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RESEARCH ARTICLE PHARMACOLOGY

PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF *CALOTROPIS PROCERA* (AIT.) R. BR. FLOWERS

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ABSTRACT

Phytochemical screening and antimicrobial activity of chloroform, acetone and methanolic extracts of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae) flowers were tested against various pathogens such as *Salmonella paratyphi, S. paratyphi A, Bacillus subtilis, Bacillus thuringiensis, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* Phytochemical screening recorded positive results for alkaloids, tannins, steroids, glycosides, saponins, phenols and flavonoids. Among which flavonoids and steroids showed maximum degree of precipitation. Negative results are obtained for fixed oils. Maximum antibacterial activity was showed against *Staphylococcus aureus* and *Bacillus subtilis.*

KEYWORDS

Calotropis procera, antibacterial activity, pathogens, MIC

INTRODUCTION

Infectious diseases are world's leading cause of premature death, killing 50,000 people every day[1]. Resistance to antimicrobial agents is emerging in a wide variety of pathogens and multiple drug resistance is becoming common in diverse organisms[2]. The microbial resistance is growing day by day and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, measures to be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient[3]. This has necessitated a search for new antimicrobial substances from other sources including plants.

Many higher plants accumulate extractable organic approaches substances in quantities sufficient to be economically management of disease. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. It is estimated that only one per cent of 2,65,000 flowering plants on earth have been studied exhaustively for their chemical composition and potential against important medicinal value[4]. There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants[5]. It is well known that plants, although lacking the typical immune response, have an in-built system for protection against biotic and abiotic stress conditions. Since plants have co-evolved with pathogens, they understandably have also developed the chemical protection pathways against the parasitic organisms. Therefore, it is reasonable to expect a variety of plant compounds with specific as well as general antimicrobial activity and antibiotic potential[6]. Over the past 20 years, there has been a lot of investigation on plants as source of new antimicrobial agents. But still there is an immediate need to identify novel substances active towards pathogens with high resistance[7].

Calotropis procera (Ait.) R. Br. (Asclepiadaceae) (Plate 1), a xerophytic shrub widely distributed in the tropics of Asia and Africa, is commonly known as "Akra" in India. Almost every part of this plant is used in various forms in different parts of the world. In the indigenous systems of medicine, the flowers are used as digestive, stomachic and tonic and are useful in asthma, cold, cough and cholera[8].

MATERIALS AND METHODS

Preparation of extracts

The flowers of *Calotropis procera* L. were collected from in and around Coimbatore and shade dried then made into coarse powder. Fifty gram of powder was successively extracted with solvents like chloroform, acetone and methanol. Then the extracts were allowed to dry and stored at 4ºC.

Screening of active compounds

Phytochemical screening was carried out using standard methods [9, 10] to detect the bioactive compounds like alkaloids, tannins, phenols, steroids, glycosides, saponins, flavanoids and fixed oils.

Pathogenic strains

Bacterial strains like *Salmonella paratyphi, S. paratyphi* A, *S. paratyphi B, Bacillus subtilis, B. thuringiensis, Proteus mirabilis, P. vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* were the pathogens selected for the study. The bacteria were maintained in nutrient agar slants and stored at 4°C.

Antimicrobial activity

Antimicrobial assay was performed using disc diffusion method[11]. Under aseptic condition, the liquid nutrient agar medium for bacteria was poured in sterilized Petri plates to a depth of 4mm. After solidification of the media the strains were swabbed on the plates. The organic flower extracts with various concentrations (25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml) were used to saturate the disc (Whatman No.1, 6mm) and placed on the seeded plates. The kanamycin 30 µg/disc was used as control. The plates were incubated for 24 hrs for at 37°C. The zone of inhibition was measured in millimeter.

Determination of Minimum Inhibitory Concentration (MIC)

The determination of the minimum inhibitory concentration of the methanol extract and the dichloromethane fraction was carried out using the agar dilution method[12]. Different concentrations of the extracts were prepared to give a final concentration in the range of 10 to 0.039 mg mL^{-1}. 2 ml of each dilution was mixed with 18 ml of Mueller-Hinton agar, poured into Petri dishes and allowed to set. The agar was streaked with an overnight broth culture of the test organisms and incubated overnight. The lowest concentration inhibiting growth was regarded as the minimum inhibitory concentration of the extracts.

RESULTS AND DISCUSSION

Since, ancient times, the plants have been a veritable source of drugs. Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. The present phytochemical analyses of various solvent extracts of *C. procera* flowers revealed positive results for alkaloids, tannins, phenols, steroids, glycosides, saponins and flavonoids (Table 1).

Table 1.
Bioactive compounds of *C. procera* flowers

Bioactive Compound	Chloroform	Acetone	Methanol
Alkaloids	+	+	+
Tannins	+	+	+
Phenols	-	+	+
Glycosides	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Coumarins	-	+	+
Fixed oil			

'+' indicates presence ***' – ' indicates absence***

Antimicrobial activity

The traditional healers use primarily water as the solvent, but plant extracts prepared with methanol and ethanol as solvents provided more consistent antimicrobial activity as also reported earlier[13]. To support this view, the methanolic extract of flowers showed good inhibitory activity against all the pathogens tested (Table 2) compared to other extracts.

Table 2

Antimicrobial activity of methanolic extract of C. procera flowers

Name of the bacteria	Chloroform			Acetone			Methanol			Control (kanamycin 30 µg/disc)
	25 mg/ml	50 mg/ml	100 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Gram positive bacteria	**Zone of Inhibition (diameter in mm)**									
Staphylococcus aureus	-	-	12.3	7.6	8.3	10	9.6	15.3	20.3	26
Bacillus thuringiensis	-	-	8.3	7.3	8	9.6	8	12.6	14.6	21
B. subtilis	-	-	11	8.3	7.6	8	10.3	11	19.3	23
Gram negative bacteria										
Salmonella typhi	-	-	9	7.3	8	9	8.3	12	14.3	25
S. paratyphi A	-	10	28.6	7.6	8.3	10	8.3	11.6	14.3	22
S. paratyphi B		9.3	10	7.6	7.3	8.6	-	8	10	25
Pseudomonas aeruginosa	-	-	11.3	9	10	9	12.3	12.6	14.3	25
Proteus mirabilis	-	-	10	-	-	9	7	12.6	14.6	21
P. vulgaris	-	-	10.3	7.6	8.3	10.3	-	8	10	22
Escherichia coli	-	-	9.3	8.3	8	9.3	8	10.3	13.3	22

Among the extracts studied, the petroleum extract showed inhibition only at higher concentration. The chloroform extract exhibited moderate inhibition against all the organisms. The methanol extract showed higher inhibitory activity against *Staphylococcus aureus* and *Bacillus subtilis* and moderate inhibition against other all pathogens especially *Salmonella paratyphi* and lowest activity against *Escherichia coli*. The minimum inhibitory concentration (MIC) of methanolic extract varies from 1.7 to 4.6 mg/ml. It is evident from the above results that the flower extracts exhibit potential activity. The results confirmed the previous results of antibacterial activity of some medicinal plants against *Bacillus subtilis, Pseudomonas* sp., *Staphylococcus aureus*[14, 15].

Antibacterial effects of these extracts on *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* showed that the flowers can be used in the treatment of gastrointestinal infection and diarrhea and skin diseases[16] and they can also be used in the treatment of urinary tract infection associated with *Proteus* sp.[17]. The extracts of these plants can be used in the treatment of boils, sores and wounds, since *Staphylococcus aureus* and

Pseudomonas aeruginosa have been implicated as causative agents of these diseases[18].

The finding showed moderately high activity on gram positive than gram negative bacteria. One of the several unique characteristics of gram-negative bacteria is the outer membrane of the cell that is responsible for protecting the bacteria from destruction of the inner membrane or cell wall (peptidoglycan). Thus, outer membranes of the gram-negative bacteria provide a selective barrier to external molecules and thereby prevent the release of metabolite-binding proteins and hydrolytic enzymes (nucleases, alkaline phosphatase) found in the periplasmic space between the outer surface of the inner (plasma) membrane and the inner surface of the outer membrane[19]. As gram positive possess single layer boundary it's being attacked easily.

Antimicrobial properties exhibited by the plant could be due to presence of phytochemicals present in the plant extracts[20]. These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Tannins bind to proline rich proteins and interfere with the protein synthesis[21]. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls[22]. Coumarins are also known act against gram positive bacteria and it is produced in carrots in response to fungal infection which could be attributed to its antimicrobial activity[23]. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell[24]. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes[25].

These screening experiments provide a preliminary platform for further phytochemical and pharmacological studies that may open the possibility of finding new clinically effective antimicrobial compounds.

Table 3

Minimum inhibitory concentrations of methanolic extract of C. procera flowers

S. No.	Name of the pathogen	MIC mg/ml
	Gram positive bacteria	
1	*Staphylococcus aureus*	1.9
2	*Bacillus thuringiensis*	2.3
3	*B. subtilis*	2.1
	Gram negative bacteria	
4	*Salmonella typhi*	2.2
5	*S. paratyphi A*	2.3
6	*S. paratyphi B*	4.6
7	*Pseudomonas aeruginosa*	1.7
8	*Proteus mirabilis*	2.4
9	*P. vulgaris*	4.5
10	*Escherichia coli*	2.5

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