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RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL ACTIVITY OF MEDICINAL ORCHID *MALAXIS RHEEDEI* SW. AGAINST SOME SELECTED PATHOGENS

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ABSTRACT

The antimicrobial activity of whole plant extracts of *Malaxis rheedei* Sw was evaluated *in vitro*, using disc diffusion method. Four different extracts were used which included chloroform, methanol, ethyl acetate and petroleum ether. Five species of bacterial strains namely *Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae* and fungus strains *Aspergillus flavus, Trichophyton rubrum, Candida albicans, Candida tropicalis, Cryptococcus neoformans* were used for the present study. In the case of bacteria the *Klebsiella pneumoniae* was the most susceptible bacteria and it showed very least activity. In case of fungus *Candida tropicalis* and *Candida albicans* exhibit very least activity. But other microbes showed good activity. Therefore the petroleum ether, chloroform, ethyl acetate, methanol extract of the whole plant part of *M.rheedei* was more potential against these microbes.

INTRODUCTION

In the recent years, the development of resistance of pathogens against antibiotics has become a difficult issue caused by the indiscriminate use of modern antibiotics (Sharmin *et al.,* 2013). About 50-75% of hospital deaths are reported due to infectious diseases (Keerthiga *et al.,* 2014). The potential for developing antimicrobial from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles (Evans *et al.,* 2002). Plant based antimicrobial have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobial. Orchids have been used as food and in traditional medicine for treating several ailments as remedy for microbial infections by different tribes all over the world (Shanmugavalli *et al.,* 2009). The first record of Indian orchids used in ayurvedic medicine is *Malaxis rhedei*, *Eulopia dabia* and *Flickingeria nodosa* which was discussed in 'Charaka Samhita', a classic ancient Indian medicinal treatise written by Charaka in Sanskrit, a few thousand years ago(Sahaya *et al*., 2013). *Malaxis* genus is distributed throughout the world. It is found in India, Bangladesh, Eastern Himalayas, Bhutan, Andaman Islands, Myanmar, Thailand, Malaysia, Combodia, China, Vietnam, Java, Sumatra, Philippines and Australia.

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In this genus include terrestrial and semi epiphytic orchids. *Malaxis acuminata* D.Don, an endangered therapeuticaly important terrestrial orchid and is one the components of 'Ayurvedic' drug preparations (Arenmongla *et al.,* 2012). *Malaxis rheedei* (Orchidaceae) commonly named as jeevakam is a rare, terrestrial, endangered and medicinal orchid. The plant distributed throughout the India mainly in Western Ghats regions. Traditionally, whole plant used by *Kattunayaka tribes* in Nilambur area, Malappuram district in Kerala. Hence the medicinal plant *Malaxis rheedei* was selected to analyse its potential efficiency against Infections caused by pathogenic bacteria and fungi.

MATERIALS AND METHODS

Collections of Plant Material

Whole plant parts of *M. rheedei* were collected from Nilambur forest, Malappuram district, Kerala, India. The voucher specimen has been deposited in Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.

Preparation of Plant Extract

The fresh whole plant parts of *M. rheedei* were washed with tap water and shade dried for two month and powdered coarsely. Then they were finely powdered mechanically using Pulverizer and passed through 40 mesh sieve and stored in airtight containers.

About 250g of powdered whole part were extracted in soxhlet apparatus with petroleum ether, chloroform, ethyl acetate and methanol. The extract was dried under reduced pressure at low temperature (40-50°C). The last traces of the solvent were removed under vacuum drier and the solid mass obtained was stored at 4°C until further use.

Antimicrobial Activity of Crude Extract

The microbiological assay of the various extracts of *M. rheedei* was done by comparing the inhibition of the growth by measured concentration of the antibiotics

Microbial stains

The bacterial strains *Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae* and fungus cultures like *Aspergillus flavus, Trichophyton rubrum, Candida albicans, Candida tropicalis, Cryptococcus neoformans* were obtained from Microbiology Department, PSG Medical Hospital, Coimbatore, Tamil Nadu. All these cultures were maintained on nutrient and potato dextrose agar plates at 4°C respectively in lab.

Preparation and standardization of inoculums

All the bacterial and fungal cultures were transferred into 100 ml of nutrient broth (NB). The inoculated broths were incubated at 37°C for 24 hours and at 27°C for 72 hours in the case of bacteria and fungi, respectively.

Antimicrobial assay

Antimicrobial activity of the various extracts of *M. rheedei* was determined by disc diffusion method. All petridishes were plated with nutrient agar and potato dextrose agar medium prepared according to the manufacturer's manual given below.

Antibacterial activity

Nutrient agar medium was prepared and transferred into sterile petriplates. 25ml of the standardized bacterial innoculum was spread on agar medium using sterile cotton swab. The disc impregnated with extracts were placed on the inoculated agar medium. Amphicillin (10µg/disc) was used as standard to determine the sensitivity of each microbial species. All the petriplates were incubated at 37°C for 24 hours. After the incubation period, diameter of zone of inhibition was measured.

Composition of nutrient agar medium for bacterial culture

S. No.	Composition	Quantity (g)
1	Peptone	5.0
2	Beef extract	3.0
3	Sodium chloride	5.0
4	Agar	15.0
5	Distilled water	1000 ml
	pH	7.0

Antifungal activity

Potato dextrose medium was prepared and transferred into sterile petriplates. 200µl of the standardized fungal inoculum was spread on agar medium using sterile cotton swab. The discs impregnated in extracts were placed on the inoculated agar medium. Tetracycline (10µg/disc) was used as reference standard to determine the sensitivity of each microbial species tested. All the petriplates were incubated at 27°C for 72 hours. After the incubation period, diameter of zone of inhibition was measured. Growth inhibition was determined as the diameter of the inhibition zones around the discs. The growth inhibition diameter was an average of 4 measurements, taken at four different directions. All the tests were performed a triplicate.

Composition of potato dextrose agar medium for fungal culture

S. No.	Composition	Quantity (g)
1	Potato (peeled)	200.0
2	Dextrose	20.0
3	Agar	15.0
4	Distilled water	1000ml
	pH	6.2

Statistical analysis

The data were reported as mean ± standard deviation (n=3) for determining the statistical significance, standard error, mean and analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The disc diffusion procedure (Kirby-Bauer method) has been widely accepted by the Food and Drug Administration (FDA) and as a standard by the National Committee for Clinical Laboratory Standards (Barry and Thornsberry, 1985; NCCLS**,** 2003). In the present study, the antimicrobial activity of different extract like Petroleum ether, chloroform, ethyl acetate and methanol extracts of the whole plant part of *M. rheedei* was carried out and the growth inhibition drug pattern was tested with the microorganisms and compared with the standard drug, Amphicillin and Tetracycline. Five bacterial strains namely *Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae and* fungus strains *Aspergillus flavus, Trichophyton rubrum, Candida albicans, Candida tropicalis, Cryptococcus neoformans* were used in the present study.

Petroleum ether extract

Petroleum ether extract of the whole plant part of *M. rheedei* showed maximum antibacterial activity against *Staphylococcus aureus* (20mm) and *Salmonella typhi* in 10mm diameter. Moderate activity was shown against *Acinetobacter baumannii* (8mm)*, Pseudomonas aeruginosa* (7.05 mm)*.* The least activity was shown against *Klebsiella pneumoniae* (6.06 mm). In the case of fungal strains Petroleum ether extract of the whole plant part of *M. rheedei* showed maximum antifungal activity against *Cryptococcus neoformans* (20mm diameter). Moderate activity was shown against *Trichophyton rubrum* (15mm) and *Candida tropicalis* (15mm) diameter. The least activity was shown against *Aspergillus flavus* (10mm) and *Candida albicans* (10mm)

Chloroform extract

Chloroform extract of the whole plant part of *M. rheedei* showed maximum antibacterial activity against *Pseudomonas aeruginosa* (25mm) and *Acinetobacter baumannii* (22mm).

Table 1. Antibacterial activity of whole plant extract of *M. rheedei* against different bacterial Strains

S.No	Bacteria	Control	Petroleum ether	Chloroform	Ethyl acetate	Methanol
1	*Acinetobacter baumannii*	15.04±0.03	8.03±0.02	22.03±0.02	10.04±0.02	20.07±0.02
2	*Pseudomona saeruginosa*	30.04±0.01	7.05±0.01	25.05±0.01	15.09±0.03	25.06±0.03
3	*Staphylococcus aureus*	18.13±0.03	20.10±0.02	20.08±0.03	10.03±0.01	25.08±0.01
4	*Salmonella typhi*	25.14±0.02	10.15±0.02	15.13±0.02	16.15±0.02	20.04±0.02
5	*Klebsiella pneumoniae*	6.13±0.06	6.06±0.01	6.03±0.02	6.02±0.01	11.37±0.02

Table 2. Antifungal activity of *M. rheedei* whole plant extract against fungal species tested by disc diffusion assay

S. No	Fungus	Control	Petroleum ether	Chloroform	Ethyl acetate	Methanol
1	*Aspergillus flavus*	40.02±0.01	10.04±0.03	15.07±0.02	40.04±0.02	30.0267±0.02
2	*Trichophyton rubrum*	12.19±0.15	15.04±0.01	5.03±0.01	_	_
3	*Candida albicans*	11.07±0.04	10.10±0.06	_	_	_
4	*Candida tropicalis*	12.35±0.10	15.04±0.02	_	_	_
5	*Cryptococcus neoformans*	36.02±0.01	20.04±0.02	20.03±0.02	20.04±0.01	20.04±0.04

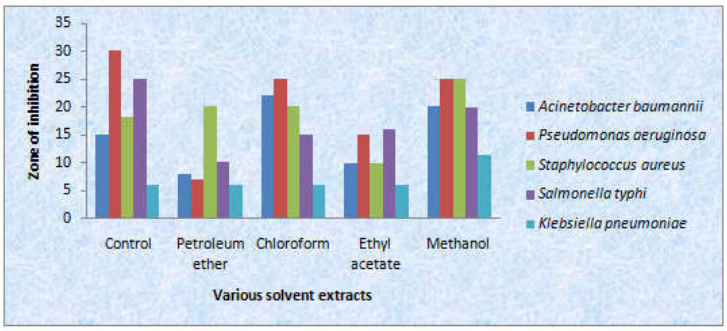

Figure 1. Antibacterial activity of whole plant extract of *Malaxis rheedei* against different bacterial strains

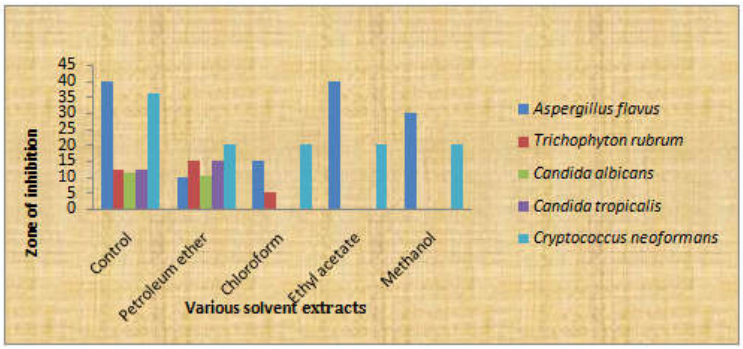

Figure 2. Antifungal activity of whole plant extract of *Malaxis rheedei* against different fungal strains

Moderate activity was shown against *Staphylococcus aureus* (20mm) and *Salmonella typhi* (15.13mm). The least activity was shown against *Klebsiella pneumoniae* (6.03mm). In case of fungal strains Chloroform ether extract of the whole plant part of *M. rheedei* showed maximum antifungal activity against *Cryptococcus neoformans* (20mm) and *Aspergillus flavus* (15mm). Moderate activity was shown against *Trichophyton rubrum* (12.19mm). No activity was shown against *Candida albicans* and *Candida tropicalis.*

Ethyl acetate extract

Ethyl acetate extract of the whole plant part of *M. rheedei* showed maximum antibacterial activity against *Salmonella typhi* (16mm) and *Pseudomonas aeruginosa* (15mm).

Moderate activity was shown against *Acinetobacter baumannii* (10.04mm) and *Staphylococcus aureus* (10.03mm). The least activity was shown against *Klebsiella pneumoniae* (6.02mm). In case of antifungal activity shown maximum effect against *Aspergillus flavus* was 40mm in diameter. Moderate activity was shown against *Cryptococcus neoformans* (20mm) and no activity was recorded against *Trichophyton rubrum*, *Candida albicans* and *Candida tropicalis*

Methanol extract

Methanol extract of the whole plant part of *M. rheedei* showed maximum antibacterial activity against *Pseudomonas aeruginosa* (25.06mm) and *Staphylococcus aureus* (25mm).

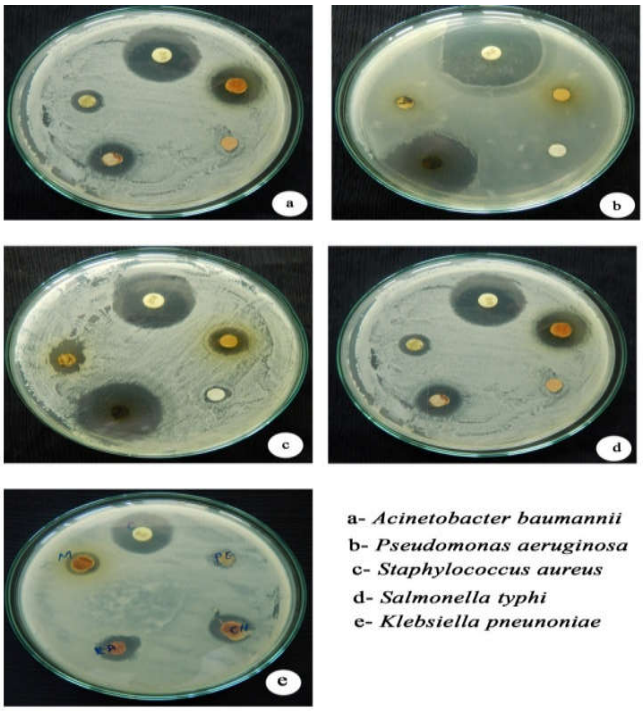

Plate 1. Plate showing comparative antimicrobial activity of different extract of *M. rheedei* against pathogenic bacteria

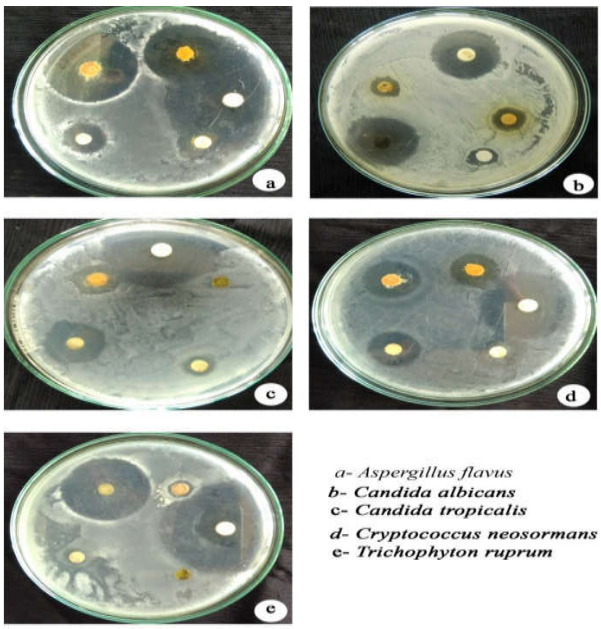

Plate 2. Plate showing comparative antimicrobial activity of different extract of *M. rheedei* against pathogenic fungus

Acinetobacter baumannii (20.07mm) and *Salmonella typhi* (20.04mm) showned moderate activity and a least activity was seen in *Klebsiella pneumoniae*. Antifungal activity was maximum in *Aspergillus flavus* (30mm) and moderate in *Cryptococcus neoformans* (20mm). No activity was recorded in *Trichophyton rubrum*, *Candida albicans*, and *Candida tropicalis.*In the present study, the whole plant extracts *M. rheedei* exhibited considerable antibacterial and antifungal activity. However, the methanolic extracts and chloroform extract of whole plant parts exhibited more significant antibacterial activity than the Petroleum ether and ethyl acetate extracts.

Pseudomonas aeruginosa and *Staphylococcus aureus* showed high activity. *Klebsiella pneumoniae* was the most susceptible bacteria, among the five bacteria tested and it showed the least activity. Ethyl acetate and methanol extract of whole plant parts exhibited more significant antifungal activity than the petroleum ether and chloroform. *Aspergillus flavus* showed the high activity. *Candida albicans* and *Candida tropicalis* were the most susceptible fungus, among the five fungus tested and these fungus showed the least activity (Table 1&2 and Fig 1&2). These results confirm the traditional knowledge on medicinal uses of *M. rheedei.*

Conclusion

M. rheedei has great potential as antimicrobial agent against selected pathogenic microorganisms due to the presence of selected alkaloid and flavonoid compounds. The present study also support the medicinal usage of the studied plants and suggest that the plant extracts like Petroleum ether, chloroform, ethyl acetate and methanol extracts of the whole plant part of *M. rheedei* possess compounds with antibacterial and antifungal properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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