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Antioxidant, Antimicrobial activities and GC-MS analysis of Calotropis gigantea white flowers

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Abstract

Calotropis gigantea white (Asclepiadaceae), is a weed plant commonly known as giant milkweed. It has one of the important traditional medicines to treat various ailments. The aim of this study to screen the phytochemicals present in the flower extract by GC-MS analysis. The results showed the presence of phytochemicals of alkaloids, tannins, phenol, flavonoids, sterols, anthraquinones, proteins and quinones in the flower extract. The GC-MS analysis of the extract revealed the presence of 4 major compounds. This study forms a basis of biological characterization and the importance of the compounds identified and creates many bioactive ingredients to treat many diseases.

Keywords: *Calotropis gigantea*, Flowers, GC-MS analysis, Antioxidant, Antimicrobial.

Introduction

Scientific investigations of medicinal plants have initiated in many countries because of their contributions to health care. The primary benefits of using plant based medicines are relatively safer than synthetic alternatives, offering marked therapeutic benefits and more affordable treatment. [1] Thus, over 50% of these modern drugs are of natural product origin and these natural products play an important role in the drug development in the pharmaceutical industry. [2] In general, bacteria have the genetic ability to transfer and gain resistance to drugs used as therapeutic agents. The only way to prevent antibiotic resistance is by using new compounds which have not based on the existing synthetic anti-microbial agents.[3] To promote the use of medicinal plants as potential sources of anti-microbial compounds, it is important to thoroughly find out their composition and activity and thus confirm their use.[4]

Calotropis gigantea white commonly known as Mudar Yercum widely distributed in Eastern and southern parts of India, The plants produce white or violet colored flower in bunches, much branched, tall, erect, large and enduring with latex throughout. Ethnobotanical and common plants serve as a rich of natural drugs for research and development.[5] *Calotropis gigantea* white is a weed plant commonly known as giant milkweed. It has scientifically reported for several medicinal properties viz. the flowers have reported to take over analgesic activity, anti-microbial and cytotoxic activity.[6] Leaves and areal parts of the plant have reported for anti-diarrheal activity[7] and anti-bacterial activity,[8] anti-oxidant activity.[9] Roots have reported to contain anti-pyretic activity.[10] Various chemical constituents have reported from different parts of the plant; calotropin, frugoside and 4′-O-β-D-glucopyranosyl frugoside has isolated from the roots of *Calotropis gigantea*. The wax contains β-amyrin and its isovalerate, calotropeols- a and b, mixture of tetracyclic triterpene, traces of sterols, C31 and C33 hydrocarbons, fatty acids and giganteol have isolated from the stem bark of *C. gigantea*. The leaf contains ascorbic acid, o-pyrocatechic acid and also contains β-amyrin, taxasterol, tarasterol and β-sitosterol. Two new cardenolides, 19-Nor and 18, 20-epoxy-cardenolides were isolated from the leaves of *C. gigantea*.[11-14]

In the present study, *C. gigantea* white flowers extract has evaluated for anti-oxidant and anti-microbial activity against different micro organisms by *in vitro* conditions. Further GC-MS analysis has carried out to identify the bioactive constituent present in the white flowers of this plant.

Materials and Methods

Plant material

Fresh flowers of *Calotropis gigantea* white growing wild have randomly collected in December-2013 from the Siva temple in Kumbakonam, Thanjavur District, Tamilnadu (India) and authenticated by Prof. N. Ramakrishnan, (Department of Botany) and voucher specimens (GACBOT-209) has deposited in the Herbarium of the Department of Botany, Government Arts College (Autonomous), Kumbakonam, Bharathidasan University, India.

Extraction and fractionation

The dried flowers of *C. gigantea* white extracted with 90% methanol (MeOH) (4 X 500 ml) under reflux. The methanol extract (76 g) has subjected to column chromatography with silica gel (60-120 mesh) as the stationary phase. The charged column has then eluted with different solvents of chloroform (4 x 250 ml) and ethyl acetate (4 x 250 ml) to yield several sub fractions. The fractions have collected and the solvent recovered by simple distillation and has concentrated *in vacuo* and left in an ice-chest for a week. The residue from chloroform fraction (37 g) and ethyl acetate (19.4 g) has taken up in Me_2CO and left in an ice-chest for two days when a brown solid separated and recrystallized from hot methanol.

GC-MS analysis

GC-MS analysis of $CHCl_3$ and EtOAc fractions have performed on a Hewlett Packard HP 6890 Gas Chromatography with Hewlett Packard 5973 mass spectrometer system equipped with a DB-5 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature has programmed from 70-240°C at the rate of 5°C/min. The ion source has set at 240 °C and electron ionization at 70 eV. Helium used as the carrier gas at a flow rate of 1 mL/min. Scanning range was 35 to 425 amu. Interpretation of the mass spectrum of the unknown part has compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials have found out.

DPPH free radical scavenging test

The DPPH free radical is a stable free radical, which has widely accepted as a tool for estimating free radical-scavenging activities of antioxidants.[15] Hydrogen or electron donation capacities of the compounds have measured from the bleaching of the purple-colored methanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This spectrophotometer test uses the stable radical DPPH as a reagent. The sample solution of material (50 µL) at four concentrations (1.0, 0.5, 0.25 and 0.125 mg / mL) has mixed with freshly prepared methanolic solution of DPPH (634 µM) and allowed to stand for 30 min at room temperature. The absorbance has measured at 515 nm using a spectrophotometer and inhibits free radical DPPH in percent (%) has calculated using the formula below:

The percent of inhibition of DPPH reduction (decolourization)

$$\% \text{ of inhibition} = \frac{A0 - Asample}{A0} \text{ X } 100$$

where (A_0) is absorbance of control (blank) and (A_{sample}) is absorbance of test compound. The compound concentration showing 50% inhibition (IC_{50}) has calculated from the plot of inhibition percentage against sample concentration. Tests have been carried out in triplicate. Samples and DPPH dissolved in methanol. L-ascorbic acid has used as positive control.

Antimicrobial activity

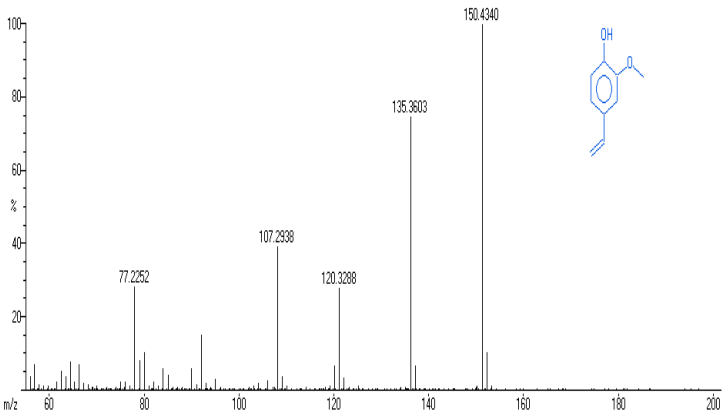
The antimicrobial activity test has carried out in the following variation of the method originally described by Bauer et al. [16] Muller Hinton ager has prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45˚C. The cooled media have poured on to a sterile petri plates and allowed for solidification. The plates with the media have seeded with the respective microbial suspension using sterile swab. The plant extracts have prepared at different dose individually placed in the each petri plates, discs and placed control and standard (Streptomycin and Amphotericin) discs. The plates have incubated at 37˚C for 24 hrs. After incubation period, the zone of inhibition surrounding the discs has measured using a transparent ruler and the diameter recorded in mm.

Statistical analysis

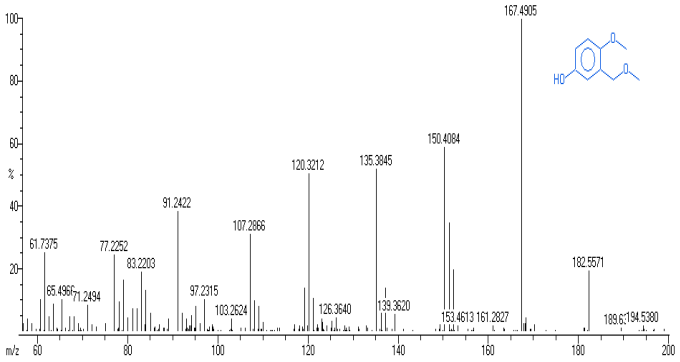
The experimental results has expressed as statistical comparisons of Mean ± SEM carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple Comparisons Test. P values less than 0.05 has considered as statistically significant.

Results and Discussion

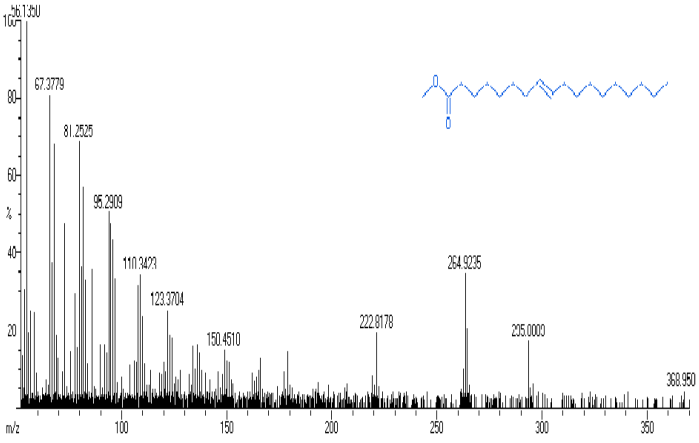
The active principles with their retention time (RT), molecular formula, molecular weight (MW), components present in the chloroform and ethyl acetate fraction of *Calotropis gigantea* white flowers have identified by GC-MS analysis (Figure 1 & 2). The GC-MS spectrums showed a molecular ion peak at m/e 150.434, 167.490, 295.080, and 150.468 follow a molecular formulas of $C_9H_{10}O_2$ (MW 150.00), $C_9H_{12}O_3$ (MW 168.00), $C_{19}H_{36}O_2$ (MW 296.00), $C_{16}H_{15}BrN_2O_3$ (MW 151.00) have identified three compounds in the chloroform extract namely (1) 2-methoxy-4-vinylphenol (RT 10.43), (2) Phenol-4-methoxy-3-(methoxy methyl) (RT 11.38), (3) 8-octadecenoic acid, methyl ester (E) (RT 19.00) and only one compound in the ethyl acetate extract namely (4) Benzhydrazide, 4-methoxy-N2-(5-bromo-2-methoxy benzylideno) (RT 2.37).



(1) 2-methoxy-4-vinylphenol (RT 10.43)

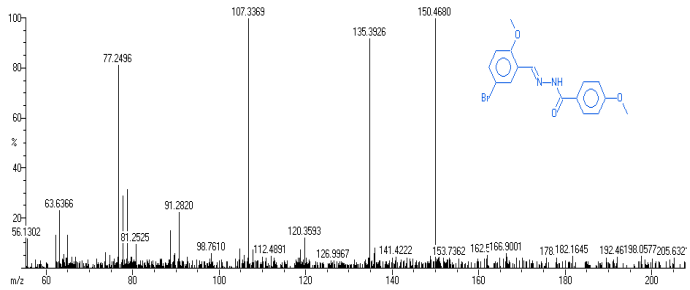


(2) Phenol-4-methoxy-3-(methoxy methyl) (RT 11.38)



(3) 8-octadecenoic acid, methyl ester (E) (RT 19.00)

Figure 1: GC-MS identification of compounds present in chloroform extract of *Calotropis gigantea* white



(4) Benzhydrazide, 4-methoxy-N2-(5-bromo-2-methoxy benzylideno) (RT 2.37)

Figure 2: GC-MS identification of compounds present in ethyl acetate extract of *Calotropis gigantea* white

Anti-oxidant activity

The anti-oxidant activity of *C. gigantea* white flowers extract in solvents of varying polarity has measured with hydrogen granting or radical scavenging ability, using the stable radical, DPPH. The method has based on reduce alcoholic DPPH solutions in the presence of a hydrogen granting anti-oxidant. The DPPH free radical scavenging activity of the Chloroform extract of *C. gigantea* white showed the highest scavenging activity (% inhibition 89.27, 86.71, 79.82 and 75.90 at 1.0, 0.5, 0.25 and 0.125 mg / ml respectively), followed by ethyl acetate extract. Methanol extract showed least DPPH radical scavenging ability with % inhibition 83.14, 75.42, 72.66 and 52.52 at 1.0, 0.5, 0.25 and 0.125 mg / ml respectively. The results of the free radical scavenging activity of *C. gigantea* white assessed by DPPH test and amount of the sample needed for 50% inhibition of free radical activity, IC_{50} values has summarized in Table 1. Lower IC_{50} value suggests higher anti-oxidant activity. Based on the results found the anti-oxidant activity of *C. gigantea* white methanol extract (IC_{50}: 93.73 µg / ml) has comparable with standard anti-oxidant of L-ascorbic acid.

Table 1: DPPH free radical scavenging activity of the flower extracts of *Calotropis gigantea* white extracts

Concentration (mg / ml)	**IC_{50} (µg/ml)**				
Samples	**1.0**	**0.5**	**0.25**	**0.125**	**IC_{50}**
	Radical scavenging effect (%)				
C. gigantea white chloroform extract	89.27 ± 0.01	86.71± 0.06	79.82 ± 0.70	75.90 ± 0.05	130.15 ± 0.01
C. gigantea white ethyl acetate extract	87.41 ± 0.15	82.08 ± 0.05	76.36 ± 0.01	65.91 ± 0.21	106.08 ± 0.60
C. gigantea white methanol extract	83.14 ± 0.70	75.42 ± 0.15	72.66 ± 0.02	52.52 ± 0.04	93.73 ± 0.20
L-ascorbic acid	98.00 ± 0.02	95.62 ± 0.20	94.80 ± 0.01	89.01 ± 0.15	70.05 ± 0.05

Values are expressed in Mean ± Standard Deviation (M±SD)

Antimicrobial activity

Anti-microbial disease is becoming common in South Asia, because of development of anti-microbial drug resistant pathogens. To resolve the problem and to detect alternative chemotherapeutic agents, the search for novel forms from newer sources is global challenges.[17] Results held in the present study revealed the plant extracts tested have potential anti-microbial activity against both Gram positive and Gram negative micro-organisms. The anti-microbial activity from the plant *C. gigantea* white examined in the present study has assessed qualitatively by measuring the inhibition zone diameters. For determining zone of inhibition, pure Gram-positive, Gram-negative and fungal strains have taken as a standard antibiotic (Ciprofloxacin, Amphotericin - B) for comparison of the results. All the test samples have screened for their anti-bacterial and anti-fungal activities against the *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* and the fungi *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*. The sets of dilutions (50 µg / ml) of test

compounds and standard drugs (Ciprofloxacin 10 µg / ml and Amphotericin B - 10 µg / ml) have prepared in double distilled water using nutrient agar tubes. The results of the anti-bacterial and anti-fungal activities have presented in Table 2.

Table 2: Inhibition zones formed by *Calotropis gigantea* white extracts and Standard antibiotics

S. No.	Microorganisms	Diameter of inhibition zones (mm / 50 µL) (M±SD)			
		Chloroform Extract	Ethyl acetate Extract	Methanol Extract	Standard (10 µg)
1	*Staphylococcus aureus*	30 ± 0.04	27 ± 0.07	21 ± 0.12	32 ± 0.65*
2	*Escherichia coli*	16 ± 0.01	14 ± 0.60	12 ± 0.04	19 ± 1.98*
3	*Klebsiella pneumonia*	18 ± 0.02	15 ± 0.05	14 ± 0.12	21 ± 1.22*
4	*Candida albicans*	19 ± 0.67	16 ± 0.02	15 ± 0.20	22 ± 1.60**
5	*Aspergillus niger*	10 ± 0.05	08 ± 0.60	06 ± 0.01	12 ± 1.04**
6	*Aspergillus flavus*	09 ± 0.01	06 ± 0.70	04 ± 0.04	12 ± 1.50**

Used concentrations: 50 µL of 10 mg / mL of *Calotropis gigantea* extracts Bacteria Standard* - Ciprofloxacin (10 µg); Fungal Standard** - Amphotericin - B (10 µg) Values are expressed in Mean ± Standard Deviation (M±SD) (n=3)

Among the samples used, widest zones of inhibition has observed at chloroform and ethyl acetate extracts showed 16 ± 0.01 and 14.0 ± 0.60 mm zone of inhibition against *E. coli* while *C. gigantea white* methanol extract showed significant inhibitory activity of 12 ± 0.04 mm against *E. coli*. On the other hand the tested compound at chloroform extract showed higher activity of 30 ± 0.04 mm inhibition zones against *S. aureus* and followed by ethyl acetate extracts showed 27 ± 0.07 mm inhibition zones against *S. aureus* (Table 2). The *C. gigantea* white methanol extract showed maximum inhibition zone of 21 ± 0.12 mm against *S. aureus*. Both chloroform and ethyl acetate extracts showed better activity than methanol extract of white *C. gigantea* white. Of the tested organisms, *S. aureus* has highly resistant followed by *E. coli*. In general, the activity of plant extracts is high on Gram-positive organism when compared to Gram-negative organism. This finding has already reported[18, 19] and could explain by the different cell wall structures of these bacteria. Gram-negative bacteria have an outer phospholipidic membrane with structural lipopolysaccharide components which have not found in Gram-positive bacteria. The ability of the extracts to reveal anti-bacterial activity against the bacteria suggested the presence of hydrophilic and hydrophobic antibacterial compounds.[20] But the present study revealed a controversy report that gram-negative bacteria have more susceptible to the test compound than gram-positive bacteria. It may be due to the presence of broad spectrum of anti-microbial compounds in the flowers of *C. gigantea* white.

The anti-fungal activities of the test compounds compared with standard drugs, the results revealed that in the chloroform and ethyl acetate extracts for fungal activity has more sensitive compared with *C. albicans* shows good result as compare with *A. niger* and *A. flavus*. The growth inhibition zone measured ranged from 4.0 to 19.0 mm for fungal strains. The results show the chloroform, ethyl acetate and methanol extract of *C. gigantea* white flowers have found to be more effective against all the microbes tested. The results have compared with standard antibiotic drugs.

Conclusion

Calotropis gigantea white has used as a very famous traditional folk medicine by many cultures, and it had the subject of extensive phytochemical and bioactive investigations, its chemical components and bioactivities have not been completely investigated yet. In our present study a new phytochemicals have identified from the genus of *C. gigantea* by GC-MS. The DPPH free radical scavenging activity of the chloroform extract of *C. gigantea* white showed the highest scavenging activity followed by ethyl acetate extract and this screening work, test compounds found to be not idle against any organism, such as Gram-positive, Gram-negative, and fungal strains have resistant to all the extracts of *C. gigantea* white.

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