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Research Article

ANTIMICROBIAL ANALYSIS OF DIFFERENT PARTS EXTRACT IN DIFFERENT SOLVENT SYSTEM OF A WASTE WEED- *CALOTROPIS PROCERA*

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ABSTRACT

Objective: In the current study, we have focused on the major secondary metabolite containing parts such as flower, leaf, and root for phytochemical extraction with three different solvent systems to make a comparative study against three virulent bacteria species which are capable of intestinal infection, pneumonia, skin infections, and food poisoning.

Methods: Antimicrobial activity of ethanol, methanol, and chloroform extracts from bark, leaves and roots of *Calotropis procera*, was examined against three virulent bacteria species: *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis* using disc diffusion method.

Results: The ethanol extract of leaf showed significant activity against *S. aureus* with a zone of inhibition ranging from 14 to 20 mm for *S. aureus.* The ethanol extract of flower was effective against *E. coli* with maximum 18 mm. Ethanol extract of root showed significant activity against *S. aureus.* Methanol extract of leaves showed moderate activity against *S. aureus* with a zone of inhibition ranging from 14 to 20 mm. Methanol extract of root showed significant activity against *S. aureus* with a zone of inhibition ranging from 12 to 22 mm. Methanol extract of flowers showed activity against *E. coli* with a zone of inhibition ranging from 11 to 20 mm. The chloroform extract of leaves showed significant activity against *S. aureus*. Chloroform extract of flower showed activity with zone of inhibition ranging from 11 to 17 mm for *S. aureus* chloroform extract of root showed activity against *E. coli* with zone of inhibition ranging from 9 to 17 mm.

Conclusion: From the above study, it can be concluded that the activity of the plant extract may be due to the secondary metabolites or broad-spectrum antibiotic compounds present in it.

Keywords: *Calotropis procera,* Antimicrobial, Weeds.

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INTRODUCTION

"Weeds" are the valueless plant that grows wildly. A plant is named to be weed when it is found abundantly or due to lack of knowledge about its significant value. But being a plant biologist baptizing a plant with such terms is a matter of regret. Now it is a challenge for every researcher to acknowledge human society with the significant value of every individual plant. Day-by-day trees are getting poached for urbanization and a very limited number of plants are seen in urban areas. In such places, the weeds are the only source of green view due to nature's green view, act as vision therapy for eyes. There are some weeds used for human consumption [1] and cattle fodders. Weeds have high pharmaceutical values [2] such as *Tridax procumbens* have antimicrobial [3,4], antiseptic, insecticidal, parasiticidal [5], and anticancerous activity [6] as its rich in secondary metabolites.

Calotropis procera or milk weed (high latex content) belongs to the family of Apocynaceae are consider as a common weed in many parts of the world. Its vegetation is widely seen in Indochina, south Asia, west Asia, North, and Tropical region of Africa. Being a weed this plant has a high significant value such as its parts such as leaf, flower, stem, and roots has been used for the treatment of common diseases such as antibacterial [7] antifungal [8], antipyretic [9], and analgesic problem [10]. Scientific reports suggest that this herb has effective treatment such as paralysis, rheumatic pain [11], expectorant, and anti-inflammatory [12,13].

In the current study, we have focused on the major secondary metabolite containing parts such as flower, leaf, and root for phytochemical extraction with three different solvent systems (ethanol, methanol, and chloroform) to make a comparative study against three virulent bacteria species (*Escherichia coli, Staphylococcus aureus,* and *Bacilus subtilis*) which are capable of intestinal infection, pneumonia, skin infections, and food poisoning. The reports available on the antimicrobial study of *C. procera* suggests that no such comparative reports of plant parts, especially the flower has not been reported yet. In the current article, we aim to find the best effective plant part and the solvent system against these pathogenic microbes.

METHODS

Plant materials

The plant materials of *C. procera* were collected from Bhubaneswar locality Ghatikia area behind SUM Hospital. A fresh sample of the plant was identified by professor Baldev Khuntia Ex-reader Botany, College of Basic Science and Humanity, OUAT, Bhubaneswar.

Pre-processing of plant materials

The plant parts were washed thoroughly under running tap water and rewashed with distilled water then the plant parts were separated and cut into small pieces which were kept in the shade for drying about 15 days. The dry plant parts were collected and were grinded in an electric grinder into fine powder. Each parts powder was collected in a different plastic pouch and clearly labeled and stored for extraction.

Preparation of plant extracts

The powered extracts of different parts were weighed up to 100 g and were taken individually in different Soxhlet apparatus (Borosil

Glasswork Limited, Worli, Mumbai, India) using ethanol as solvent. The temperature of the apparatus was set to 40°C for 18–20 h. The extract was collected and filtered through Whatman no. 1 filter paper then the crude extract was concentrated at room temperature. The crude extract was collected and was stored at 4°C until the further analysis. The above procedure was repeated for both methanol and chloroform solvent.

Test microorganisms and growth media

Bacteria strains of *E. coli*, *S. aureus,* and *B. subtilis* were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from the Department of Microbiology, College of Basic Science and Humanities, OUAT, Bhubaneswar, were used for evaluating antimicrobial activity. The bacterial stock cultures were incubated for 24 h at 37°C on nutrient agar and potato dextrose agar (PDA) medium (HiMedia), respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the fungal strains were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

Antimicrobial test

Antimicrobial activities of ethanolic, methanolic, and chloroform extracts of *C. procera* were determined by filter paper disc diffusion method.

Disc diffusion method

Sterile filter disc (diameter 4 mm, Whatman paper No. 3) was placed in Petri dishes (diameter 90 mm) filled with MHA and seeded with 0.3 ml of the test organism. The disc was impregnated with test concentrations (25, 50, 75, and 100 µg/ml) of the compounds investigated dissolved in dimethyl sulfoxide (DMSO). The zones of growth inhibition around the discs were measured after 24 h of incubation at 37°C. Each microorganism was tested in triplicate, and the solvent (DMSO) was used as a control, while streptomycin was used as a positive control.

Minimum inhibitory concentration (MIC)

Different concentrations of the flower, leaf, and root extracts of *C. procera* were prepared to obtain 2.5 mg/ml, 5.0 mg/ml, and 7.5 mg/ml. Three drops of overnight broth culture of the test organisms were inoculated into the dilutions and incubated at 37°C for 24 h. The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the MIC.

Statistical analysis

Results obtained were reported as the mean±standard deviation of triplicate measurements. Significance differences for multiple comparisons were determined by one-way analysis of variance followed by Duncan test with p=0.05 using SPSS (version 19).

RESULTS

The result of antibacterial sensitive of ethanol, methanol, and chloroform extracts of flower, leaves, and root of the plant under study against the three different bacterial strains are interpreted in Tables 1-3.

The ethanol extract of leaf showed significant activity against *S. aureus* and *E. coli* with a zone of inhibition ranging from 14 to 20 mm for *S. aureus* and 10.11 for *E. coli*. *B. subtilis* is not resistant, and the zone of inhibition was not seen. The ethanol extract of flower was effective against *E. coli* with maximum 18 mm at 100 µl (20 mg). *S. aureus* also showed significant zero of inhibition. *B. subtilis* is not resistant to ethanol extract of root showed significant activity against *S. aureus* and 10–20 mm for *E. coli*. Zone of inhibition was not seen in *B. subtilis*.

Methanol extract of leaves showed moderate activity against *S. aureus* with a zone of inhibition ranging from 14 to 20 mm (25–100 µl) and *E. coli* 12–18 mm (25–100 µl). *B. subtilis* was not resistance against the extract. Methanol extract of root showed significant activity against *S. aureus* with zone of inhibition ranging from 12 to 22 mm (25–100 µl) and *E. coli* 11–19 mm (25–100 µl). *B. subtilis* was not resistant against the extract. Methanol extract of flowers showed activity against *E. coli* with a zone of inhibition ranging from 11 to 20 mm (25–100 µl). *B. subtilis* was not resistant against the extract.

The chloroform extract of leaves showed significant activity against *S. aureus* and *E. coli*. Chloroform extract of flower showed activity against *S. aureus* and *E. coli* with zone of inhibition ranging from 11 to 17 mm for *S. aureus* and 10–16 mm for *E. coli*. Chloroform extract of root showed activity against *E. coli* with zone of inhibition ranging from 9 to 17 mm (25–100 µl) and *S. aureus* 9–16 mm (25–100 µl) *B. subtilis* was not resistant against the extract.

The results of MIC are interpreted in Table 4.

DISCUSSION

Drug resistance of human pathogenic bacteria has been reported all over the world, and the situation is alarming in developing as well

Table 1: Antimicrobial activity of different solvent such as ethanol, methanol, and chloroform extracts of *C. procera* (flower)

Name of the solvent extracts	Concentration of the extract	Diameter of zone of inhibition (mm±SD)		
		Name of the organism		
		Bacterial species		
		E. coli	*S. aureus*	*B. subtilis*
DMSO	NC	-	-	-
Ethanol	25 µl (5 mg)	09±0.08	10±0.08	-
	50 µl (10 mg)	14±0.03	12±0.02	-
	75 µl (15 mg)	16±0.09	15±0.08	-
	100 µl (20 mg)	18±0.11	17±0.05	-
Methanol	25 µl (5 mg)	11±0.01	09±0.07	-
	50 µl (10 mg)	15±0.07	11±0.10	-
	75 µl (15 mg)	17±0.06	14±0.08	-
	100 µl (20 mg)	20±0.04	19±0.05	-
Chloroform	25 µl (5 mg)	10±0.16	11±0.01	-
	50 µl (10 mg)	12±0.03	13±0.04	-
	75 µl (15 mg)	13±0.07	16±0.06	-
	100 µl (20 mg)	16±0.08	17±0.06	-
Streptomycin	PC (10 µg)	22±0.10	20±0.30	12±0.20

Values are expressed as mean zone of inhibition (mm)±SD of three replicate. NC: Negative control, PC: Possitive control (streptomycsin), -: No activity, *C. procera: Calotropis procera, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis*, SD: Standard deviation, DMSO: Dimethyl sulfoxide

Table 2: Antimicrobial activity of different solvents such as ethanol, methanol, and chloroform extracts of *C. procera* (Leaf)

Name of the solvent extracts	Concentration of the extract	Diameter of zone of inhibition (mm±SD)		
		Name of the organism		
		Bacterial species		
		E. coli	*S. aureus*	*B. subtilis*
DMSO	NC	-	-	-
Ethanol	25 µl (5 mg)	10±0.08	14±0.10	-
	50 µl (10 mg)	10±0.25	16±0.03	-
	75 µl (15 mg)	10±0.08	17±0.03	-
	100 µl (20 mg)	11±0.15	20±0.12	-
Methanol	25 µl (5 mg)	12±0.06	14±0.10	-
	50 µl (10 mg)	14±0.02	16±0.05	-
	75 µl (15 mg)	16±0.20	19±0.07	-
	100 µl (20 mg)	18±0.10	20±0.21	-
Chloroform	25 µl (5 mg)	08±0.01	09±0.02	-
	50 µl (10 mg)	09±0.02	12±0.12	-
	75 µl (15 mg)	11±0.11	14±0.15	-
	100 µl (20 mg)	14±0.21	17±0.20	-
Streptomycin	PC (10 µg)	20±0.10	22±0.10	12±0.02

Values are expressed as mean zone of inhibition (mm)±SD of three replicate. NC: Negative control, PC: Positsive control (stresptomycin), -: No activity,
C. procera: Calotropis procera, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, SD: Standard deviation, DMSO: Dimethyl sulfoxide

Table 3: Antimicrobial activity of different solvents such as ethanol, methanol, and chloroform extracts of *C. procera* (root)

Name of the solvent extracts	Concentration of the extract	Diameter of zone of inhibition (mm±SD)		
		Name of the organism		
		Bacterial species		
		E. coli	*S. aureus*	*B. subtilis*
DMSO	NC	-	-	-
Ethanol	25 µl (5 mg)	10±0.02	10±0.09	-
	50 µl (10 mg)	13±0.07	15±0.02	-
	75 µl (15 mg)	15±0.06	18±0.03	-
	100 µl (20 mg)	20±0.05	21±0.04	-
Methanol	25 µl (5 mg)	11±0.03	12±0.10	-
	50 µl (10 mg)	12±0.06	14±0.04	-
	75 µl (15 mg)	15±0.07	18±0.09	-
	100 µl (20 mg)	19±0.05	22±0.06	-
Chloroform	25 µl (5 mg)	09±0.02	09±0.06	-
	50 µl (10 mg)	10±0.07	10±0.05	-
	75 µl (15 mg)	14±0.40	12±0.03	-
	100 µl (20 mg)	17±0.10	16±0.07	-
Streptomycin	PC (10 µg)	22±0.30	24±0.10	12±0.30

Values are expressed as mean zone of inhibition (mm)±SD of three replicate. NC: Negative control, PC: Positive control (streptomycin), -: No activity,
C. procera: Calotropis procera, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, SD: Standard deviation, DMSO: Dimethyl sulfoxide

Table 4: Minimum inhibitory concentration (mg/ml) of different parts of *Calotropis procera*

Test organisms	Ethanolic leaf extracts of *Calotropis procera* (mg/ml)	Ethanolic root extracts of *Calotropis procera* (mg/ml)	Ethanolic flower extracts of *Calotropis procera* (mg/ml)
Bacteria			
E. coli	5.0	5.0	5.0
B. subtilis	-	-	-
S. aureus	5.0	5.0	5.0

E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis

as developed countries due to in the discriminate use of antibiotics. Plants are an important source of potentially useful structures for the development of novel chemotherapeutic agents and the first step toward this goal is the *in vitro* antibacterial assay [14].

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such bacteria, fungi, or protozoan. Antimicrobial agents either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Various parts of medicinal plants such as the leaves, flowers, fruits, roots and the bark extract, infusion, decorations, and powders have proven useful in curing a wide range of health-related issues [15]. The present study was conducted to analyze the antibacterial activity of *C. procera* with optimized conditions.

Further work was only carried out the used parts of samples *C. procera* were leaves root and flowers with three solvents, i.e. methanol, ethanol, and chloroform. A weak antibacterial property of ethanolic extracts of *C procera* leaves and latex against *E. coli, S. aureus, Salmonella* sp., and *Pseudomonas* species was recorded using paper-disc diffusion and broth dilution techniques. The results obtained revealed that ethanol

was the best extractive solvent for a fraction with antibacterial activity. Furthermore, ethanol was reported for its efficiency for extracting the antimicrobial active substances from calotropis compared to other solvents [16]. The used solvent is an important factor for the isolation of selective bioactive compounds [17]. In our results, methanol extracts of leaves exhibited much more bioactivity than other extracts. This is close agreement with Manilal *et al*. 2009, Rangaiah *et al*. 2010 [18,19].

Pandey *et al*. [20] also reported the maximum zone of inhibition was recorded in case of leaves of methanol extract of *C. procera* against *E. coli* ranging 25.5 mm of zone of inhibition. Ranjit *et al.* [21] reported flowers of *C. procera* shows antibacterial action against Gram-positive and Gram-negative microorganism. Some other reports are also reported that various parts of this plant show that antimicrobial activities [8,16,22,23]. Some other reports are also reported that various part of this genera shows that antimicrobial activity [24].

In the present study, both ethanol and methanol extracts were effective against some bacterial strains. Methanol extract was more effective against the bacteria compared to ethanol extract. The extracts were not effective against *B. subtilis*. From the above study, it can be concluded that the activity of the plant extract may be due to the secondary metabolites or broad-spectrum antibiotic compounds present in it. The polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity [25].

CONCLUSION

As the searches for new drugs are in demand, plant extracts may provide an attractive alternative source against various infections and chronic disease. Furthermore, due to multidrug-resistant microorganisms and side effects of the synthetic drugs, these studies can be helpful in discovering new therapeutic agents with less or no side effects. It can finally be concluded that weeds are valuable medicines and should be protected. Therefore, there is huge room for research in the direction of more pharmacological activities of plant and to elucidate the mechanism of action of same in future.

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CONFLICTS OF INTERESTS

we declare that we have no conflict of interest

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