

MANGROVE PLANT DERRIS TRIFOLIATA- EVALUATION OF ANTIBACTERIAL PROPERTYR. SUGANYA^{1*} AND M. THANGARAJ²¹Jeppiaar Engineering College, Jeppiaar Nagar, Rajiv Gandhi Salai, Chennai., 2 CAS in Marine Biology, Annamalai University, Parangipettai, Chidambaram., *Research Scholar, Department of Biotechnology, Sathyabama University, Chennai. Email: sugi11187@gmail.com

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ABSTRACT

OBJECTIVE: Natural products such as plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. Secondary metabolites from plants especially mangroves is traditionally being used widely as antimicrobials. Hence, present study was aimed to determine the antibacterial property of *Derris trifoliata* against Gram positive organism: *Staphylococcus aureus*; Gram negative organisms: *Escherichia coli* and *Klebsiella pneumonia*.

METHODS: The *Derris trifoliata* leaves were collected, cleaned, shade dried and grounded into a crude powder. The methanol leaf extracts were isolated. The antibacterial assay of the leaf extracts was performed by Agar well Diffusion method [8, 9]. MIC and MBC of the isolated compounds were performed by broth dilution method [12].

RESULT: The methanol extracts of *Derris trifoliata* showed the highest bactericidal activity against the *Staphylococcus aureus* and *Escherichia coli* but not against *Klebsiella pneumonia*. **CONCLUSION:** This present study was conducted to prove the medicinal application of the mangroves especially *Derris trifoliata*. This can lead to the discovery of antibacterial properties of other mangroves and their pharmaceutical importance.

Keywords: *Derris trifoliata*, mangrove, *Staphylococcus aureus*, *Escherichia coli*, antibacterial assay.

INTRODUCTION

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [1]. The chemical constituents of the medicinal plants, particularly the secondary metabolites have pronounced pharmacological actions on animal systems and organs. Several bioactive compounds were isolated from the plant sources such as digoxin, digitoxin, morphine, reserpine, taxol, vinblastine, quercetin [2] etc, which have different pharmacological and nutritional properties. Research work on mangrove plants is intensified and information on these plants be exchanged. This thought will go a long way in the exploration of medicinal compounds for the benefit of man and is likely to decrease the dependence of drugs [3]. *Derris trifoliata* is one of the most commonly used insecticides. The leaves contain the chemical compound rotenone, a poison that kills a wide range of creatures from insects to earthworms and fish [4,5]. This poison is used in catching the fishes. The leaves of *Derris trifoliata* were proved to have many therapeutic uses including stimulant, antispasmodic, counter-irritant, rheumatism, chronic paralysis, and dysmenorrhoea. The decoction of roots is used against fever and internally against sores. The roots or stem are used as laxative, carminative and anti-arthritis agent [6]. In the present study, antibacterial potentiality of *Derris trifoliata* was investigated against the gram positive and gram negative organisms.

MATERIALS AND METHODS**Collection of Plant materials**

Fresh mangrove leaves of *Derris trifoliata* were collected from Rameshwaram in 2012. They were washed with distilled water to remove the adhering dust particles and were shade dried at room temperature (29±2°C).

Extraction and fractionation of plant material

The shade dried leaves were then grounded into a fine coarse powder. The plant powder (50 g) was extracted with methanol (2 lit) in an air tight, clean flat bottomed container [7] for 7 days at room temperature with occasional stirring and shaking.

The filtrate was concentrated using a Rotary evaporator [] at low temperature (39°C) and low pressure. The weight of the crude extract was 30 gm.

Antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*:

Gram positive and Gram negative bacteria were isolated from urine specimens and the isolates were maintained in nutrient agar slant at 4°C.

Gram positive bacteria: *Staphylococcus aureus*

Gram negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*

The obtained compounds were reconstituted by dissolving 250 mg. of the respective compounds isolated in 1ml. of dimethyl sulfoxide in screw capped tubes in case of primary screening of plant solvent extracts. For determining the MIC of plant extracts stock solution was prepared at a concentration of 800 mg. of the respective compounds isolated in 1 ml. of dimethyl sulfoxide in screw capped tubes. They were then stored at 4°C.

Primary screening for antibacterial activity

The antibacterial assay of the isolated compounds was performed by agar well diffusion method [8, 9]. The media (Mueller Hinton Agar No.2), along with the inoculums (108 cfu/ml), was poured into the petriplate (Hi Media). The cultures were grown for 24 hours, and the turbidity of the culture was maintained according to the 0.5 McFarland Standards. For the agar well diffusion method, 5 wells were prepared in the plates with a cup-borer (0.8 cm) and different quantity of the stock solution i.e. 250 mg./ml was pipette directly into each well (200µl, 150µl, 100µl, 50µl). Controls were maintained in the fifth well that comprised pure solvents instead of the extract [10]. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition surrounding bacterial growth. Gentamycin, Erythromycin, Tetracyclin, Penicillin, Ceftriazone and Ceftazimide (10-32 µg) were used as reference standards [11].

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

Broth dilution method [12] was used to determine the MIC and MBC of the compounds isolated from the plant extracts. 800mg/ml. of the stock solution was taken and then serially diluted to get various concentrations viz., 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml. 10µl. of test bacterial strain was inoculated in the tubes and incubated at 37°C for 18 hours. Observations were performed at least in duplicate and results were expressed as the lowest concentration of plant extracts that produced a complete suppression of colony growth, MIC. Minimal bactericidal concentration using agar dilution method in petri plates with Millipore filter was performed with the extracts that gave significant MIC values against each bacterial strain.

RESULTS

In the present study *Derris trifoliata* leaf extracts extracted in methanol were investigated at four different concentrations for their

antimicrobial potentiality against three clinically important microbial strains isolated from urine specimen. The antibacterial assay of the leaf extracts was performed by agar well diffusion method in which different concentrations of the extracts were used [8, 9]. But in the present study different quantity of the stock solution was used.

Name of the extract	Quantity of extract (µl)	Bacteria (inhibition zone in mm)		
		<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
Methanol extract	200	15	14	Nil
	150	14	12	Nil
	100	12	10	Nil
	50	9	8	Nil

The methanol extract of *Derris trifoliata* exhibited antibacterial activity against *S. aureus* and *E. coli*, but not against *K. pneumoniae*. The antibacterial activity of the standards were tested and reported.

Antibiotic discs used	Test Organism (zone in mm)			Control Organism (zone in mm)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i> MTCC 1407	<i>E. coli</i> MTCC 443	<i>K. pneumoniae</i> MTCC 109
Gentamycin	25	11	23	25	13	23
Erythromycin	20	25	Nil	20	22	Nil
Tetracycline	15	9	11	15	9	11
Penicillin	20	12	Nil	20	12	Nil
Ceftriaxone	24	18	24	25	18	24
Ceftazimide	16	15	22	17	15	22

MIC and MBC was done [12]. In this case, different concentration of the extracts was prepared. The MBC values were in the range of 200mg/ml. to 400mg/ml. The lowest MBC values of 400mg/ml. was recorded by the methanol extract against *S. aureus* and *Escherichia coli*. The highest MBC value of 200mg/ml. was recorded by the methanol extract against *S. aureus*. There was no bactericidal activity concerned with the extract against *E. coli*.

Bacterial agent	Extract Concentration (mg/ml)					
	400	200	100	50	25	12.5
<i>S. aureus</i>	-	-	+	+	+	+
<i>E. coli</i>	-	+	+	+	+	+

Plants parts are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which were found in vitro to have antimicrobial properties[14-18]. While scientists have placed a high value on the ecological function of mangrove ecosystems for some time, only recently has the broader community come to recognise the multi-faceted role that mangroves play in the environment. Mangroves are used by a vast array of organisms as breeding, nursery and feeding areas. This present study was conducted to prove yet another medicinal application of the mangroves especially *Derris trifoliata* [19, 20]. This can lead to the discovery of antibacterial properties of other mangroves and their pharmaceutical importance.

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