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Research Article

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A study on antimicrobial and anthelmintic activity of methanolic leaf extracts of *Syzygium malaccense* (L.) Merr. & Perry

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ABSTRACT

Traditional plants are the potential sources of many potent and powerful drugs. They represent a rich source of antimicrobial and anthelmintic activity. The development of resistance and high cost of conventional synthetic drugs have paved the way to the evaluation of alternative source of medicines. The present study was undertaken to evaluate antimicrobial and anthelmintic activity of methanolic extract of leaves of Syzygium malaccense (L.) Merr. & Perry. Antimicrobial activity was evaluated against clinically important Proteus bacteria and Candida albicans using Kirby-Bauer disk diffusion method. The result showed that extract have moderate degree of both antibacterial and antifungal activity. The anthelmintic activity was carried as per method of Ajaiyeoba et al, which involves determination of time of paralysis and time of death of earthworm (Pheretima posthuma). The result showed that extract, revealed a concentration dependent anthelmintic activity.

Keywords: Anthelmintic activity, Methanolic Extract, Myrtaceae, antimicrobial, Syzygium malaccense

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INTRODUCTION

Syzygium malaccense (L.)Merr. & Perry is a small tree indigenous to regions of Malaysia and South East Asia [1]. It is mainly found in lowland to mountain rain forest [2], [3]. The tree is valued for its large edible fruits. The fruit of the plant is often called as 'Malay Apple'. The fruit of the plant is often called as 'Malay Apple'. They are widely used in traditional medicines for mouth infections, throat infections, stomach ache and abdominal ailments [4], [5]. Crushed leaves are used as antiemetic, purgative and also to treat bronchitis, tongue inflammation, dysentery, constipation, diabetes, cough, pulmonary cataract, headache and other ailments [6]. Since no report has been found on anthelmintic and anti microbial property, the present study is conducted to evaluate these properties.

EXPERIMENTAL SECTION

Collection and Authentication

The leaves of the plant *Syzygium malaccense* (L.)Merr. &Perry (Family: Myrtaceae) was collected from Ernakulum district, Kerala, India in September 2014. The fresh leaves were taxonomically identified by Botanist. The authentification of the plant was done by Mrs.Leela.M, Botany Department, Paliyam Government Higher Secondary School, Paravur , Cochin, Kerala, India.

Preparation of plant extract
The leaves were dried under the shade and then powdered (40 mesh size) with a mechanical grinder and stored in air tight container. The dried powdered material of the leaves was then extracted with methanol in soxhlet apparatus. The extract is evaporated by using a rotary evaporator.

Chemicals
All the Chemicals and reagents were procured from (S.D. Fine chemicals, Mumbai, India). The solvents used for the study were of analytical grade. The drug Piperazine citrate was procured from Glaxo smithkline Ltd., Mumbai.

Antimicrobial Activity
The antimicrobial activity was carried out by Kirby Bauer disk diffusion method i.e. Disc Diffusion Method [7]. The media used for the study is Yeast Peptone Dextrose Agar Medium (YPD).The micro organisms used for the study are *Proteus mirabilis* (gram negative bacteria) and *Candida albicans* .YPD plates were prepared and allowed to solidify for 5 min and 0.1% of inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 min. The different concentrations of extracts (1, 2, and 4 mg/disc) were loaded on 5mm sterile individual discs. The loaded discs were placed on the surface of the medium and the compound was allowed to diffuse for 5min and plate was kept for incubation at 37℃ for 24 hours. Negative control was prepared by using respective plant extract and Ciprofloxacin and Clotrimazole were used as positive control for *Proteus and Candida albicans* respectively. After 24 hours of incubation, inhibition zones formed around the disc evidence the antimicrobial activity. Diameters of the zone of inhibition were measured with transparent ruler in millimeter.

Anthelminitic Activity
The anthelmintic activity was carried as per method of Ajaiyeoba et al method [8], [9]. The assay was performed in-vitro using adult earthworm (pheretima posthuma) owing to its anatomical and physiological resemblance with intestinal round worm parasites of human beings. Earthworm *Pheretima posthuma* (pheretimidae) have been widely used for the initial evaluation of anthelmintic compounds *in vitro*, because of their easy availability. Indian adult earth worm (*Pheretima posthuma*) was collected from the water logged areas of soils and washed with normal saline to remove all filthy matter. The earth worm of 3-5 cm in length and 0.1- 0.2 cm in width were used for all the experimental protocol. The collected worm was authenticated by Anitha P.B, Department of Zoology, GHSS Ernakulum, Kerala.

Test samples and standard solution were prepared by dissolving or suspending 3g of each extract in 30 ml of distilled water to obtain a stock of 100mg/ml. The different working dilutions in concentration range of 10,20,30,40, and 50mg/ml were prepared from above stock solution. Standard used in the assay is Piperazine citrate 10mg/ml. Six adult earth worms were placed in petridish which contains different concentrations 10,20,30,40, and 50 mg/ml. The time taken for paralysis and death was noted. The mean of paralysis time was observed and noted .The observations were made for time taken for the paralysis, when no movement of any sort could be shown except when the worm were shaken vigorously. Time for death of the worms were recorded after ascertaining that worm neither moved when shaken vigorously nor when dipped in warm water(50°C).

RESULTS AND DISCUSSION

The study has shown that the leaf extract is effective against both *Candida albicans* & *Proteus* bacteria. The zone of inhibition of methanolic leaf extract of *Syzygium malaccense (L.) Merr .&Perry* for *Candida albicans* ranged from 0.4mm to 0.9mm and for *Proteus* bacteria, ranged from 0.5mm to 0.9 mm and showed a moderate degree of antimicrobial activity. Sensitivity of bacterial strains to standard antibiotic (Ciprofloxacin) showed a zone of inhibition of 1.4 mm and sensitivity of fungal strains to standard (Clotrimazole) showed a zone of inhibition of 1.2 mm which is depicted in Figure 1 and 2 .The results are shown in Table 1 and 3. Minimum Inhibitory Concentration (MIC) is the lowest concentration at which no growth of test strain was observed. The MIC of methanolic extracts of *Syzygium malaccense* (*L.)Merr.&Perry* for *Candida albicans* was found to be 10µg/ml and are shown in Table 2 and 4. The study also reveals a concentration dependent anthelmintic activity. The methanolic extracts of *Syzygium malaccense* (*L.)Merr. &Perry* exhibited activity [time for paralysis(P)of 60.86min and time for death (D) of 71.60 min] at 10mg/ml concentration compared with standard Piperazine citrate [(P) of 25.87 min] at same concentration. The results are shown in Table 5. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that MIC of the methanolic extract for *Proteus* bacteria was found to be 10µg/ml. The results leads to muscle relaxation and flaccid paralysis. So, the methanolic extract of *Syzygium malaccense* (*L.)Merr. &Perry* showed significant antimicrobial activity and anthelmintic activity.

Table 1. Antibacterial Activity by Zone of Inhibition

Microorganism	Sample taken (mg)	Concentration (µg/ml)	Zone of inhibition of Test sample(cm)	Zone of inhibition of Ciprofloxacin(cm) (Concentration 10µg/ml)
Proteus	0.05	5	0	1.4
	0.1	10	0.5	
	0.15	15	0.7	
	0.2	20	0.9	

Table 2: MIC of the Sample preparation

Organism	Minimum inhibitory concentration of sample (µg/ml)
Proteus	10

Table 3: Antifungal Activity by Zone of Inhibition

Microorganism	Sample taken (mg)	Concentration (µg/ml)	Zone of inhibition of Test sample(cm)	Zone of inhibition of Clotrimazole(cm) (10µg/ml)
Candida albicans	0.05	5	0	1.2
	0.1	10	0.4	
	0.15	15	0.6	
	0.2	20	0.9	

Table 4: Minimum Inhibitory Concentration of the sample preparation

Organism	Minimum inhibitory concentration of sample (µg/ml)
Candida albicans	10

Table 5: Anthelmintic activity of methanolic leaf extract

Sl. No	Test Substance	Concentration (mg\ml)	Time taken for paralysis & Death (min)	
			Paralysis	Death
1	Control (Distilled Water)	-	-	-
2	Piperazine Citrate (Standard)	10	25±0.87	-
3	Group I	10	60±0.86	71±0.60
4	Group II	20	48±0.63	62±0.52
5	Group III	30	31±0.30	47±0.88
6	Group IV	40	24±0.86	32±1.05
7	Group V	50	18±0.42	30±0.03

[Values are expressed as Mean ±SEM (N=6)]

Figure 1: Activity against Proteus bacteria

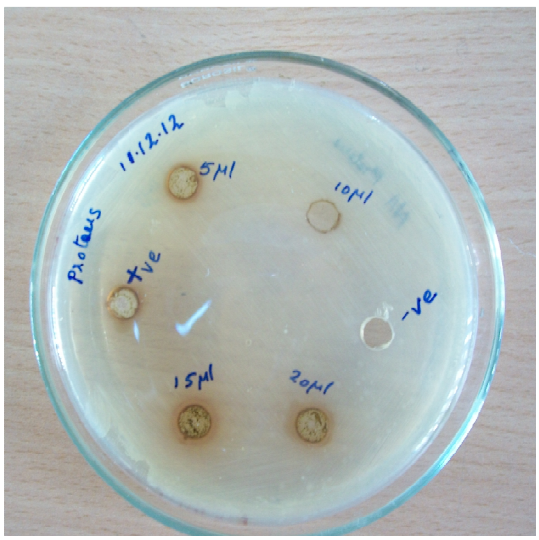
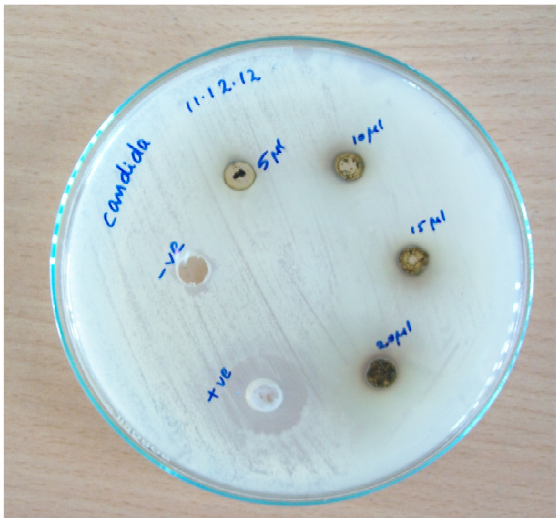


Figure 2: Activity against Candida



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