



Antibacterial and phytochemical analysis of stem and root extracts of *Calotropis gigantea* against selected pathogens

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ABSTRACT

The extracts of *Caliotropis gigantea* (stem and root extracts) were tested against clinical isolates of *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholerae* and *Staphylococcus aureus*. The agar well diffusion assay method was used to access the activities of plant extract against the isolated organisms. All the extracts of stem and root showed good antimicrobial activity against the isolated organisms. The ethanol extract of stem are more effective to the organism. The methanol extract of root are more effective to the organism. The phytochemical analysis can be carried out on the ethanol extract of stem and methanol extract of root shows the presence of quinones, steroids, terpenoids, thin layer chromatography (TLC) have been carried out on ethanol extract (stem), methanol extract (root) of *Caliotropis gigantea*, which show different Rf values and possible combinations of chromatography solvents for separation of these phytochemicals.

Keywords: *Caliotropis gigantea*, medicinal plants, Antibacterial activity, phytochemicals, thin layer chromatography

1. INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The native systems of medicine like Ayurveda and Siddha plays an important role in the human health care system in India and also suggested to increase the natural resistance of the body to diseases [1]. Plants contain many potent biologically active molecules with different medicinal uses [2, 3]. India is precise rich in natural possessions and the knowledge of traditional medicine and the practice of plants as source to treat various dreadful diseases. During the past era the need and usage of traditional treatment has extended universally and is getting popularity and herbal medicines assist the health needs of about 80% of the universal population. The medicinal value of these

plants lies in some chemical substances which produce a definite physiological action on the human body. Medicinal plants are the fundamental for traditional therapy [4]. In addition, the old-fashioned medicine related to treatment of both human and animal mycoses with plant-derived preparations is considered a valuable knowledge for the discovery of new drugs [5].

The bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [6]. These compounds are synthesized by primary or rather secondary metabolism of living organism. Secondary metabolites are chemically and taxonomically found diverse compounds with

obscure function and used for human therapy [7]. Traditionally pharmacological analysis of compound-s from natural or synthetic derivation has been the source of innumerable therapeutic compounds. Communicable infections, specifically skin and mucosal infections, are usual in most of the common people. An important group of these skin pathogens are the fungi, among which dermatophytes and *Candida* spp., besides certain pathogenic bacteria are the most frequent [8, 9]. Moreover, in the past few years, the ratio of immunosuppressed and immunocompromised patients are affecting more due to multidrug resistance.

Calotropis gigantea L. is a medium-sized shrub, 2 to 3 meters in height with the young parts being covered with white hairs. The bark are pale in nature. The leaves are obovate or oblong, 10 to 20 centimeters long, 3 to 8 centimeters wide, pointed at the tip and heart shaped at the base. The corolla is 1.5 to 2.5 centimeters across and is usually white though sometimes dull-purple or purplish-lilac; the lobes are ovate-lanceolate and spreading [10]. *C. gigantea* is commonly known as milk weed or swallow – wort, is a common waste land weed. This plant is a native of Bangladesh, Burma, China, Indonesia, Malaysia, Pakistan, Philippines, Thailand and Srilanka. *C. gigantea* is frequently available in India and used for several medication purposes in traditional medicinal system to cure several diseases and medicinal proved scientifically [11, 12]. The flowers of the *C. gigantea* are used in stomachic, bechic, antiasthmatic, analgesic activity [13]. Root is used for the treatment of lupus, tuberculosis, leprosy and syphilitic ulceration and also contains anti-pyretic activity [14].

This plant is popularly known because it produces large quantity of latex. Latexes are source of various biologically active compounds, including glycosides, tannins and many proteins. Leaves and areal parts of the plant are used in the treatment of external swellings and diarrhoea [15]. Latex is reported to contain purgative properties of wound healing activity [16]. One of the important medicinal plant *C. gigantea* used to screen for the in vitro antibacterial activity and phytochemical analyses of the plant. This study attempts to determine the phytochemical analysis and antimicrobial effect of *C. gigantea*. In this report, we provide new information on the antimicrobial activities of *C. gigantea* using known microbial pathogens as tested organisms.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The *C. gigantea* (stem and root) were collected from local area at karungalikuppum (village), Vellore district. The samples were collected in sterile plastic bags and transported aseptically to the laboratory.

2.2. Processing of samples

The freshly collected stem and root were washed with sterile distilled water and dried in shade at room temperature for 10-15 days. Then the stem and root were powdered by using mortar and pestle. The finely powdered stem and root were used for extraction of antimicrobial compounds.

2.3. Preparation of extract

2.3.1. Solvent extract

The solvents used for extraction includes Ethanol, Methanol, Acetone, Hexane, Ethyl acetate. One gram of dried plant Stem and Root powder was taken in a separate conical flask and 20ml of ethanol was added into it. This content was mixed well using glass rod. Then the conical flask was covered with aluminium foil and kept in a shaker for extraction at room temperature for minimum two days [17].

2.3.2. Separation of crude extract

After extraction is completed the solvent mixture is transferred to a centrifuge tube and centrifuged at 3000 rpm for 10 minutes. Then the supernatant of solvent extract were transferred to clean 25ml beaker crude compound was concentrated by evaporation at room temperature. Crude extracts were transferred to small vials and kept at 4°C until further use.

2.3.3. Test pathogens

The isolates were confirmed by morphological, cultural and biochemical studies. The pathogen includes *E. coli*, *P. aeruginosa*, *V. cholera*, *S. aureus* and *S. typhi*.

2.3.3. Antibacterial activity of plant extracts

Antibacterial activity of various solvent extract of stem and root were studied by well diffusion using Muller Hinton Agar plates. Muller Hinton Agar medium was prepared in Erlenmeyer flask and sterilized by autoclaving at 121°C for 15 minutes. After sterilization 20ml of medium was added into each sterile petriplate in a aseptic condition and allowed to solidify. 18 hours old bacterial broth culture was prepared and inoculated into MHA plates using sterile cotton swabs and make well by using

well cutter. To that well add 50µl of solvent extract by using pipette. All the plates were incubated at 37°C for 24 hours. After incubation the plates were observed for zone of inhibition [18].

2.3.4. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations of active bands from the plant (stem-ethanol, root-methanol) extracts were determined by tube dilution method [19]. Two sets of four test tubes were taken to which 360 µl of 18 hours old bacterial inoculum at 10⁻⁴ dilution was added. To the first tube of first set, 40 µl of plant extract was added and mix well take 20 µl from the first tube were serially diluted up to fourth tube. The test tubes were then incubated for an hour. 50 µl of the suspension was taken from each tube and spotted on the medium in a petridish divided already into four equal quadrats. The plates were incubated for 24 hours at 37°C. Observations were made for the visible growth of the organisms. The tube containing the lowest concentration of the extract which when streaked on the plate did not show any visible growth of organism after 24 hrs was considered as minimum inhibitory concentration.

2.4. Phytochemical analysis of crude extract

Various phytochemical investigations performed using obtained extracts. The procedures are already reported by number of workers and used without any modification or alteration [20]. The phytochemical name and investigation methods are as follows:

2.4.1. Phenols

To test the Phenol phytochemical presence, in a test tube 1ml of extract and 2 ml of distilled water were added followed by few drops of 10% ferric chloride (FeCl₃). Appearance of blue or green colour indicates presence of phenols.

2.4.2. Flavonoids

To test the flavonoid phytochemical presence, in a test tube 1 ml of extract a few drops of dilute sodium hydroxide (NaOH) added. An intense yellow colour was produced in the plant extract which becomes colourless on addition of few drops of dilute acid indicates the presence of flavonoid.

2.4.3. Quinones

To test the quinone phytochemical presence, in a test tube 1ml of extract and 1ml of concentrated

sulphuric acid (H₂SO₄) was added. Formation of red colour shows the presence of quinones.

2.4.4. Tannins

To test the tannin phytochemical presence, in a test tube 1 ml of 5% ferric chloride added to solvent free extract. The presence of tannin is indicated by the formation of bluish black or greenish black precipitate.

2.4.5. Saponins

To test the saponin phytochemicals presence in various extract, the extract was diluted with 20 ml distilled water and was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicates the presence of saponin.

2.4.6. Cardiac Glycosides

To test the cardiac glycoside phytochemicals presence, in a test tube 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride (FeCl₃) solution. Afterwards it was underplayed with 1 ml concentrated sulphuric acid (H₂SO₄). A brown ring of the interface indicates a deoxy sugar characteristic of cardenolites.

2.4.7. Steroids

To test the presence of steroid phytochemicals, 1 ml of extract dissolved in 10 ml chloroform and equal volume of concentrated sulphuric acid (H₂SO₄) added by sides of test tube. The upper layer turns red and sulphuric acid layer shown yellow with green fluorescence. This indicated the presence of steroids.

2.4.8. Terpenoids

To test the presence of terpenoid phytochemicals, in a test tube 5 ml of each extract was mixed with 2 ml of chloroform. 3ml of concentrated sulphuric acid (H₂SO₄) was then added to form a layer. A reddish brown precipitate coloration at the interface formed indicated the presence of terpenoids.

2.5. Partial purification of crude extract by thin layer chromatography (TLC)

2.5.1. Preparation of TLC plate

TLC plate was prepared by spreading the slurry of silica gel-G evenly on the glass plate (size) of 2mm thickness. The plate was air dried and activated at 100°C for 15 minutes. The crude extract was spotted at the bottom of the silica gel coated plate. Then the TLC plate was placed in the glass tank filled with

solvent in different proportion. After running, the TLC plate was kept in closed iodine chamber to visualize the separation [21].

The TLC run plates were observed under bright light and the separated spots were marked and the respective Relative front values were calculated using the following formula. Rf value =

$$\frac{\text{Distance moved by solute from the origin}}{\text{Distance moved by solvent from the origin}}$$

3. RESULTS

3.1. Identification of test organisms

The isolated microorganisms are identified by using various morphological and biochemical tests, the isolated organism are identified as *E. coli*, *P. aeruginosa*, *V. cholera*, *Salmonella sp.*, and *S. aureus*. The results were tabulated in the Table 1.

3.2. Antibacterial activity

Conferring to Mock *et al.* agar-based techniques are not suitable for analyzing the bioactive compounds because some of the compounds diffusion ability is very less [22]. However, the disk diffusion and E-test methods have shown a good correlation with the broth-based reference methods for testing organism. Hence, agar disc diffusion method was performed in the present study to investigate the antibacterial activity of *C. gigantea* latex extract. Results obtained in the present study relieved that the tested medicinal plants extracts possess potential antibacterial activity against the test pathogens. The measurement of zone of inhibition observed for the plant were tabulated in the table, the maximum inhibitory action was seen in ethanol stem extract of *C. gigantea* and methanol root extract of *C. gigantea* respectively (Figure 1). The results were tabulated in the Table 2. These two extracts were selected for further studies.

3.2.1. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) study shows the inhibition at 10µl of ethanol stem extract of *C. gigantea*, 0.5µl methanol root extract of *C. gigantea* against test pathogens.

3.3. Phytochemical analysis of crude extract

Various phytochemical tests performed on the ethanol extract of *C. gigantea* (Stem) and methanol

extract of *C. gigantea* (Root). The results are reported in the Table 3.

Figure 1. Antibacterial activity of *Pseudomonas aeruginosa*

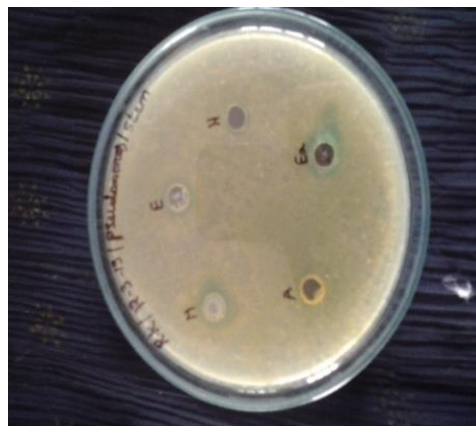


Table 3. Phytochemical Analysis of *Calotropis gigantea*

| Phyto-chemicals | <i>C. gigantea</i> (Stem) | <i>C.gigantea</i> (Root) |
|--------------------|---------------------------|--------------------------|
| Phenols | - | - |
| Flavonoids | - | - |
| Quinones | + | + |
| Tannins | - | - |
| Saponins | - | - |
| Cardiac glycosides | - | - |
| Steroids | - | + |
| Terpenoids | + | + |

3.4. Partial purification of crude extract by thin layer chromatography (TLC)

Both the selected two extracts was to begin with, checked by Thin layer chromatography (TLC) on analytical plates over silica gel-G of 0.2 mm thickness. TLC of plant extract in ethanol of stem and methanol of root reports their spots for various phytochemicals. The separated spots were marked and the respective Relative front (Rf) values were calculated. The results are reported in the Table 4.

Table 4. Rf value of ethanol and methonal extract of *C. gigantea*

| <i>C. gigantea</i> | Rf Value | |
|--------------------|----------|------|
| | Stem | Root |
| Ethanol extract | 0.84 | 0.68 |
| Methanol extract | 0.96 | 0.88 |

Table 1. Characteristics of selected pathogens

| Characteristics | <i>E. coli</i> | <i>P. aeruginosa</i> | <i>V. cholera</i> | <i>S. typhi</i> | <i>S. aureus</i> |
|--------------------------|----------------------------|---|---------------------------------|------------------------|--|
| Gram staining | Gram (-), rod | Gram (-), rod | Gram (-), rod | Gram (-), rod | Gram (+), cocci |
| Motility | motile | motile | motile | motile | non- motile |
| Capsule staining | Non capsulated | Non capsulated | Non capsulated | Non capsulated | Non capsulated |
| Colony morphology | | | | | |
| Nutrient agar | White colony | Translucent pigment bluish green colony | Translucent Bluish tinge colony | Grayish white colony | Circular, smooth, shiny Golden Yellow colony |
| MacConkey agar | Lactose fermenting | Non lactose fermenting | Lactose fermenting | Non lactose fermenting | Lactose fermenting |
| Selective media | EMB – green metallic sheen | Cetrimide green fluorescent colony | TCBS Yellow | SS agar Black colony | MSA – yellow, shiny colony. |

Table 2. Antibacterial activity of *Calotropis gigantea*

| S.No | Test organism | Zone of Inhibition (mm) | | | | | | | | | |
|------|----------------------|-------------------------|------|---------|------|---------|------|--------|------|---------------|------|
| | | Methanol | | Ethanol | | Acetone | | Hexane | | Ethyl Acetate | |
| | | stem | root | stem | root | stem | root | stem | root | stem | root |
| 1 | <i>S. aureus</i> | 10 | 9 | 11 | 10 | 7 | 8 | 7 | 7 | 8 | 10 |
| 2 | <i>E. coli</i> | 9 | 8 | 11 | 10 | 7 | 7 | 6 | 8 | 8 | 11 |
| 3 | <i>V. cholerae</i> | 12 | 12 | 11 | 12 | 6 | 7 | 9 | 8 | 11 | 6 |
| 4 | <i>P. aeruginosa</i> | 8 | 12 | 7 | 11 | 8 | 10 | 6 | 9 | 7 | 11 |
| 5 | <i>S. typhi</i> | 10 | 9 | 8 | 5 | 13 | 16 | 7 | 7 | 8 | 6 |

4. DISCUSSION

Plants are main substrate of potentially beneficial structures for the expansion of unique chemotherapeutic agents. Plant extract has been used traditionally to treat a number of infectious diseases including those caused by bacteria, fungi, protozoa and viruses [23]. Multidrug resistance is an emerging problem in healthcare industry, as various pathogenic microbes are reported to develop resistance towards the wide variety of drugs [24]. These multi drug resistant organisms are very lethal and difficult to treat [25, 26]. Infection rate and the severity of the multi-drug resistant organisms are very high in immune compromised patients [27]. To overcome these problems, discovery and development of new drug molecules are very essential. Phytochemical elements such as aromatic compounds, tannins, alkaloids, flavonoids and other bioactive compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores [28].

The ethanol stem extract of *C. gigantea* inhibit high antibacterial activity against selected pathogens compare to other solvent extract. So that the ethanol extract was highly effective, maximum range (8mm-11mm). Methanol root extract of *C. gigantea* inhibit high antibacterial activity against selected pathogens compare to other solvent extract. So that the methanol extract was highly effective, maximum range (9mm-12mm), hence these extract was selected for further studies. Phytochemical analysis for these extract was done, ethanol stem extract of *C. gigantea* contains Quinones, Terpenoids. Methanol root extract of *C. gigantea* contains Quinones, Terpenoids and Steroids. Partial purification of these two extracts such as ethanol stem extract of *C. gigantea* and methanol root extract of *C. gigantea* shows that it contains two and three different components respectively. So that the root extract of methanol has effective activity against the pathogens. Thus, they can be used in the management of infectious diseases caused by resistant microbes. The demonstration of antimicrobial activity of *C. gigantea* latex extract against bacterial species may be a symbolic of the existence of wide range potent bioactive compounds [29].

Conflict of Interest

The authors declare that they have no conflicts of interest.

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