water, add iodine for another minute, and rinse again. Briefly treat with alcohol to separate bacterial types, rinse immediately, then apply the final stain with safranin. After a rinse and gentle drying, examine under a microscope: bacteria that remain purple are Gram-positive, and those that turn pink are Gram-negative.

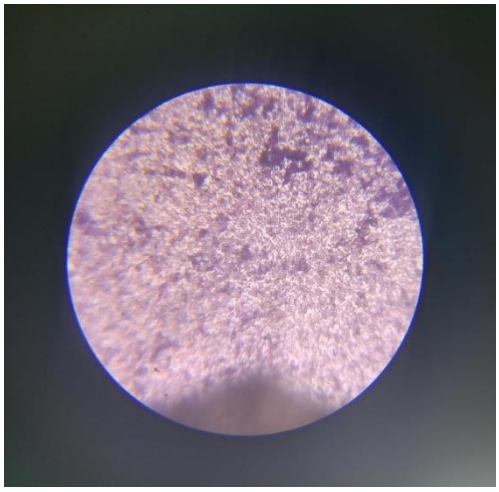

Figure.4: Gram positive bacteria

3.Biochemical tests-

Biochemical tests using Calotropis plant extract are conducted to evaluate enzymatic and metabolic activities, with standardized principles for each test

3.1. Amylase test-

- Weigh and mix the following for 1 litter of medium:
    - Soluble starch: 10–20 g

    - Agar: 12–15 g
    - Distilled water: up to 1 Liter
- Optionally, add nutrients such as peptone (5 g), NaCl (5 g), and meat extract (3 g) for enriched media.
- Heat gently with stirring until both agar and starch dissolve fully. Avoid boiling too long, as excess heat may hydrolyse the starch before the
- Autoclave the mixture at 121°C for 15 minutes to sterilize.
- After cooling to about 40–45°C, pour 20–30 mL into each sterile petri dish.
- Let the agar solidify at room temperature. Store plates in the refrigerator if needed.
- add iodine; iodine colours starch blue-black
- blue-black stained agar indicates positive amylase activity.

Figure 5: Amylase test

3.2. Urease Test

- Weigh and mix the following for 5ml distilled water-
- K2HPO4 – 2g/l
- Peptone – 1g/l
- Dextrose – 1g/l
- NacL – 5g/l
- Agar – 15g/l
- urease test detects the hydrolysis of urea into ammonia and CO2.
- Autoclave the mixture at 121°C for 15 minutes to sterilize.
- The extract is introduced into a urea-containing medium with phenol red as a pH indicator.
- After autoclave Make a slant of this mixture in 10 min UV in laminar air hood.
- A colour changes from yellow to bright pink or magenta indicates positive urease activity.

3.3 Citrate test

- Weigh and mix the following for 5 ml distilled water:
    - Sodium citrate – 2 g/l
    - Ammonium dihydrogen phosphate – 1 g/l

- Sodium chloride – 5 g/l
- Magnesium sulphate – 0.2 g/l
- Dipotassium phosphate – 1 g/l
- Agar – 15 g/l
- Bromothymol blue (indicator) – 0.08 g/l

- The citrate test detects the ability of bacteria to utilize citrate as a sole source of carbon with ammonium phosphate as a nitrogen source.
- Autoclave the mixture at 121°C for 15 minutes to sterilize.
- After autoclaving, pour into tubes and allow to solidify as slants in a laminar air hood (UV for 10 min if desired).
- Inoculate the slant with the test organism using a straight wire and incubate at 35–37°C for up to 48 hours.
- A colour changes from green to blue, along with visible growth, indicates positive citrate utilization; no colour change means negative

3.4. Carbohydrate (Fermentation) Test

- **Weigh and mix the following in test tube –**

Figure 6: Urease test

- Beef – 10g
- Peptone – 3g
- NaCl - 5g
    - Dextrose, maltose, D-mannitol, and sucrose respectively in test tube – 10g
    - This biochemical test evaluates the plant extract's components for their ability to ferment carbohydrates, leading to acid/gas production.
    - After incubation with a carbohydrate fermentation medium (containing a pH indicator and a specific sugar), a colour change (usually from red/orange to yellow) indicates acid production by fermented sugars.

Figure 7: Test for- 1. Sucrose 2. Maltose 3. D-mannitol 4. Dextrose

3.5. Casein (Protease) Test

- Weigh and mix the following –
  Peptone – 5g/l
  Beef – 3g/l
  Dextrose – 10g/l
- The casein hydrolysis (protease) test determines the ability to degrade casein protein.
- The extract is introduced onto a skim milk agar plate.
- A clear zone surrounding the application area after incubation signifies positive protease (caseins) activity by hydrolysing casein. Measurement of absorbance at 280 nm can also be used for quantitative estimation.

3.6. Methyl Red (MR) and MRVP (Methyl Red Voges- Proskauer) Tests

- The MR test checks for stable acid production from glucose fermentation. Extract is incubated in MRVP broth, then methyl red is added.
- A red colour (pH<4.4) indicates positive mixed acid fermentation.
- In the VP part of the MRVP test, after incubation, alpha-naphthol and KOH are added. A red colour signals acetoin production (a neutral product).
- Both MR and MRVP tests help differentiate fermentation pathways that could be influenced by phytochemical compounds in Calotropis.

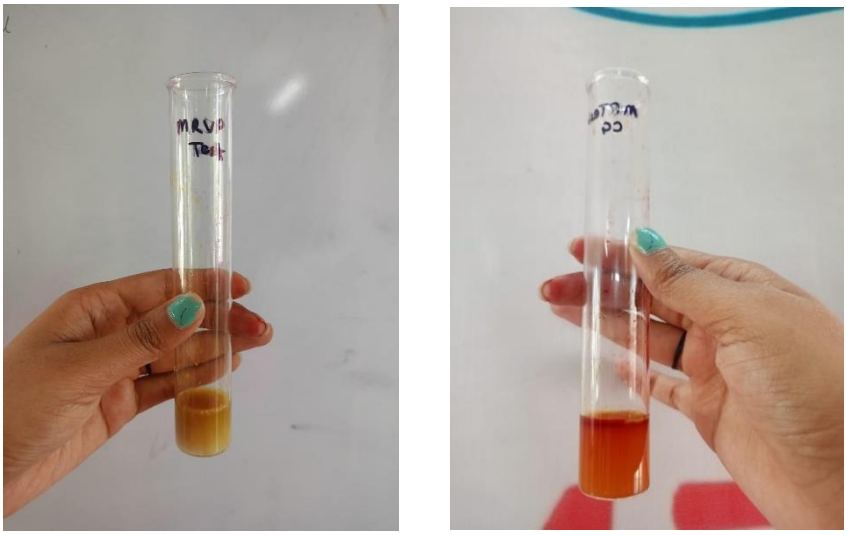

Figure 8: MRVP test 2. MR test

4. Phytochemical test -

The phytochemicals present in the powdered leaves and stems were extracted by percolation method using widely used Soxhlet extraction method.[15] The presence of phytochemicals was confirmed by the appearance of specific colours as visualized by microscope.[16] These include cardenolides, flavonoids, terpenoids, alkaloids, and non-protein amino acids. These phytochemicals are known to have antimicrobial, antiviral, antiallergic and anti-inflammatory properties [17]

4.1. Alkaloid test

4.1.1. Wagner's/Wegner's Test)-

- Take 0.5 mL of the plant extract and dissolve in 1 mL of 1% hydrochloric acid.
- Add 3 drops of Wagner's reagent (iodine-potassium iodide solution).
- Observation: A reddish-brown precipitate confirms the presence of alkaloids.

4.1.2. Mayer test:

- Take 1-2 mL of the plant extract in a test tube.
- Add a few drops of Mayer's reagent, which is a solution made by dissolving mercuric chloride and potassium iodide in water.
- Mix gently.
- A positive result is indicated by the formation of a cream-colored, yellowish, or white precipitate.

4.2. Tannin Detection:

4.2.1 Ferric Chloride Test:

- Add few drops of 1% ferric chloride to the extract.
- Blue-black or greenish colour indicates tannins.

4.3. Lead Acetate Test:

- Add 3 mL of 10% lead acetate solution.
- Formation of white precipitate confirms tannins.

4.4. Steroid Test (Salkowski's Test)

- Add 2 mL acetic anhydride to the extract, followed by 2 mL concentrated sulfuric acid.
- Observe colour change from violet to blue or green indicating steroids.

4.4. Flavonoid Test:

- Sodium Hydroxide Test:
- Add a few drops of sodium hydroxide.
- Yellow colour appearing and disappearing upon acid addition indicates flavonoids.
- Shinoda Test:
- Add magnesium ribbon pieces and concentrated HCl.
- Colour shift from orange to red shows presence of flavanol glycosides.

4.5. Lead Acetate Test for Phenolics:

- Add 3 mL of 10% lead acetate to the extract.
- White precipitate confirms phenolic compounds, including tannins.

4.6. Terpenoid Test (Salkowski's Method)

- Mix 2 mL chloroform with 2 mL concentrated sulfuric acid in the extract.
- Reddish-brown interface formation indicates terpenoids.

4.7. Saponin Test (Foam Test)-

- Dilute 2 mL extract with 5 mL distilled water and shake for 15 minutes.
- Persistent stable foam or honeycomb-like froth indicates saponins.

5. Antimicrobial resistance test:

Anti-microbial agents are anything that inhibits or kills the growth of these micro-organisms and prevent from any kind of disease.[18]. Antimicrobial activity of C. gigantea leaves fractions was measured by disc diffusion method as described.[19]

5.1.MIC [minimum inhibitory concentration

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of phytochemical which inhibits the visible growth of bacterium in liquid medium. [20] Disc Diffusion and Microdilution Broth Approach. The minimum inhibitory concentration (MIC) of the fractions was determined using the broth microdilution method described previously. Plant extracts showing more than15 mm inhibition zone were selected for MIC. [21]. We diffusion a minimum inhibitory concentration in agar plate against pseudo monas, bacillus bacteria with C. Gigantea plant extract. The plates were incubated at 37 °C for 24 hours for bacterial strains and at 30 °C for 72 hours for fungal strains. After incubation, each well was observed for a colour change from yellow to red or purple, which indicated microbial growth. latex extracts showing more than 15 mm inhibition zone were selected for MIC. Selective broth medium was used for dilutions as well as preparing inoculums.[22]

6.Results:

Table 1: Biochemical test result

Biochemical test	Colours	Test result
Amylase test	Blue black	+
Urease Test	pink	+
Citrate Test	Green	+
Carbohydrate	Red/orange to yellow	+
Casein	Blue-black	+

MR test	Red	+
MRVP test	Yellow	-

Table 2: Phytochemical screening of C. gigantea:

Phytochemicals	Colours	M (L)	M (S)	H(L)	H(S)
Polyphenols	Greenish Blue	+	+	+	+
Alkaloids	Reddish grey	+	+	-	-
Carbohydrates	Violet	+	+	-	-
Terpenoids	Reddish grey	+	-	+	+
Steroids	Yellowish	-	+	+	+
Saponins	Light Maroon	+	+	+	+
Tannins	Dark Maroon	+	-	+	+
Flavonoids	Orange	+	+	+	+

Figure 9: (a) Bacillus (b) Pseudomonas

7. Conclusion:

Calotropis gigantea is a valuable medicinal plant with a rich history in traditional medicine and strong scientific support for its many health benefits. Its natural compounds provide effective antimicrobial and anti-inflammatory effects, making it a promising candidate for new treatments, especially in the fight against drug-resistant infections. This plant offers a natural, safer alternative that bridges traditional healing wisdom with modern medical needs, highlighting the importance of continued research and development for its therapeutic use.

Acknowledgement

The author would like to express her sincere gratitude to Rapture Biotech, Dehradun, and the entire team for their invaluable support and guidance throughout the preparation of this research. We also gratefully acknowledge the financial assistance provided by Rapture Biotech, Dehradun, which made this work possible.

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