Aqueous stem extracts have more antibacterial and antifungal efficacy than its other parts: A *Calatropis gigantea* as a case study

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The present study was aimed to evaluate the phytochemical, antibacterial, and antifungal properties of the plant extracts obtained from different parts of the plant *Calotropis gigantea* such as leaves, stem, and flowers using different solvents. We evaluated the antibacterial and antifungal properties of these extracts by performing several tests based on colorimeter and by Kirby-Bauer diffusion methods. These extracts were tested against different pathogenic bacterial and fungal strains such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger*, and *Aspergillus flavus* respectively. We observed that antimicrobial efficacies of the extracts among different parts of *Calotropis gigantea*, the aqueous stem extracts have greater anti-microbial efficacy on all the selected test bacterial and test fungal strains. The efficacy is greater than even that of the standard controls such as Ampicillin and Itraconazole. We conclude that the stem of *Calotropis gigantea* has potent and diverse medicinal constituents than that of other plant body parts, where these phytochemical constituents may serve as novel drugs for treating microbial infection in future.

Keywords: *Calotropis gigantea*; different solvents; aqueous stem extracts; phytochemicals; novel drugs.

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Introduction

In the present era, there are rapid advancements in the fields of medical and pharmaceutical sciences. Even though there is a rapid advancement, equally there is emergence of new diseases caused by different microbial organisms. Extensive use of the existing drugs leads to emergence of antibiotic resistance pathogens [1]. There were several reports that the pathogenic bacteria were evolving and becoming resistant to the drugs over the time. Pathogenic organisms cause many infections in human beings such as pulmonary, respiratory, cutaneous, nosocomial and several other communicable infections [2-5]. Majority of the times, we observe these infections in immune deficient patients who are sensitive and more likely to be affected by these pathogens [6, 7]. Chemically synthesized drugs are very effective against the infectious diseases but, on the other hand, they are found to have many side effects [8]. Therefore, there is a need to look for alternative drugs for the synthetic and chemical drugs.

Plants serve as major sources of potential drugs [9]. They are biocompatible and have no side effects [9]. Plants produce secondary metabolites which act in defense mechanisms of the plant at the time of pathogen invasion [10]. These secondary metabolites are also called as phytochemicals. As plants produce phytochemicals which has medicinal properties,

they are used for the curing and betterment of human and animal health. *Calotropis gigantea* is one of such plant which possess medicinal properties [11].

Calotropis gigantea is a commonly known as a xerophytic weed plant which belongs to the family *Asclepiadaceae*. Different parts of this plant possess different therapeutic properties such as antipyretic, analgesic, anti-asthmatic, wound healing, antioxidant, anti-diarrheal, anti-microbial activities, and many more [11]. The latex produced by this plant exhibits good pro-coagulant activity and in wound healing [12, 13]. *Calotropis gigantea* leaves have metabolites which have therapeutic properties such as central nervous system (CNS) depressant activity and hepato-protective activity [14-16]. The roots of this plant exhibit pregnancy interceptive activity and antipyretic activity [17]. The flowers were also found to have medicinal properties such as analgesic activity. The presence of the ingredient anhydrosophoradiol-3-acetate in flowers show anti-tumor activity [18, 19]. *C. gigantea* is reported to exhibit mosquito repellent properties against the mosquito species such as the *Culex gelidus* and *Culex tritaeniorhynchus* which serve as vectors and spread diseases such as Japanese encephalitis [20]. Till date, there are many reports available in the literatures, which describe about the antibacterial and antifungal efficacy of alcoholic extracts of different plant parts of *Calotropis gigantea,* but, to the best of our knowledge, there is no report describing about antibacterial and antifungal activity of aqueous stem extract of *Calotropis gigantea* [21]. Thus, in this report, we made an attempt to make comparative study of the antimicrobial properties of extracts derived from different parts of *Calotropis gigantea* in different solvents and identify the plant part with best antimicrobial efficacy.

Materials and Methods

Collection and processing of plant material

The leaves, stem, and flowers of *Calotropis gigantea* were collected. The plant material was washed properly with water to remove the contaminants. Later, the collected plant material was dried under the shade at room temperature for 14 days till the entire moisture content was lost from the samples. Finally, the dried plant material was grinded into powder and was stored separately in air tight containers.

Preparation of solvent extract

We prepared a total of nine extracts using different solvents from the stored plant material obtained from the earlier step. Water, ethanol, and chloroform were used as solvents. 10 g of each plant material i.e. leaves, stem, and flower were dissolved in 100 ml of each solvent to prepare 10% of the plant extract. At the time of extraction, we used Soxhlet extractor, where water solvents were kept at 100°C for 1-2 hrs, and ethanol and chloroform solvents were kept at 50-55°C and 45-50°C for 45-50 minutes respectively. All these solvents were concentrated by placing them on rotary shaker with 120 rpm at room temperature for 24 hours.

Phytochemical screening of plant extracts

Qualitative analysis of these plant extracts was performed to identify the following bioactive compounds through standard methods as described in Table 1.

Inoculum preparation

The bacterial strains of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* and the fungal strains of *Aspergillus niger* and *Aspergillus flavus* were used as test organisms to demonstrate the antibacterial and antifungal efficacy of the selected plant extracts. Under the sterile conditions, using laminar air flow chamber, we have inoculated a total of 100 µl of each test bacterial culture strains and fungal culture strains into 10 ml of nutrient broth and 10 ml of Czapekdox broth respectively. After inoculation, the test tubes were set for incubation at 37°C. The bacterial test cultures were kept for incubation for 3-5 days and the fungal cultures

Table 1. Name of the phytochemical analysis test used for the identification of the presence or absence of the phytochemicals.

Name of the test	Method	Significance
Alkaloid test	For 2 ml of each extract picric acid solution was added	Formation of orange color indicates the presence of Alkaloids
Flavonoid test	5 ml of diluted ammonia solution was added to 1 ml of each plant extract followed by few drops of H_2SO_4	Formation of yellow color indicates the presence of flavonoids
Steroid test	For each 1 ml of plant extract, few drops of acetic acid were added. The resultant mixture was gently warmed and cooled under tap water. One drop of concentrated H_2SO_4 was added along the sides of the test tube.	Appearance of green color indicates the presence of steroids.
Terpenoid test	To 5 ml of each plant extract, 2 ml of chloroform and 3 ml of concentrated H_2SO_4 was added	Formation of reddish-brown color at the interface indicates the presence of terpenoids.
Tannin test	1 ml of 5% ferric chloride was added to the 1 ml of each plant extract.	The formation of bluish black or greenish black precipitate indicates the presence of tannins.
Saponin test	Each plant extract was diluted with 20 ml of distilled water and was agitated for 15 minutes in a test tube.	The formation of foam specifies the presence of Saponin.
Phenol test	To 1 ml of each plant extract, 2 ml of distilled water was added followed by few drops of 10%ferric chloride.	Appearance of blue or green color specifies the presence of phenols.
Quinone test	To 1 ml of plant each extract 1 ml of concentrated H_2SO_4 was added.	Formation of red color specifies the presence of quinones.
Anthraquinone test	To 1 ml of each plant extract we added 1 ml of sodium hydroxide solution	Blue green or red color indicates the presence of anthraquinones.
Resin test	For 2 ml of each extract 10 ml of acetic anhydride was added then a drop of concentrated H_2SO_4 was added to the resultant mixture	Appearance of purple color, which rapidly changes to violet, shows the presence of resins.
Carbohydrate test	To 1 ml of each plant extract 4 ml of anthrone reagent added were mixed in a test tube and heated on a water bath for 10 minutes.	Appearance of green color indicates the presence of carbohydrates

were incubated for 5 days. After observing the sufficient growth in the test tubes, those cultures were sub-cultured once in every 30 days.

Antibacterial and Antifungal sensitivity test

The antibacterial and antifungal sensitivity of each plant extracts were compared against the standard discs of Ampicillin (10 mg/ml) and Itraconazole (30 mg/ml) respectively by following Kirby-Bauer diffusion method. We took about 15-20 ml of molten agar and Czapekdox media onto sterile petri plates for the inoculation of test bacterial and fungal strains respectively. As the media cools down, about 200 µl of the bacterial and fungal cultures were spread separately in separate Petri dishes. Two wells were punched onto the media at a corner and 100 µl of different plant extracts was added into the wells with an antibiotic disc opposite to the two extracts on these plates. This procedure was repeated for all the other plant extracts. The petri plates were sealed with paraffin and incubated 3 days for bacteria and 5 days for fungal cultures at 37°C. After incubation, the zones of plant materials and the antibiotics were recorded and compared. Experiment was carried out in triplicates for each test organism.

Results and Discussion

Comparative results of phytochemical screening of *Calotropis gigantea* leaves, stem, and flower extracts using different solvents such as water,

Table 2. The tests performed for the confirmation of the presence of phytochemicals in the selected plant parts extracts (LW: leaves in water; LE: leaves in ethanol; LC: leaves in chloroform; FW: flower in water; FE: flower in ethanol; FC: flower in chloroform; SW: stem in water; SE: stem in ethanol; SC: stem in chloroform).

Test	Combination of Plant part and solvent								
	LW	LE	LC	FW	FE	FC	SW	SE	SC
Alkaloid Test	+	+	-	+	+	-	-	-	-
Flavonoid Test	+	+	+	+	+	+	+	+	+
Steroid Test	+	+	+	+	+	+	+	+	+
Terpenoid Test	+	-	-	+	+	+	-	+	+
Tannin Test	+	+	+	+	+	-	-	-	-
Saponin Test	+	+	+	+	+	+	+	-	+
Phenol Test	-	+	+	-	+	-	-	-	-
Quinone Test	-	-	-	+	+	+	+	+	+
Anthraquinone Test	-	-	-	-	-	-	-	-	-
Resin Test	-	-	-	-	-	-	-	-	-
Carbohydrates Test	-	+	+	-	+	-	+	+	-

Table 3. Antibacterial sensitivity of *Calotropis gigantea* leaf, stem, and flower extracts in different solvents (AE: aqueous extract; EE: ethanol extract; CC: chloroform extract; "-": no activity).

Bacterial organism	Zone of inhibitions (Diameter in mm)									Ampicillin (10 mg)
	Leaf extract			Stem extract			Flower extract			
	AE	EE	CE	AE	EE	CE	AE	EE	CE	
Bacillus subtilis	-	10.5±0.2	-	37.5±0.5	12±0.25	-	-	12±0.4	-	16.1±0.67
Klebsiella pneumoniae	-	11±0.75	-	19±0.4	10±0.4	-	10.2±0.7	11±0.8	10±0.4	15±0.6
Staphylococcus aureus	-	-	-	43±0.25	-	-	13±0.6	12±0.3	14±0.5	28±0.2
Pseudomonas aeruginosa	-	-	-	-	-	-	-	20±0.65	12.6±0.2	15±0.07

ethanol, and chloroform are summarized in Table 2. Different solvents have various degrees of solubility for different phytochemicals. Aqueous leaf extract revealed the presence of alkaloids, flavonoids, steroids, terpenoids, tannins, and saponins. Ethanol and chloroform leaf extracts contain alkaloids, flavonoid, steroids, Tannins, saponins, phenols, and carbohydrates. Aqueous stem extracts contain flavonoids, steroids, saponins, quinones, and carbohydrates. Ethanol stem extracts has flavonoids, steroids, and terpenoids.

Antibacterial activity of the extracts

Antibacterial sensitivity of *Calotropis gigantea* leaf, stem, and flower extracts was screened against gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Table 3). Among them, the aqueous stem extract gave greater zone of inhibition than the standard antibiotic disc of ampicillin, which indicated that there was a greater antibacterial efficacy of the aqueous stem extract towards the tested bacterial pathogens. Moreover, in-depth analysis revealed that the aqueous stem extract had more antibacterial efficacy against *Staphylococcus aureus* (43±0.25 mm) than that of *Klebsiella pneumoniae* (19±0.4 mm). Flower extract in ethanol solvent inhibited *Pseudomonas aeruginosa* (20±0.65 mm) more than Ampicillin (15±0.07 mm). Ethanol extracts

Table 4. Antifungal sensitivity of *Calotropis gigantea* leaf, stem, and flower extracts in different solvents (AE: aqueous extract; EE: ethanol extract; CC: chloroform extract; "-": no activity).

Fungal organism	Zone of inhibitions (diameter in mm)									Itraconazole (30mg)
	Leaf			Stem			Flower			
	AE	EE	CE	AE	EE	CE	AE	EE	CE	
Aspergillus niger	18±0.25	-	-	31±0.5	-	-	-	13.1±0.2	10±0.4	18±0.75
Aspergillus flavus	20±0.4	13±0.25	-	27±0.4	-	-	-	17.2±0.5	12±0.8	16±0.2

of leaf and stem, aqueous extracts of stem, and flower extracts of all the solvents showed antimicrobial activity. There were no zones of inhibition for aqueous leaf extracts and chloroform leaf and stem extracts indicating lack of antibacterial property against bacterial strains.

Antifungal activity of the extracts

Antifungal sensitivity of *Calotropis gigantea* leaf, stem, and flower extracts were screened against *Aspergillus niger* and *Aspergillus flavus* strains using different solvents and are presented in Table 4. The aqueous stem extract gave maximum zones of inhibition comparing to standard antibiotic disc of Itraconazole (30 mg) in both fungal strains. Aqueous leaf extract, ethanol leaf extract, ethanol flower extract, and chloroform flower extracts gave zones of inhibition against tested fungal strains. Out of all the extracts, the aqueous stem extract gave maximum inhibition was observed towards *A. niger* (31±0.5 mm). There were no zones of inhibition for the extracts of chloroform leaf, chloroform stem, ethanol stem, and aqueous flower extracts indicating lack of efficacy antifungal activity towards fungal strains.

Conclusion

From the present study, it can be concluded that aqueous stem extract of *Calotropis gigantea* possess high antibacterial and antifungal activity comparing to other extracts of leaves, stems, and flowers of *Calotropis gigantea*. The photochemical constituents of aqueous stem extract of *Calotropis gigantea* can act as a basis of production of novel drugs in pharmaceutical.

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