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Preliminary Phytochemical Analysis and Antimicrobial Activity of *Calotropis procera*

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Abstract
Herbal plants are an important part of our natural wealth. They are being used from very ancient times till the present day. The remarkable therapeutic diversity of herbal plants is one of the main reasons of their distinct position. To revitalize traditional medicines, the efficacies claimed for their applications need to be proved significantly. Even though many plants provide relief but only those with pharmacologically conformed activity, should be recommended for use in "primary health care". The present study was carried out to screen potent phytochemical constituents from aqueous and ethanolic leaf extracts of *Calotropis gigantea* and to determine antimicrobial activity.

Keywords
Herbal plants, *Calotropis procera*

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1. INTRODUCTION
Herbal plants are an important part of our natural wealth. They are being used from very ancient times till the present day. The remarkable therapeutic diversity of herbal plants is one of the main reasons of their distinct position. To revitalize traditional medicines, the efficacies claimed for their applications need to be proved significantly. Even though many plants provide relief but only those with pharmacologically conformed activity, should be recommended for use in "primary health care". (Jia and Zhang, 2005**).**

Calotropis gigantea is also a plant of herbal importance. This plant belongs to Apocynaceae family which includes latex bearing plants. ***Calotropis gigantea*** is known for its utilization in traditional medicinal system for various properties to cure a variety of diseases. Herbal medicines have less side effects and man can get the herbs easily from nature. Medicinal plants contain many antioxidants such as vitamins, carotenoids, flavonoids, polyphenols, saponins, enzymes and minerals.

Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, antimutagenic, anti-tumour, and hepatoprotective properties. Phenols, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts, the antioxidant activities of which play an important role in the absorption or neutralization of free radicals (Prabha and Vasantha, 2011).

In India *Calotropis* occupies special importance because of its large industrial uses and economic values. It has various medicinal properties. Different parts of the plant have immense potential to cure various diseases and disorders like asthma, cold, epilepsy, fever, indigestion, leprosy, piles, skin

diseases etc., and exhibiting activities that are anti-inflammatory, anthelmintic, anticancer and antitumor; as observed in various polyherbal preparations (Tenpe *et al.,*. 2007). It is a highly potential plant resource and different parts of this plant are used for multi purposes. *Calotropis gigantea* is seen susceptible to infection by several viruses viz. *Groundnut bud necrosis virus* (GBNV) (Reddy *et al.,*, 2011) and a *Begomovirus* (Prajapat *et al.,*, 2012). Phytochemical constituents are important to understand their roles in the plants and to devise strategies for rapid diagnosis and control.

The present study was carried out to screen potent phytochemical constituents from aqueous and ethanolic leaf extracts of *Calotropis gigantea* and to determine antimicrobial activity.

2. MATERIALS AND METHODS

2.1. COLLECTION OF PLANT MATERIALS AND IDENTIFICATION

Fresh leaves of *Calotropis gigantea* used in this study, which were collected from the campus of Hindustan college of Arts and Science, Coimbatore-641028, Tamil Nadu.

The collected plant material was identified as *Calotrophis gigantea* by Scientist E, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu - 641003.

2.2 PHYTOCHEMICAL ANALYSIS

Phytochemical tests were performed to identify the active chemical compounds such as Alkaloids, Flavonoids, Tannins, Saponins and phenolic compounds in different extracts such as Aqueous, Methanol and Ethanol by following procedure.

2.3. REAGENTS REQUIRED

- Mayer's reagent
- Ferric chloride
- 5% NaOH
- 10% HCl
- lead acetate solution
- Distilled water.

2.4. PREPARATION OF REAGENTS

- **Preparation of Ferric chloride solution**

5g of ferric chloride was dissolved in 100 ml of Distilled water.

- **Preparation of lead acetate solution**

10g of Lead acetate powder was dissolved in 100 ml of Distilled water.

- **Preparation of 5% NaOH**

5g of NaOH was dissolved in 100 ml of Distilled water.

- **Preparation of 10% HCl**

10 ml of concentrated HCl was diluted with 90 ml of Distilled water

2.5. PROCEDURE

Test for Alkaloids (Mayer's test)

- Take 2 ml of extract in a clean and dry test tube.
- Add two drops of Mayer's Reagent by the side of the test tube.
- A white or creamy precipitate indicates the test as positive.

Test for Flavonoid (5%NaOH,10%HCL)

- Take 1 ml of extract in a clean and dry test tube.
- Add 1 ml of 10% HCl and 5% NaoH into the test tube.
- The yellow colour solution solution turns into colourless indicates the test postive.

Test for Saponin (Foam test)

- 1 ml of extract was diluted with the same amount of distilled water.
- The suspension was shaken well.
- A 2 cm layer of Foam indicates the test positive.

Test for Tannins (Ferric chloride test)

- Take 1 ml of extract in a clean and dry test tubes.
- Add 2 drops of neutral 5% Ferric chloride solution.
- A dark green colour indicates the test positive.

Test for Phenolic Compounds (Lead acetate test)

- 1 ml of extract in a clean and dry test tube.
- Add 2 drops of lead acetate solution.
- Formation of white precipitate indicates the test as positive

2.6. ANTIMICROBIAL ACTIVITY

An antimicrobial is the substance that kills or inhibits the growth of microorganisms such as bacteria, fungi and protozoans. An antimicrobial drug either kills microbes (microbicidal) or prevents the growth of microbes (microbiostatic). Disinfectants are antimicrobial substance used on non-living objects or outside the body. In recent years, multiple drug resistant in human pathogenic microorganisms had been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases.

2.6.1. ANTIBACTERIAL ACTIVITY

PREPARATION OF THE MEDIUM

The well diffusion method was used to determine the antibacterial activity of the extracts prepared from the *Calotropis gigantea* leaves using standard procedure. The culture plates were prepared by pouring 20 ml of Muller Hinton Agar (MHA) medium into sterile petri plates. In this method, first the test bacteria broth of bacteria are used to inoculate on the nutrient agar plates with the help of sterile cotton swabs to develop the lawn culture. Then to these plates 6 mm diameter well are punched in agar plates pre-inoculated with test microorganisms. Undiluted over night broth cultures should never be used as an inoculum. Routine direct application of suitably diluted leaf extracts of *Calotrophis gigantea* which were Aqueous, and Ethanol with different concentration such as 25, 50, 75 ,100 µl were used.

The test microorganism used were *Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae.*

AGAR WELL DIFFUSION METHOD

The antibacterial effect of Aqueous, and Ethanol extracts of *Calotropis gigantea* leaves on three bacterial organisms (*Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae*) were determined using agar well diffusion method. The Muller Hinton Agar (MHA) medium (HI-Media, Mumbai) which was already swapped with the test organism was incubated at 37°C for 16 - 18 hours. The zone of inhibition in mm was measured and the results were given in the table.

2.6.2. ANTIFUNGAL ACTIVITY

Antifungal activities of plant extract were tested by agar diffusion method. The culture plates were prepared by pouring 20 ml of Sabouraud's Dextrose Agar (SDA) medium into sterile petri plates. The inoculum suspension was spread uniformly over the agar medium using sterile cotton swabs to get uniform distribution of fungi. Wells (6mm in diameter) were punched in the agar. Different leaf extract of Calotrophis gigantea which were Aqueous and Ethanol with different concentrations such as 25, 50, 75, 100 µl were used.

The test microorganisms: *Aspergillus flavus* and *Candida albicans* were used for the antifungal studies.

AGAR WELL DIFFUSION METHOD

The antifungal effect of Aqueous and Ethanol extracts of *Calotropis gigantea* leaves on two fungal organisms (*Aspergillus flavus* and *Candida albicans*) were determined using agar well diffusion method. The Sabouraud's dextrose agar medium which was already swapped with the test organism was incubated at 37°C for 72 hours. The zone of inhibition mm was measured, and the results were given in the table.

3. RESULTS

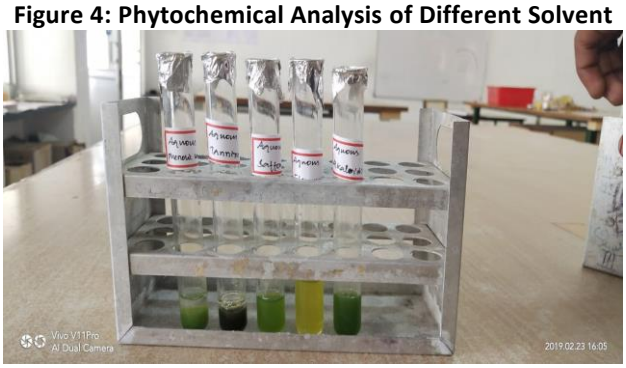
3.1 PHYTOCHEMICAL ANALYSIS

The data in Table 5 and Figure 4 shows screening of different extracts which are Methanol, Ethanol and Aqueous of *Calotropis gigantea* leaf sheath based on phytochemical tests. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes.

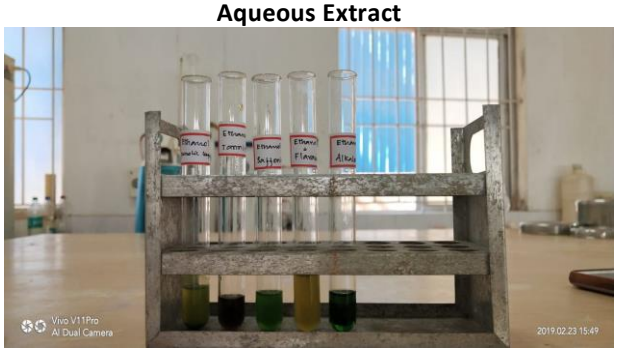
Table 5: Phytochemical Constituents of leaf extracts

PHYTO CHEMICALS	AQUEOUS	METHANOL	ETHANOL
Alkaloids	-	-	-
Flavanoids	-	-	-
Saponins	+	+	+
Tannins	+	-	+
Phenolic compounds	-	-	+

Figure 4: Phytochemical Analysis of Different Solvent



Aqueous Extract



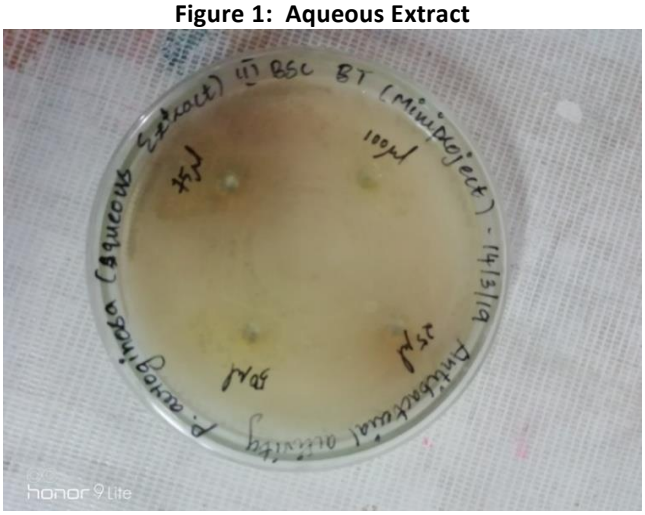
Ethanol Extract

Methanol Extract

4.2.1. ANTIBACTERIAL ACTIVITY

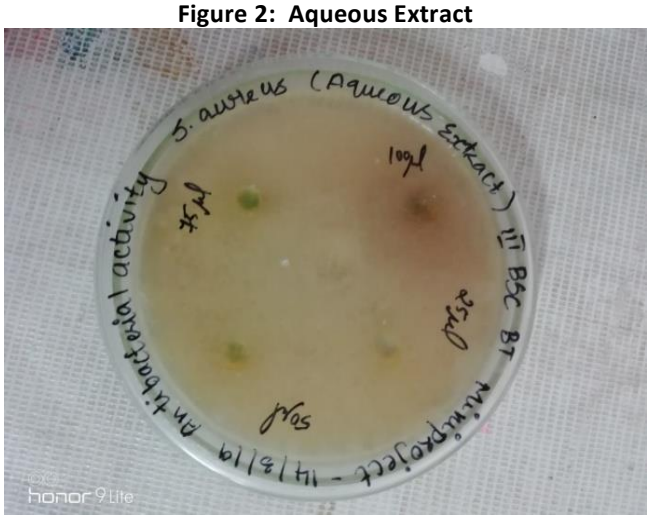
The antibacterial effect of two extracts like Aqueous and Ethanol of leaves on three microorganisms viz., *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were determined using agar well diffusion methods. The zone of inhibition representing the antibacterial activity for all the solvents of *Calotropis gigantea* are represented below. (Table 1 and Table 2; Figure 1 and Figure 2).

Figure 1: Aqueous Extract



Pseudomonas auroginosa

Figure 2: Aqueous Extract



Staphylococcus aureus

TABLE 1: Antibacterial activity of Aqueous extract of Calotropis *gigantea* (Fresh leaves)

S.NO	MICRO ORGANISM	ZONE OF INHIBITION (mm)			
		25µl	50µl	75µl	100µl
1	*Staphylococcus aureus*	-	-	-	13
2	*Klebsiella pneumonia*	-	-	-	-
3	*Pseudomonas aeruginosa*	-	6	9	11

TABLE 2: Antibacterial activity of Ethanolic extract of Calotropis *gigantea* (Fresh leaves)

S.NO	MICRO ORGANISM	ZONE OF INHIBITION (mm)			
		25µl	50µl	75 µl	100µl
1	*Staphylococcus aureus*	-	-	-	-
2	*Klebsiella pneumonia*	-	-	-	-
3	*Pseudomonas aeruginosa*	-	-	-	-

4.2.2. ANTIFUNGAL ACTIVITY

The antibacterial effect of two extracts like Aqueous and Ethanol Calotropis gigantea. leaves on three microorganisms like C*andida albicans and Aspergillus flavus* were determined using agar well diffusion methods. The zone of inhibition representing the antifungal activity for all the solvents of *Calotropis gigantea*. are represented below (Table 3 and Table 4; Figure 3).

Figure 3: Ethanol extract

Aspergillus flavus

TABLE 3: Antifungal activity of Aqueous extract of Calotropis *gigantea* (Fresh leaves)

S.No	MICROORGANISM	ZONE OF INHIBITION (MM)			
		25µl	50µl	75µl	100µl
1.	*Aspergillus flavus*	-	-	-	-
2.	*Candida albicans*	-	-	-	-

TABLE 4: Antifungal activity of Ethanol extract of *Calotropis gigantea* (Fresh leaves)

S.no	MICROORGANISM	ZONE OF INHIBITION (MM)			
		25µl	50µl	75µl	100µl
1.	*Aspergillus flavus*	5	7	8	9
2.	*Candida albicans*	-	-	-	-

The phytochemical screening of *Calotropis gigantea.* revealed the presence of various bioactive secondary metabolites which might be responsible for this medical attribute. Results showed five phytochemicals screened were present. They are Alkaloids, Flavonoids, Saponins, Tannins and Phenolic compounds. In all, more Phytochemicals that is Saponins, Tannins and phenolic compounds were found present in Methanol. Ethanol extracts showed the presence of two Phytochemicals such as Tannins. Aqueous extract showed the presence of three Phytochemicals such as Saponins and Tannins. In the entire phytochemical test, Saponins were found in most of the solvents. Naturally saponins may serve as a antifeedants and protects the plant against microbes and fungi.

Kumar *et al.,* (2011) has reported that C. *gigantea* is extensively studied for its medicinal properties by advanced scientific techniques and a variety of bioactive compounds have been isolated from the different parts of the plant and were analysed pharmacologically. The plant is reported for analgesic activity, antimicrobial activity, antioxidant activity, anti-pyretic activity, insecticidal activity, cytotoxicity activity, hepatoprotective activity, pregnancy interceptive properties, purgative properties, procoagulant activity and wound healing activity. The medicinal properties of this plant represent it as a valuable source of medicine

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