Gouri Sankar K. *et al.* / *International Journal of Biological & Pharmaceutical Research.* 2014; 5(4): 308-312.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological
&
Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

ANTI-MICROBIAL INVESTIGATION AND PHYTOCHEMICAL ANALYSIS ON ORGANIC SOLVENT LEAF EXTRACTS OF *CALOTROPIS GIGANTEA*

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ABSTRACT

The leaves extract of *Calotropis gigantea* were screened for its anti microbial and phytochemical activities. The solvents used for the leaves extraction were Acetone, Chloroform and Petroleum ether. The extract was tested against infectious diseases causing fungal pathogens such as *Aspergillus niger, candida albicans Aspergillus fumigatus* and bacterial pathogens such as *Bacillus cereus, Proteus vulgaris*, and *Escherichia coli* using the Agar well diffusion method. The Acetone extract of *Calotropis gigantea* showed more activity against fungus like *Candida albicans* zone of diameter 14.5mm, and bacteria like *Escherichia coli* zone of diameter 17.5mm, when compared to other solvent extracts. The results confirmed that presence of Antifungal and Antibacterial activity in the shade dried extract of *Calotropis gigantea* against the human pathogenic organisms.

Key Words: Organic solvent, Leaf Extract, Phyto-chemical Analysis, Antimicrobial Activity.

INTRODUCTION

Calotropis gigantea commonly known as milkweed or swallow-wort belongs to the family *Asclepiadaeae* a shrub about 6 M high which includes 280 genera and 2,000 species is widely distributed in Eastern and southern parts of India, Ceylon, Eastern Asia and other parts of tropics. In India the genus is represented by two species. Viz., *Calotrpis gigantea* and *Calotropis procera*. The plants produces white or violet coloured flower in bunches, much branched, tall, erect, large and perennial with latex throughout (Baskaran C *et al.,* 2012). Different plant parts have shown biological activities viz., antipyretic, anti-inflammatory, wound healing, analgesic, antidiarrhoeal, antioxidant and as an anti-diabetic, antinociceptive, fibrinogenolytic, anti-coagulant. Latex has good ovicidal and larvicidal properties etc (Chandrabhan S *et al.,* 2011). The prevalence of invasive, opportunistic microbial and fungal infections has increased at an alarming rate especially in immune-compromised individuals. Although it appears to be a great array of antimicrobial and antifungal drugs, there is at present a quest for new generations of antimicrobial and antifungal compounds due to the low efficacy, side effects or resistance associated to the existing drugs (Falguni K *et al.*, 2011; Juncker T *et al.*, 2009). This plant has potential antimicrobial properties against microbial infections. Commercially available antimicrobial agents (antibiotics) are now used to treat diseases arising from microbial infections (Krishnaraj C *et al.*, 2010; Murti Y *et al.*, 2010). A major problem encountered with antibiotics in clinical use is drug resistance, which mostly leads to treatment failure. Other problems with antibiotics include toxicity, high cost, low cost efficacy, etc (Nenaah EG *et al*, 2011; Okiei W *et al.,* 2009). This necessitates a continuous search for new antimicrobial agents. Medicinal plants have no doubt remained the major sources of traditional medicine worldwide. This study attempts to determine the phytochemical analysis and antimicrobial effect of

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Calotropis gigantea. In this report, we provide new information on the antimicrobial activities of *C. gigantea* using known microbial pathogens as tested organisms**.**

MATERIALS AND METHODS

Collection of Plant Material

Calotropis gigantea leaf were collected from botinical garden in vagdevi college of pharmacy, Gurazala, Guntur(Dt), Andhra Pradesh, India and used for this study.

Extraction of plant material

Collected leafs were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were shade dried then made into fine powder, this powdered samples (100g/100ml) in Acetone, chloroform and petroleum ether for overnight at room temperature., soxhlet apparatus are used for this extraction. The extract from these solvents are soaked and evaporated under pressure. The leaf extracts were concentrated at 50°C and the residue obtained was stored at 4°C.

PHYTOCHEMICAL ANALYSIS OF THE EXTRACT

Specific qualitative tests were performed to identify bioactive compounds of pharmacological importance through standard methods. In brief, the phytochemicals such as tannins, alkaloids, saponins, flavonoids, terpenoids, and phenols/polyphenols were qualitatively determined as following:

Test for alkaloids (mayer's test)

2.0ml of extract was measured in a test tube to which picric acid solution was added. The formation of orange coloration indicated the presence of alkaloids.

Test for cardiac glycosides (keller-killani test)

5ml of plant extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer which shows the presence of Cardiac glycosides.

Test for tannins

The substance (extracts) mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of Tannins.

Test for saponins

Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H2SO4. Formation of yellow color observed in each extract indicated the presence of flavonoids.

Test for Steroids

One gram of the test substance (plant extracts) was dissolved in a few drops of acetic acid. It was gently warmed and cooled under the tap water and a drop of concentrated sulphuric acid was added along the sides of the test tube. Appearance of green colour indicates the presence of Steroids.

Test for terpenoids (salkowski test)

5ml of each plant part extract was mixed in 2 ml of chloroform, and concentrated H2SO4 (3ml) was carefully added to form a layer. Formation of reddish brown coloration at the interface shows the positive results for presence of terpenoids.

Test for Reducing Sugars

One gram of the aqueous extract was weighed and placed into a test tube. This was diluted using 10 ml of de-ionised distilled water. This was followed by the addition of Fehling's solution. The mixture warmed to 40ºC in water bath. Development of brick-red precipitate at the bottom of the test tube was indicative of the presence of a reducing sugar. Same procedure was repeated using dimethylsulphoroxide (DMSO) as the diluent for the ethanolic extract.

Test for Resins

Two grams of the ethanolic extract was dissolved in 10ml of acetic anhydride. A drop of concentrated sulphuric acid was added. Appearance of purple colour, which rapidly changed to violet, was indicative of the presence of resins. Same procedure was repeated using the aqueous extract of the plant material.

Test organisms

The bacterial species used for the test were *Bacillus cereus, Salmonella typhi, Proteus mirablis, Escherichia coli* and *Pseudomonas aeruginosa.* The fungus species used for the test were *Aspergillus flavus, Aspergillus niger, Aspergillus fumigates, Candida tropicalis,* and *Candida albicans.* All the stock cultures were obtained from biotechnology lab, vagdevi college of pharmacy, Gurazala, Guntur(Dt), Andhra pradesh, India.

Antibacterial activity

The leaf extracts obtained above were screened

for their antibacterial activity in comparison with standard antibiotic ciprofloxacin (100mg/ml) in vitro by well diffusion method. Lawn culture was used using the test organism on Muller Hinton Agar (MHA). The inoculated plates were kept aside for few minutes using well cutter, four wells were made in those plates at required distance. In each step of well cutting the well cutter was thoroughly wiped with alcohol. A fixed volume (0.1ml) of the *Calotropis gigantea* extract was then introduced into the wells in the increasing concentration. The plates with bacteria were incubated at 37˚c for 24 hours. The activity of the extract was determined by measuring the diameters of zone of inhibition (Sharma AK *et al.,* 2011).

Antifungal Activity

The leaf extracts were also screened for their antifungal activity in comparison with standard antibiotic Ketoconazole (10mg/ml) in vitro by well diffusion method. Lawn culture was prepared using the test organism on Sabouraud's Dextrose Agar (SDA). The inoculated plates were kept aside for few minutes using well cutter, four wells were made in those plates at required distance. A fixed volume (0.1ml) of the *Calotropis gigantea* extract was then introduced into the wells in the increasing concentration. The plates with fungi were incubated at room temperature for 48 hours. The activity of the extract was determined by measuring the diameters of zone of inhibition (Subramanian SP *et al.*, 2010; Watkins John V *et al.*, 2005; Yesmin MN *et al.,* 2008).

RESULTS AND DISCUSSION

Phytochemical Analysis of Bioactive Compound In Different Solvent Extracts Of Calotropis Gigantea

The plant leaf extracts in different solvents were screened for the presence of various bioactive phytochemical compounds. The analysis revealed the presence of cardiac glycosides, saponins, flavonoids, steroids, and terpenoids in most prominent amount while alkaloids and tannins is less amount. Resins absent in organic solvents. These were documented in Table 1.

Antibacterial Efficacy Analysis of Different Organic Solvent Extracts Of Calotropis Gigantea

Antibacterial efficacy of different solvent extracts of *Calotropis gigantea* is shown in the table2. The Acetone extract have shown better activity against these pathogenic organisms. Acetone extract was more effective against *Escherichia coli*. Among these three extracts Acetone shows better activity than Chloroform and petroleum ether against the standard drug Ciprofloxacin. The results of antibacterial activity are shown in the table 2 and figure1.

Antifungal Efficacy Analysis of Different Organic Solvent Extracts Of Calotropis Gigantea

Antifungal efficacy of different solvent extracts of *Calotropis gigantea* is shown in the table3. The Acetone extract have shown better activity against these pathogenic organisms. Acetone extract was more effective against *Candida albicans.* Among these three extracts Acetone shows better activity than Chloroform and petroleum ether against the standard drug Ketoconazole. The results of antifungal activity are shown in the table 3 and figure2.

Antimicrobial activity of different solvent extracts of *C. gigantea* showed varying degrees of antibacterial and antifungal activity against all microorganisms tested. There are many reports of plants in the family Asclepiadaceae possessing anti-microbial activity. From this study it can be said that, Acetone shade dried leaf extract of *Calotropis gigantea* showed wide range of Antibacterial and Antifungal activity can be used and administered in the ethno medical practice. The present study has shown a spectrum of antimicrobial activities which provides a support to some tradition uses of these few medicinal plants. But the effective biomolecules which act as antimicrobial have to be identified isolated and subjected to extensive scientific and pharmacological screening that can be used as sources for new drugs.

Table 1. Phytochemical Screening

Phytochemicals	Acetone extract	Chloroform extract	Pet.ether extract
Alkaloids	+	+	+
Cardiac Glycosides	+	+	+
Saponins	+	+	+
Tannins	+	+	-
Flavnoids	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Reducing Sugars	+	-	-
Resins	-	-	-
Anthraquinone	-	-	-

(+) = Positive (-) = Negative

Table 2. Inhibition zone diameter different extracts of *calotropis gigantea* against different bacteria (mean) (mm)

S.No	Microorganisms	Ciprofloxacin	Acetone extract	Chloroform extract	Petroleum ether extract
1	Bacillus cereus	21.91	14.22	10.1	8.6
2	Proteus vulgaris	18.06	8.4	6.8	4.2
3	Escherichia coli	26.32	17.15	12.05	10.7

Table 3. Inhibition Zone Diameter Different Extracts of *Calotropis Gigantea* Against Different Fungus (mm)

S.No	Microorganism	Fluconazole	Acetone	Chloroform	Petroleum ether
1	Aspergillus niger	17.2	11.3	8.1	5.2
2	Candida albicans	16.4	14.5	6.7	3.1
3	Aspergillus fumigatus	18.7	13.8	9.2	4.4

Fig 1. Antibacterial activity of different solvent leaf extracts of *Calotropis gigantea* against different organisms

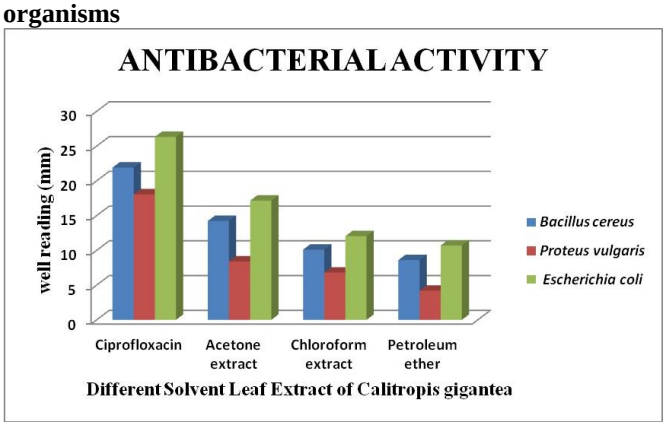
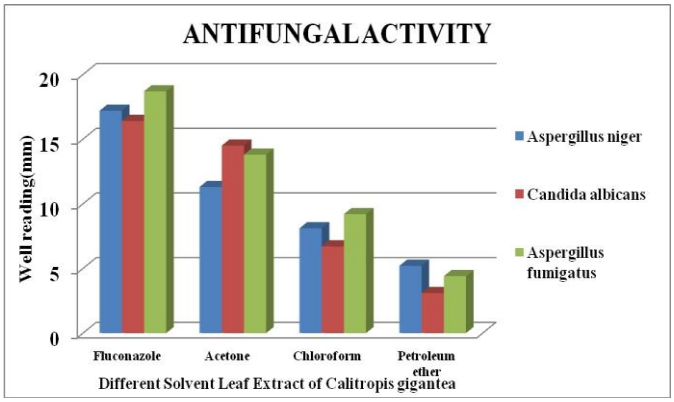


Fig 2. Antifungal activity of different solvent leaf extracts of *Calotropis gigantea* against different organisms



CONCLUSION

The phytochemical analysis revealed the bioactive compounds which are responsible for the Invitro antimicrobial of *C. gigantea* over all fungi and bacteria strains in all extracts could be alkaloids, cardiac glycoside, tannins, saponins, flavonoids, steroids, terpenoids, reducing sugar and resins. The result of this work suggests that the whole plant extract of C. gigantea has number of medicinal properties. From this work it can be said that the shade dried *Calotropis gigantea* leaf extract of Acetone has more effective against these pathogenic organisms and can be used for the future references for various other diseases.

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