

Biopesticidal Potential of Stem Extracts of *Calotropis gigantea* R.Br. (Linn.)

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ABSTRACT

Calotropis gigantea R.Br, though commonly known as a waste weed plant, is highly regarded for its medicinal potential especially for its efficacy as an antimicrobial. Plant diseases are important to humans because they cause damage to plants and plant products, commonly with an associated economic effect, either positive or negative. The popularity of botanical pesticides has been increasing considerably and several plant extracts are being used globally in green pesticides. Plant extracts have now well established the influence commercially in botanical pesticides as bioinsecticides, biofungicides and bionematicides. Recently phytochemicals such as sitosterol found in medicinal plants have also been used as antimicrobials against plant pests because of their relatively safe status and wide acceptance by the farmers. The GCMS analysis for constituent phytochemicals of *Calotropis gigantea* Stem Methanolic Extract (CGSME) evidenced the presence of Sitosterol for which earlier researchers have already proved its potential for best antimicrobial activity including phytopathogens. The results of these studies provide scope towards exploitation of CGSME in its application in novel therapeutics for plant protection. This biopesticidal application of CGSME would serve as a novel biotechnological approach for enhancement of crop yield and quality.

KEY WORDS

Calotropis gigantea, Phytopathogens, Phytoextracts

1.INTRODUCTION

Biological Control of Phytopathogens

The control of plant diseases biologically includes the use of beneficial microbes and plant extracts to control phytopathogens that could be considered as an alternative to chemical pesticides (Heydari et al). Of late, across the globe, attention has been given due regard towards higher plant products being exploited as novel chemotherapeutics in plant protection. The popularity of botanical pesticides has once again been increasing resulting in some plant products being used globally as green pesticides (Gurjar et al).

Biopesticides are well known for their advantages of environmental safety, target specificity, improved resistance management, reduced residue and compatibility with organic farming.

The principal aim of current research in this field has been the development of alternative control strategies for the reduction of dependency on synthetic pesticides. Plants possess the capability for synthesis of aromatic secondary metabolites, which include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Cowan et al). The herbal constituents with phenolic structures such as thymol, eugenol, and carvacrol, were found to possess highly activity against the phytopathogens. These groups of compounds exhibit antimicrobial effect and serve as ideal plant defense mechanisms against pathogenic microorganisms (Das et al).

Antimicrobial efficacy of CGSME

Plants contain thousands of constituents which are rich with valuable sources of novel and biologically active molecules possessing antimicrobial properties. (Gu jrar et al)

Calotropis possesses multiple antibacterial and antifungal properties. Extracts from different parts of *Calotropis gigantea* plant leaf, latex, flower and root have been found to possess efficacy against several plant pathogenic microorganisms without imposing any ill or its side effects. Antimicrobial assays have revealed that *Calotropis gigantea* inhibited *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus* and *C. albicans*. The phytochemicals that denature microbe proteins and disrupt their functionality were studied to result in the antimicrobial activity of *C. gigantea*. (Gaurav Kumar et al)

CGSME could prove helpful in botanical pesticides as alternative tools for integrated pest management since they have positive impacts on environmental preservation, low toxicity and low risk of developing resistance in target pests. (Alam et al)

Role of β -sitosterol in controlling phytopathogens

Plants are very much exposed to a large number of pests and pathogens, which results in variations in its metabolism, including sterol profiles. Sterols are biological molecules which have an important role in several biological processes. In addition to their essential function in the support of cell membrane and fluidity, they also serve importantly as hormone precursors and are very much involved in biotic and abiotic stress responses (Arnqvist L et al). Sterols of plants are composed of campesterol (campesterol), β -sitosterol (beta-sitosterol), and stigmasterol (Stigmasterol).

Changes in metabolism in stigmasterol and β -sitosterol levels also have been associated with fungal or bacterial infection and were associated with the induction of signaling pathways resulting in the synthesis of molecules of antimicrobial activity and changes in membrane permeability (Hartmann M.A. et al).

Plants synthesize these molecules; but animals acquire them through diet (Bouic P et al).

High control activity is exhibited by β -sitosterol against different plant diseases without resulting in environmental pollution.

2.MATERIALS AND METHODS

2.1Sample Collection, Identification and Preparation

C. gigantea leaves were collected from the different localities of Namakkal district. Identification of plant was done with the help of Botanical Survey of India, TNAU Campus, Tamilnadu. *C. gigantea* stem pieces were brought and shade dried for 2 weeks. Pieces of dried stem were surface sterilized using 1% sodium hypochlorite. These pieces of stem were washed with distilled water in order to remove the excess sodium hypochlorite and dried in an oven at 40°C to remove the moisture. Dried stem pieces were then powdered with the help of an electric grinder.

2.2Preparation of CGSME

C. gigantea leaves and root powders were subjected to Soxhlet extraction. Methanol was used for phytochemical extraction. The extraction was performed over one whole night to obtain the concentrated CGSME extract. Following which concentration of the CGSME extract was carried out using a rotary evaporator and then it was stored at 4°C for further use.

2.3 Phytochemical Assay of CGSME

Phytochemical evaluation of the CGSME extract was performed using the standard procedures. Different phytochemicals, namely alkaloids, phenols, flavonoids, saponins, steroids, terpenoids, tannins, carbohydrates and glycosides were screened.

2.4 Antimicrobial Assay of CGSME

The modified agar disc diffusion method was employed. 24hrs pram positive and gram negative bacterial cultures and fungal cultures were inoculated on to Muller Hinton agar (MHA) media (Hi Media, Mumbai) by spread plate technique. Sterile discs of 6mm diameter were impregnated in various concentrations of CGSME extracts and placed onto MHA plates. The CGSME Extracts were prepared by dissolving the concentrates in DMSO (Dimethyl Sulfoxide) to obtain 62.5, 125, 50 and 500 µg/mL. The plates were sealed and incubated at $32 \pm 2^\circ\text{C}$ for 24 hrs. Readings of the diameter of zone of inhibition was recorded on completion of the incubation period. All of the above experiments were performed in triplicates and the values were recorded as mean $\pm$ standard deviation.

2.5 GC MS Analysis for Constituent Phytochemicals

GC-MS analysis was carried out using a GC Clarus 500 Perlin Elmer system which includes a AOC-20i auto sampler in addition to a gas chromatograph that was interfaced with a mass spectrophotometer (GC – MS) instrument employing the following requirements: column Elite – 1 fused silica capillary column (30 x 0.25 mm ID x 1 EM df, consisting of 100% Dimethyl polysiloxane) that operated in the mode electron impact at 70 eV where in helium (99.999%) was employed as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 EI was used (split ratio of 10:1 with an injector temperature 250°C and ion-source temperature as 280°C . The oven temperature was set with the aid of programming from 110°C (isothermal for 2 minutes) with an increase rate of $10^\circ\text{C}/\text{min}$, to 200°C then $5^\circ\text{C}/\text{min}$ to 280°C , concluding with a 9 min isothermal at 280°C . The mass spectra were recorded at 70 eV; in a scan interval of 0.5s and fragments from 40 to 550 Da.

GC-MS mass spectrum interpretation was conducted with reference to the National Institute Standard and Technology (NIST) database which is in possession of more than 62,000 patterns. The spectrum of the unknown constituent was compared with the spectrum of the known constituents stored in the NIST library. The name, molecular weight and structure of the constituents of the test materials were ascertained.

3. RESULTS AND DISCUSSION

3.1 Phytochemicals present in CGSME

Calotropis gigantea plant possesses richly several bioactive compounds. Tests were done to check the presence of different phytochemicals like phenols, alkaloids, steroids, saponins, flavanoids, terpenoids, carbohydrates, tannins and glycosides. Phytochemical tests showed the presence of alkaloids, phenols, glycosides and saponins whereas steroids were not detected.

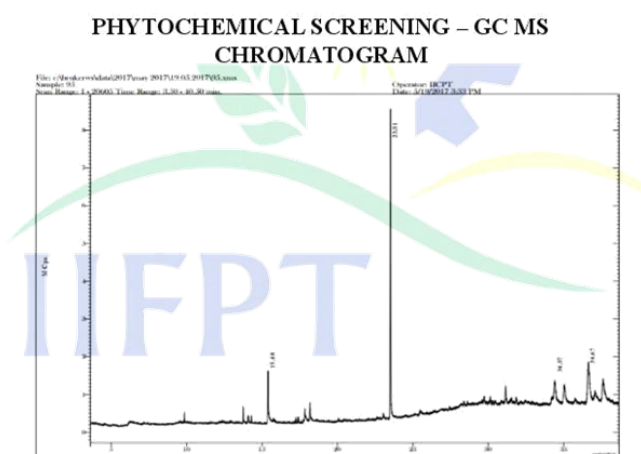
3.2 Antimicrobial Assay of CGSME

The methanolic extract of *C. gigantea* stem in this study was assessed qualitatively by measuring the diameter of the inhibition zone. To determine the zone of inhibition, Gram positive, Gram negative were analysed against standard antibiotics (ampicillin) for comparison of results. The results of this study revealed that the extracts of the plants tested had potential antimicrobial activity against Gram-positive and Gram-negative bacteria and fungal microorganisms.

CGSME demonstrated antibacterial activity against *Streptococcus* and *Bacillus* species and *Fusarium* fungal species and also the activity increased with increasing concentrations of the methanol extract of *C. gigantea* stem followed by an increase in the corresponding inhibitory zones.

3.3GC MS Analysis for Constituent Phytocompounds

Phytochemical components in CGSME by GC-MS report. The GC-MS analysis revealed the presence of 8 compounds (Table) from the methanol leaf extract of *Calotropis gigantea* (Figure 1). The GC-MS (Gas Chromatography-Mass Spectrometry) analysis of the extract discovered a number of phytocomponents with β -sitosterol as one of the major bioactive constituents for which earlier researchers have evidenced its antifungal, antibacterial properties and growth inhibitory potential against other selected insecticidal pests. The other major constituents were phenol, 2,4- bis (1,1-dimethyl)-, 3- tetradecyne, n-hexadecanoic acid, 2- (Nonyloxycarbonyl) benzoic acid, Campesterol, Stigmasta- 5, 22- dien-3-ol, acetate, (3 β)- and α -amyrin.



No.	RT	Name of Compound	Molecular Formula	Molecular Weight	Peak Area %
1	9.85	Phenol, 2,4- bis (1,1- dimethyl)-	C ₁₄ H ₂₂ O	206	1.69
2	13.76	3- tetradecyne	C ₁₄ H ₂₆	194	2.32
3	15.40	n-hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	10.98
4	23.51	2- (Nonyloxycarbonyl) benzoic acid	C ₁₇ H ₂₄ O ₄	292	66.47
5	34.37	Campesterol	C ₂₈ H ₄₈ O	400	6.08
6	35.05	Stigmasta- 5, 22- dien-3-ol, acetate, (3 β)-	C ₃₁ H ₅₀ O ₂	454	2.91
7	36.67	β -sitosterol	C ₂₉ H ₅₀ O	414	5.81
8	39.03	α -amyrin	C ₃₀ H ₅₀ O	426	3.75

CONCLUSION

This study investigates Methanolic Stem Extract of *Calotropis gigantea* for its antimicrobial properties against selected phytopathogens. CGSME demonstrated significant inhibitory effects against these bacterial and fungal phytopathogens.

GC-MS Analysis of CGSME demonstrated β -sitosterol as one of the major bioactive constituents and the other major constituents were phenol, 2,4- bis (1,1- dimethyl)-, 3- tetradecyne, n-hexadecanoic acid, 2- (Nonyloxycarbonyl) benzoic acid, Campesterol, Stigmasta- 5, 22- dien-3-ol, acetate, (3 β)- and α -amyrin.

The presence of β -sitosterol, an anti-fungal and anti-bacterial phytocompound as one of the major bioactive constituent in CGSME demonstrates the potential for antimicrobial efficacy of *Calotropis gigantea* R.Br. against phytopathogens and further for its biopesticidal application as insecticides, fungicides and nematicides.

Collaborations constituting an efficient order with pharmacologists and medical doctors, plant pathologists and microbiologists are very much crucial in influencing the complete development of an interesting lead compound into an exploitable product.

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