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ANTI BACTERIAL ACTIVITY OF LEAF EXTRACTS OF *CALOTROPIS GIGANTEA* LINN. AGAINST CERTAIN GRAM NEGATIVE AND GRAM POSITIVE BACTERIA

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ABSTRACT

The fresh dried leaves of the plant *Calotropis gigantea* were successively extracted with chloroform, ethyl alcohol, ethyl acetate and dichloromethane using Soxhlet extractor. Aqueous extract was prepared by cold maceration method. Well plate method was employed to determine the antibacterial activity against certain Gram positive bacteria like *B.subtilis* NCIM 2063, *Micrococcus luteus* NCIM 2704, *Staphylococcus aureus* NCIM 2079 and Gram negative bacteria namely, *K.pneumoniae* NCIM 2719, *P. vulgaris* NCIM 2027 and *E.coli* NCIM 2118. Ethyl acetate and dichloromethane extracts showed better and broader spectrum of activity when compared to other extracts. Ciprofloxacin (10 µg/well) was used as the standard antibacterial agent.

Key words: *Calotropis gigantea*, Well plate method, Antibacterial activity, Ciprofloxacin.

INTRODUCTION

Herbal medicines, also referred to as botanical medicine or phytomedicine, include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients. The World Health Organization has estimated that 80% of people in some Asian and African countries rely on herbal medicines for some part of their primary health care. Finding healing powers in plants is an ancient idea, especially in India. Currently 25 to 50% of all pharmaceuticals dispensed around the world are of plant origin and only few among them have been used as antimicrobials.

Ethnopharmacologists, botanists, microbiologists and natural-products chemists have been exploring the earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. Plants are rich in a wide variety of secondary metabolites,

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such as tannins, terpenoids, alkaloids and flavonoids, which have been found to have *in-vitro* antimicrobial properties. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited. New sources, especially plant sources, are also being investigated. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. The substances present in the plants serve as plant defence mechanism against predation by microorganisms, insects and herbivores[1].

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 is pale. The leaves are obovate or oblong, 10 to 20 centimetres 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[2].

Based on folklore claims, the present study was aimed to evaluate the antibacterial activities of *C.gigantea* leaf extracts against prominent human pathogenic bacteria by well diffusion method. Besides, the leaf extracts has also been qualitatively analyzed for the presence of different phytochemicals using standard test procedure.

EXPERIMENTAL

Materials and methods

Collection and preparation of samples

The plant *Calotropis gigantea* L. was collected and authenticated in the Department of Botany, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Voucher specimen (BSI/SC/5/23/10-11/Tech, Dated 13.04.2010) the leaves of *Calotropis gigantea* L. were collected during the month of March, 2010 from Coimbatore district of Tamil Nadu.

Aqueous extract

Leafs (100 gm) of plant were thoroughly washed, blotted dry and macerated with 100 mL distilled water in a blender. The macerate was first filtered through double layered muslin cloth and then centrifuged at 4000 rpm for 30 min. The supernatant was filtered through Whatmann No. 1 filter paper. This served as the mother extract.

Solvent extract

Thoroughly washed mature leaves were shade dried for 20 days and then powdered with the help of a blender. Twenty-five grams of the powder was filled in the thimble and extracted successively with chloroform, ethyl alcohol, ethyl acetate and dichloromethane using a Soxhlet extractor for 48 h. All extracts were concentrated by evaporating the solvent at 30°C and preserved at 5°C in airtight light resistant bottle until further use.

Phytochemical screening of *C. gigantea* leaf extract

The phytochemical screening of the extracts of *C.gigantea* was performed qualitatively for the presence of alkaloids, glycosides, flavonoids, tannins, saponins, sterols and triterpenes using standard procedure as reported earlier[3].

Procedure

All the extracts were subjected to antibacterial activity against the test organisms by cup plate method[4]. Mueller Hinton agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37^{o}C before inoculation.

The organisms were inoculated in the plates prepared earlier, by dipping a sterile swab in the previously standardized inoculums, removing the excess of inoculums by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times, rotating the plates through an angle of 60^{o} after each application. Finally the swab was pressed round the edge of the agar surface. It was allowed to dry at room temperature, with the lid closed. The wells were made by using sterile cork borer (8 mm diameter). 30 µL of saturated solution of the extracted drug, standard drug and solvent blank were placed on the respective wells and kept in the refrigerator for one hour to facilitate uniform diffusion. The plates were incubated at 37°C for 24 h.

RESULTS AND DISCUSSION

The literature survey on the botanical and ethnopharmacological aspects on *Calotropis gigantea* Linn. revealed availability of limited information[5]. The results of our study, focused on these aspects are presented below.

Antibacterial screening

The antibacterial activity study was carried out using cup plate technique because

this method allows better diffusion of the extracts into the medium. Ethyl Acetate extract showed better antibacterial activity against the tested strains than other extracts. The bacterial strains used in this present study were chosen on the basis of their clinical importance. For example *S. aureus* is the most common species found in wound. It acquires resistance due to the presence of penicillin-binding protein of high molecular weight and has very low affinity for β-lactam antibiotics[6].

Table 1: Anti-bacterial activity of leaf extracts of *C. gigantea* Linn.

Samples tested	Diameter of zone of inhibition (mm) n = 3					
	Gram positive bacteria			Gram negative bacteria		
	Staphylococcus aureus	***Micrococcus luteus***	***B. subtilis***	***E. coli***	***P. vulgaris***	***K. pneumoniae***
Chloroform extract	-	-	12 ± 0.48	-	-	15 ± 0.12
Ethanolic extract	-	-	-	-	-	18 ± 1.13
Ethyl acetate extract	13 ± 0.35	11 ± 0.67	15 ± 0.78	15 ± 0.67	14 ± 0.43	-
Dichloromethane extract	18 ± 0.67	12 ± 0.59	14 ± 0.48	-	18 ± 1.23	-
Aqueous extract	-	-	-	-	-	-
Ciprofloxacin	27 ± 0.56	23 ± 0.12	28 ± 0.43	25 ± 0.45	27 ± 0.34	28 ± 0.12
Solvent	-	-	-	-	-	-

(-) Indicates no activity; Values are mean ± S.E.M (n = 3)

From the results it is evident that the ethyl acetate and dichloromethane extracts showed better and broader spectrum of activity compared to other extracts. Ethanolic extract was active against one Gram negative bacteria. Chloroform exhibited moderate activity against one Gram negative bacteria and one Gram positive bacteria. The solvent blank and aqueous extract did not show any activity against tested bacteria.

The rapid emergence of resistance to antibiotics amongst pathogens generates visions of the 'potential post-antibiotic era threatening present and future medical advances'. In view of the crossover of resistance across related compounds the future can see sharply depleting antibiotic resources. Laboratories around the world have literally screened thousands of phytochemicals having *in vitro* inhibitory effect against a wide

spectrum of microbes. In most of the screened plant extracts, the most active fraction is known but individual active compounds are not characterized. An interesting observation is that majority of the active crude extracts and their fractions are almost equally active both against drug resistant and sensitive bacterial strains. Multi target based approaches of screening of medicinal plant extracts and herbal drugs are expected to yield novel activities[7,8].

The prepared extracts of *Calotropis gigantea* showed activity comparable with standard drug Ciprofloxacin inhibiting the growth of most of the above tested bacterial strains. The demonstration of antibacterial activity of *C. gigantea* extract against both Gram positive and Gram negative bacteria may be an indicative of the presence of broad spectrum antibacterial components. This indicates that the plant may be a useful source for the development of novel antibiotics against pathogenic bacteria. The extract did not exhibit any activity against the tested fungal strains.

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