



Preliminary Phytochemical screening, Anti-bacterial and Thrombolytic activity of *Cleome gynandra* aqueous extract.

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Abstract

For the past few decades plants have been the basis of treatment for many human diseases. Plant based medicines have been used in different formulations. In this study traditional plant *Cleome gynandra* was selected and its preliminary phytochemical screening, antibacterial and thrombolytic activities were evaluated in aqueous leaf extract. From this study it was clear that *Cleome gynandra* possess significant antibacterial activity with 20mm zone of inhibition against *Pseudomonas aeruginosa* and also showed moderate activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* with 14mm and 12mm zone of inhibition respectively. The thrombolytic activity was found to be increased with increase in the concentration of the sample. It showed 53% of clot lysis at 100 μ l concentration of the sample. The results of this study revealed that the *Cleome gynandra* leaf extract possess good antibacterial and thrombolytic activity.

Keywords: *Cleome gynandra*, Medicinal plants, Phytochemicals, Antibacterial activity, Thrombolytic activity.

Introduction

For many years, plants are used as crude material for drugs. In India, rich knowledge about medicinal importance of plants is available to the common people. About 3,500 plant species are used as a source of crude drug in India. Medicinally important plants are about 2,500 in number. Some plant species are considered as weed, but they are medicinally important too. In various traditional and modern methods of therapy, plant based products are currently used[1]. Plant derived substances have recently become a great interest owing to their versatile applications. Medicinal plants are the richest bioresources for drugs in traditional systems of medicine, nutraceuticals, food supplements and pharmaceutical intermediates [2].

Cleome gynandra of Cleomaceae (Capparaceae) family is an annual herb, widely spread in many tropical and sub-tropical parts of the world. It is an erect glandular-pubescent annual herb, popularly used in the Ayurveda, Siddha, Folk and Tibetan systems of medicine[3]. This wild leafy vegetable is indigenous to the tropical and pan tropical regions and plays an important role in agricultural and nutritional system of these regions[4]. Both leaves and flowers of this plant are edible. The leaves have a strong bitter, sometimes peppery flavor similar to mustard greens.

Cleome gynandra has been used for the treatment of pain, swelling, fever, cough, asthma, skin and urinary diseases. Seed powder of this plant have carminative (relieves flatulence), antiseptic and antihelminthic (destroy parasitic worms) properties. Leaves have anti-inflammatory and sudorific (causing sweating) properties. Traditionally, few drops of leaves juice is put in ear to treat ear pain. For painful joints, leaves and seeds poultice is used [5]. The present study deals with the phytochemical screening, anti-bacterial and thrombolytic activity of *Cleome gynandra*.

Materials and Methods

Collection of plant material:

The *Cleome gynandra* leaves were collected from different localities of Coimbatore District, Tamil Nadu and authenticated by the Botanical Survey of India (BSI) in "Tamil Nadu Agriculture University" Coimbatore, Tamil Nadu, India. A voucher specimen (No.BSI/SRC/5/23/2017/Tech/2841) has been deposited at the Herbarium of the Botany Department of "Tamil Nadu Agriculture University" for future reference.

Preparation of *Cleome gynandra* aqueous extract:

The collected leaves were shade dried and powdered. 5g of leaf powder was dissolved in 50ml of distilled water and kept in rotatory shaker for 72 hours, with occasional stirring. After 3 days, the suspensions were filtered and evaporated to dryness at low temperature (< 40°C) under reduced pressure in a rotary evaporator and it was used for further analysis.

Phytochemical screening of *Cleome gynandra* aqueous extract:

Qualitative phytochemical screening of aqueous extract of *Cleome gynandra* was carried out to identify the presence of secondary metabolites including alkaloids, carbohydrates, terpenoids, flavonoids, cardiac glycosides, phenols, proteins, amino acids, saponins, steroids and tannins.[5]

1. Test for alkaloids [Wagner's reagent]

1ml of crude extract was treated with 3-5drops of Wagner's reagent and observed for the formation of reddish brown precipitate (or colouration) which shows the presence of alkaloids.

2. Test for flavonoids [Alkaline reagent test]

1ml of crude extract was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

3. Test for carbohydrates [Fehling's test]

1ml of crude extract was hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

4. Test for proteins [Biuret test]

1ml of crude extract was added to equal volume of 5% solution of sodium hydroxide and 1% copper sulphate. Appearance of pink or purple colour indicates the presence of proteins.

5. Test for cardiac glycosides [Keller Killani test]

To 1ml of extract, 2ml of glacial acetic acid containing one drop of 5 % ferric chloride and 1ml of concentrated sulphuric acid were added. Appearance of reddish brown colour at the junction of the two liquid layers indicates the presence of cardiac glycosides.

6. Test for saponins [Foam test]

1ml of extract and 3ml of water was taken in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

7. Test for tannins [Ferric chloride test]

1ml of crude extract was mixed with ferric chloride solution which would give blackish red colour indicates the presence of tannins.

8. Test for phenols [Ferric chloride test]

1ml of crude extract was treated with aqueous 5% ferric chloride and observed for the formation of deep blue/ black colour.

9. Test for steroids [Liebermann-Burchard test]

1ml of crude extract was mixed with chloroform. To this 2ml of acetic anhydride and 2 drops of concentrated sulphuric acid was added along the sides of the test tube. Initially, the formation of red colour and later green colour shows the presence of steroids.

10. Test for amino acids [Ninhydrin test]

1ml of crude extract when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple colour suggesting the presence of free amino acids.

11. Test for Terpenoids [Salkowshi test]

1ml of crude extract was dissolved in 2ml of chloroform and evaporated to dryness. 2ml of concentrated sulphuric acid was then added and heated for about 2 minutes. Development of a grayish colour indicates the presence of terpenoids.

Antibacterial activity test

The antibacterial activity of *Cleome gynandra* was tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by disc diffusion method and the zone of inhibition was measured. The test organisms were spread on the Muller-Hinton Agar. Whatmann filter paper (No. 1) discs of 6 mm diameter were made by punching the paper and the blank discs were sterilized in hot air oven at 160°C for one hour. Then the sterile filter paper discs were impregnated with *Cleome gynandra* aqueous extract. The discs were left to dry in vacuum so as to remove residual solvent, which might interfere with the experimental result. The impregnated discs with extract were then placed on the top of the inoculated agar medium by sterile forceps. Tests were performed in duplicate with streptomycin as standard. The plates were then incubated at 37°C for 24 hours. After the incubation period the antibacterial activity

was evaluated by measuring the average of inhibition zones in millimeter[6].

Thrombolytic assay

2.5 ml of venous blood drawn from healthy volunteers was distributed in 5 different pre weighed sterile micro-centrifuge tube (0.5 ml/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each micro-centrifuge tube containing pre-weighed clot, 100µl of different concentration of *Cleome gynandra* aqueous extract (20,40,60,80,100µl) was added. For control, 100µl of distilled water was added. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference in weight taken before and after clot lysis was expressed as percentage of clot lysis[7,8].

$$\text{Percentage of clot lysis} = \frac{\text{weight of released clot}}{\text{clot weight}} \times 100$$

Results and Discussion

Preliminary phytochemical screening

Phytochemicals are responsible for the pharmacological activities of *Cleome gynandra*, which in turn provide useful tools to determine their role as therapeutic agents. The qualitative phytochemical screening of the plant extract was shown in Table-1. It revealed the presence of alkaloids, flavonoids, phenols, proteins, amino acids, carbohydrates, steroids, saponins and tannins. Whereas terpenoids and cardiac glycosides were absent. The beneficial medical effects of plant materials typically result from the combination of secondary metabolites present in the plant. [5]

Table-1: Preliminary phytochemical screening of *Cleome gynandra* aqueous extract:

Phytochemical compounds	Aqueous extract of <i>Cleome gynandra</i>
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Steroids	+
Proteins	+
Amino acid	+
Carbohydrates	+
Phenols	+
Terpenoids	-
Cardiac glycosides	-

‘+’ indicates presence, ‘-’ indicates absence

Various extracts of *Cleome gynandra* was performed and it revealed the presence of tannins, phenols, flavonoids, cardiac glycosides, steroids whereas saponins were found in methanol, ethanol and acetone extracts. Terpenoids were not found in all the tested extracts[9]. Chloroform extract of *Cleome gynandra* showed the presence tannins, terpenoids, saponins and proteins whereas flavonoids and quinones were absent. Water extract showed only tannins and saponins. In acetone extract, tannins, flavonoids and quinones were absent and terpenoids and saponins were present. On the other hand in ethanol extract it showed the presence of tannins, terpenoids and proteins are present whereas flavonoids, saponins and quinones were absent [10].

From the results it was evident that all the solvent extracts were found to contain selected phytochemicals. However, compared with different solvent extracts aqueous extract was found to contain more active phyto-constituents. Many of these compounds have been recognized to possess wide range of medicinal properties such as antioxidant, antihepatotoxic and anticancer effects [5].

Antibacterial activity

The antibacterial activity of the *Cleome gynandra* was screened against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* and was shown in Figure-1 and Table-2.

Figure -1: Antibacterial activity of *Cleome gynandra* aqueous extract



Table-2:Antibacterial activity of *Cleome gynandra* aqueous extract

Sl no	Bacteria	Zone of inhibition by streptomycin (mm)	Zone of inhibition by leaf extract (mm)
1	<i>Pseudomonas aeruginosa</i>	30	20
2	<i>Staphylococcus aureus</i>	25	14
3	<i>Klebsiella pneumoniae</i>	23	12

The antibacterial activity of aqueous extract of *C. gynandra* was assessed by their zone of inhibition (IZ) values which revealed that the plant showed significant antibacterial activity against *Pseudomonas aeruginosa* (20mm) followed by *Staphylococcus aureus* (14mm) and *Klebsiella pneumoniae* (12mm).

The methanolic extract of *C. gynandra* showed maximum antibacterial activity on *Staphylococcus aureus* with 22mm zone of inhibition followed by *Bacillus subtilis* with 21mm[7]. The methanol, chloroform and petroleum ether leaf extract showed moderate inhibition against *E.coli*, *E.faecalis* and *P.vulgaris*. The aqueous extract exhibited less inhibition against *E.coli*, *E.faecalis* and *P.vulgaris* [11]. Aqueous extract of *C. gynandra* showed comparatively more zone of inhibition of about 13±0.9 mm against *S. pneumoniae*. The aqueous extract of

C. gynandra was also used to control the growth of *Salmonella typhimurium* and *S. enteritidis*[12]. The ethanolic extracts of *C. gynandra* produced higher zone of inhibition of about 18.6±0.7 mm against *S. pneumoniae*. Methanolic extract of *C. gynandra* leaves and stems were used potentially to control the growth of *Xanthomonas campestris* [13].

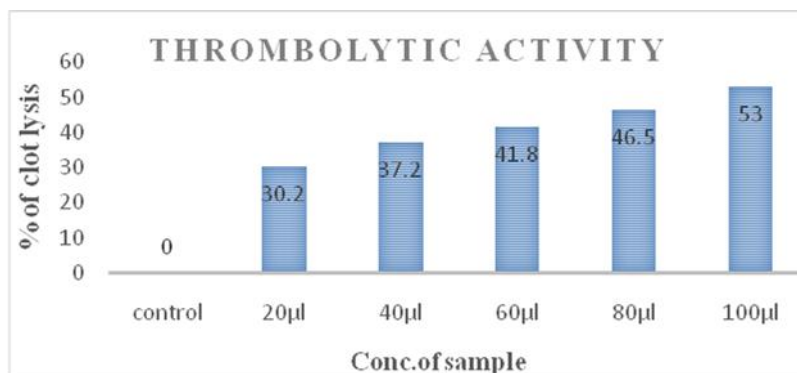
Thrombolytic activity

Cardiovascular disease caused by blood clot (thrombus) formation is one among the most severe diseases which are increasing at an alarming rate in the recent years [14]. Thrombolytic agents are used to dissolve clot in patients but their use is associated with specificity [15]. The results (Figure-2 and 3) showed that the *Cleome gynandra* plant extract at 100 µl was found to have highest percentage (53%) of clot lysis.

Figure-2:Thrombolytic activity of *Cleome gynandra* aqueous extract



Figure 3: Thrombolytic activity - % of clot lysis.



Similar results were obtained for the hydroethanolic extract of *Cleome viscosa* which showed higher % of clot lysis [8]. Aqueous crude extracts of *Punica granatum*, *Zingiber officinale* and *Phyllanthus emblica* at 10mg/ml concentration have exhibited 37.42%, 30.03%, 34.31% of clot lysis activity respectively [16]. Methanolic extract of *Withania somnifera* revealed thrombolytic activity of 68.14%, whereas ethanol and chloroform extracts of *Withania somnifera* displayed moderate (21.15% and 17.46%) thrombolytic activities [17].

Conclusion

The present study concluded that the aqueous extract of *Cleome gynandra* showed potent antibacterial activity against *Pseudomonas aeruginosa* which may be due to the presence of phytochemical constituents. It also showed the significant thrombolytic activity of *Cleome gynandra*. The clot lysis activity was found to be increased with increase in the concentration of the sample. Therefore it is evident that the *Cleome gynandra* aqueous extract can be used to treat bacterial infections and to induce clot lysis.

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