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Original Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *LEUCAS MARRUBIOIDES* DESF. ROOT EXTRACTS

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ABSTRACT

Objective: The number of multidrug-resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics forced scientists to search for new antimicrobial agents from plants. The present study was aimed to evaluate the phytochemical constituents and antimicrobial potential of *Leucas marrubioides* Desf. root extracts.

Methods: The roots of *L. marrubioides* was extracted with solvents of varying polarity, namely pet-ether, chloroform, and methanol. The phytochemical screening was carried out qualitatively to identify the major phytoconstituents present in the extracts. The antimicrobial activity of extracts was evaluated against five Gram-positive bacteria, five Gram-negative bacteria and six fungal strains by agar well diffusion method. The Minimum Inhibitory Concentration (MIC) of the extracts was determined by Broth dilution method.

Results: The results of the study indicated that the pet-ether and chloroform extracts of the plant were highly effective towards most of the Gram-positive, Gram-negative bacteria and fungal strains. While, the methanol extract was moderately active towards all the tested organisms. The pet ether extract had lowest MIC of 1.562 mg/ml against *V. cholerae* and *Curvularia sp* and chloroform extract had lowest MIC of 1.562 mg/ml against only *C. neoformans*. The methanol extract showed MIC of 1.562 mg/ml against the organisms *S. pyogenes*, *C. neoformans* and *C. albicans*.

Conclusion: The potent antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungal strains may be due to the presence of broad spectrum antibiotic compound in the extracts. The study validates the use of the plant in the treatment of bacterial and fungal infections.

Keywords: *Leucas marrubioides* Desf., Antimicrobial activity, Agar well diffusion, MIC

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INTRODUCTION

Plants are the important source of new chemical substances with potential therapeutic effects. This is due to the fact that, plants have an almost limitless ability to synthesize chemical compounds of therapeutic value. The plant-based medicines are believed to be more acceptable to the human body when compared modern synthetic drugs. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, steroids, tannins and phenolic compounds [1]. The strength of biological activities of medicinal plants is dependent on the diversity and quality of these bioactive constituents.

Medicinal plants represent a rich source of antimicrobial agents [2]. Plants are known to produce a variety of compounds to protect themselves against a variety of their own pathogens and therefore can be considered as potential source of different classes of antimicrobial substances [3]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [4, 5]. They are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [6].

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to the indiscriminate use of broad-spectrum antibiotics, an immuno-suppressive agent, intravenous catheters, organ trans-plantation and ongoing epidemics of HIV infection [7, 8]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions [9]. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance [10], there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants.

L. marrubioides Desf. (fig. 1) belongs to Lamiaceae family. It is an aromatic herbaceous plant growing about a meter height and widely distributed in India, Srilanka and Indonesia. It is used in traditional medicine for the treatment of pain, wound, chronic skin eruptions, inflammation, and asthma. The leaves are used for the treatment of cobra venom [11]. In the traditional medicine, the plant is being used for the treatment of chronic skin eruptions, which may occur due to bacterial and fungal infections. Moreover, no scientific reports on antibacterial and antifungal activity of the plant have been reported. Thus, the scientific screening of the plant for antibacterial and antifungal activity will prove the medicinal efficacy of the plant. The objective of this study is to evaluate the antimicrobial potential of pet-ether, chloroform, and methanol extracts from roots of *L. marrubioides* against bacterial and fungal pathogens by agar diffusion method and broth dilution method.

Fig. 1: The plant *L. marrubioides* Desf

MATERIALS AND METHODS

Collection and identification of plant material

L. marrubioides was collected in Tunga river basin of central Western Ghats of Karnataka. The plant was authenticated in Dept. of studies and research in Applied Botany, Kuvempu University, Jnana Sahyadri, Shankaraghatta and voucher specimen (KU/SD/ TI/135) was deposited in the department for future reference.

Extraction of plant material

The roots of *L. marrubioides* were washed thoroughly 2 to 3 times with running tap water and once with sterile water. The material was shade dried, coarsely powdered and used for extraction. Weighed amount (500g) of the material was successively extracted using solvents of varying polarity namely, petroleum ether (pet-ether; 60-80 °C), chloroform and methanol using soxhlet extractor. Each extraction was carried out nearly 48 cycles. The extracts were filtered and concentrated using rotary flash evaporator under reduced pressure and at a controlled temperature. The extracts obtained were dried, packed and stored at 4 °C in the refrigerator [12].

Phytochemical analysis

All the extracts were subjected to preliminary phytochemical analysis using standard procedure to identify the various phytoconstituents [13].

Test microorganisms

All the microorganisms used were produced from National Collection of Industrial Microorganisms (NCIM), Pune, India. *S. aureus* (NCIM 2079), *B. cereus* (NCIM 2106), *S. pyogenes* (NCIM 2608), *S. epidermidis* (NCIM 2493), *B. subtilis* (NCIM 2699) were the five Gram-positive bacteria while, *P. mirabilis* (NCIM 224), *K. pneumoniae* (NCIM 7427), *E. aerogenes* (NCIM 2692), *S. flexneri* (NCIM 5265), *V. cholera* (NCIM 5316) were five Gram-negative bacteria. The fungal strains used were *A. flavus* (NCIM 524), *A. niger* (NCIM 501), *C. neoformans* (NCIM 3378), *Curvularia sp* (NCIM 905), *Trichosporon sp* (NCIM 3369) and *C. albicans* (NCIM 3100).

In vitro antibacterial assay

The antibacterial activity of the extracts was determined by agar well diffusion method. The 24-hr old nutrient broth cultures of test bacteria were swab inoculated on the surface of solidified nutrient agar plates. The agar wells of 8 mm diameter were made by using sterile cork borer. About 100-200 µl of 10% solution (100 mg/ml) of each extract in Dimethyl Sulphoxide (DMSO) were dispensed in separate well with the help of a micropipette. The plates are then incubated at 37 °C for 24 h. Penicillin and methicillin (1 mg/ml in DMSO) were used as positive controls for Gram-positive bacteria while, streptomycin and tetracycline (1 mg/ml in DMSO) were used as positive controls for Gram-negative bacteria. The zone of inhibition was measured in millimeters in millimeter scale after incubation [14]. The experiments were conducted in triplicates and the results were represented as mean±standard deviation.

In vitro antifungal assay

The antifungal activity of the extracts was determined by agar well diffusion method, but the nutrient mediums used were Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA). The agar well plates were swabbed with 72-hr old broth cultures of the respective fungi. The agar wells of 8 mm diameter were made by using sterile cork borer. About 100-200 µl of 10% solution (100 mg/ml) of each extract in DMSO was added into the separate well using a micropipette and allowed for diffusion at room temperature for 2 h. The plates are then incubated at 28 °C for 48-72 h. Griseofulvin and fluconazole (1 mg/ml in DMSO) were used as positive controls. The zone of inhibition was measured in millimeters after incubation [15]. The experiments were conducted in triplicates and the results are represented as mean±standard deviation.

Determination of minimum inhibitory concentration (MIC)

To assess the Minimum Inhibitory Concentration (MIC) of all the extracts, broth dilution test was carried out with the concentration range 0.781, 1.562, 3.125, 6.25, 12.5 and 25 mg/ml in DMSO respectively. Series of 5 ml nutrient broth tubes were inoculated separately with 1 ml of broth cultures of test organisms. 1 ml of different concentrations of all the extracts was transferred separately to each set of tubes. For bacteria, the tubes were incubated at 37 °C for 24 h and for fungi, the tubes were incubated at28 °C for 48-72 h. Nutrient broth tubes with extracts and without extracts were used as controls. The absorbance of each tube was measured at 600 nm by using colorimeter (Equiptronics EQ-652, India). Increase or decrease in turbidity of the tubes was considered for determining the MIC. The lowest concentration with less absorbance was taken as MIC of that extract.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of crude extracts of *L. marrubioides* revealed the presence of various phytochemical constituents. The analysis showed the presence of alkaloids, steroids, triterpenes in pet ether extract, alkaloids, flavonoids, steroids, triterpenes, tannins and phenolics in chloroform extract, while flavonoids, triterpenes, tannins, phenolics, carbohydrates and glycosides in methanol extract.

Antibacterial activity

The results of antibacterial activity of pet-ether, chloroform, methanol extracts of *L. marrubioides* and the standard antibiotics are as shown in table 1. The pet ether extract was very much effective against Gram-positive bacteria *B. cereus*, *S. pyogenes*, *B. subtilis* and Gram-negative bacteria *P. mirabilis*, *K. pneumoniae*, *V. cholerae*. The pet ether extract showed considerable activity towards *S. aureus*, *S. epidermidis*, *E. aerogenes* and *S. flexneri*. The chloroform extract was potent against Gram-positive bacteria *S. pyogenes*, *S. epidermidis*, *B. subtilis*, Gram-negative bacteria *P. mirabilis*, *K. pneumoniae* and *V. cholerae*. The extract was also effective against *S. aureus*, *B. cereus*, *E. aerogenes* and *S. flexneri*.

The methanol extract showed a moderate growth inhibitory activity against all the tested organisms when compared to pet-ether and chloroform extracts.

Table 1: Antibacterial activity of *L. marrubioides* root extracts against gram positive and gram negative bacteria

S. No.	Type of bacteria	Zone of inhibition in mm						
		Pet-ether extract	Chloroform extract	Methanol extract	Penicillin	Methicillin	Streptomycin	Tetracycline
1	*S. aureus*	18.3±0.88	20.6±0.58	14.6±0.88	00	12.6±0.67	-	-
2	*B. cereus*	24.6±1.20	21.6±0.58	13.6±0.33	10.6±0.67	00	-	-
3	*S. pyogenes*	22.3±0.33	26.0±1.15	16.0±1.0	12.6±1.20	14.3±0.33	-	-
4	*S. epidermidis*	19.3±0.67	27.3±0.67	13.6±0.67	00	28.0±1.53	-	-
5	*B. subtilis*	21.3±0.88	31.0±1.73	12.6±1.20	17.3±0.88	00	-	-
6	*P. mirabilis*	23.3±1.45	26.3±0.88	13.0±0.58	-	-	00	25.0±1.0
7	*K. pneumoniae*	23.0±1.15	24.0±1.73	12.6±0.67	-	-	16.0±1.0	00
8	*E. aerogenes*	12.3±0.88	19.3±1.45	11.3±0.58	-	-	15.3±0.33	12.6±0.67
9	*S. flexneri*	14.6±0.67	17.3±0.33	10.3±0.33	-	-	16.6±0.67	00
10	*V. cholerae*	23.0±0.58	28.3±1.45	13.3±0.33	-	-	23.0±0.58	00

Zone of inhibitions are mean±standard deviation of triplicates. The diameter of well plates was 8 mm, '-': Not tested

Antifungal activity

The results of antifungal activity of all the extracts and standard antibiotics are given in table 2. The pet-ether, chloroform and methanol extracts of *L. marrubioides* were found to be most effective against the fungal strains *C. neoformans*, *Curvularia sp* and *C. albicans*. All the three extracts also showed considerable activity against *A. flavus*, *A. niger* and *Trichosporon sp*.

Table 2: Antifungal activity of *L. marrubioides* root extracts against fungal strains

S. No.	Type of fungi	Zone of inhibition in mm				
		Pet-ether extract	Chloroform extract	Methanol extract	Griseofulvin	flucanazole
1	*A. flavus*	18.6±1.15	22.3±1.45	21.6±0.58	00	10.6±1.15
2	*A. niger*	17.3±0.88	16.0±1.0	21.3±.088	15.6±1.20	00
3	*C. neoformans*	26.0±1.0	30.6±1.76	22.3±0.33	12.3±0.58	14.3±0.67
4	*Curvularia sp*	40.3±2.03	41.3±2.03	30.6±1.20	00	25.3±1.45
5	*Trichosporon sp*	20.3±0.88	23.3±1.52	28.0±1.00	00	28.3±1.45
6	*C. albicans*	32.0±1.15	37.3±1.45	29.6±1.20	17.3±1.52	00

Zone of inhibitions are mean±standard deviation of triplicates. The diameter of well plates was 8 mm, '-': Not tested

Minimum inhibitory concentration (MIC)

The MIC of all the extracts was assessed by broth dilution test with the concentration range 0.781 to 25 mg/ml. Only those organisms, which were highly susceptible to the extracts, were selected for determining the MIC. Therefore, the MIC of all the extracts on bacterial strains *S. pyogenes*, *B. subtilis*, *V. cholerae* and fungal strains *C. neoformans*, *C. albicans* and *Curvularia sp* were determined. The MIC values of all the three extracts are given in table 3.

Table 3: Minimum inhibitory concentration (MIC) of *L. marrubioides* root extracts on bacterial and fungal strains

Extracts	MIC (mg/ml)					
	Bacteria			Fungi		
	B. subtilis	*S. pyogenes*	*V. cholerae*	*C. neoformans*	*Curvularia sp*	*C. albicans*
Pet-ether	3.125	6.25	1.562	3.125	1.562	3.125
Chloroform	6.25	6.25	3.125	1.562	12.5	6.25
Methanol	3.125	1.562	6.25	1.562	6.25	1.562

The results of pet ether extract indicated that the *V. cholerae* and *Curvularia sp* were the most sensitive organisms with the lowest MIC value of 1.562 mg/ml followed by *B. subtilis*, *C. neoformans* and *C. albicans* with MIC 3.125 mg/ml. The chloroform extract was highly susceptible against *C. neoformans* and *V. cholerae* with MIC of 1.562 mg/ml and 3.125 mg/ml respectively. *B. subtilis*, *S. pyogenes*, *C. albicans* were moderately sensitive to chloroform extract with MIC of 6.25 mg/ml whereas, *Curvularia sp* was less sensitive with MIC of 12.5 mg/ml. The methanol extract was highly sensitive towards *S. pyogenes*, *C. neoformans*, *C. albicans* with lowest MIC value of 1.562 mg/ml and on *B. subtilis* with MIC of 3.125 mg/ml. *V. cholerae* and *Curvularia sp* have MIC of 6.25 mg/ml indicated that they were moderately sensitive to methanol extract when compared to other organisms.

DISCUSSION

The antimicrobial activity of various plants has been reported by many researchers [16]. Phyto-constituents present in plants namely alkaloids, flavonoids, tannins and triterpenoids are producing an exciting opportunity for expansion of modern therapies against a wide range of microorganisms [17]. In the present study, a variety of Gram-positive, Gram-negative bacteria, and fungal strains were selected for screening antimicrobial effect of three extracts to perceive antimicrobial spectrum. The results of this study showed that the pet-ether and chloroform extracts of the plant *L. marrubioides* were highly effective towards most of the Gram-positive bacteria, Gram-negative bacteria, and fungal strains. But, the methanol extract was moderately active towards all the organisms tested. When the MIC values of extracts are compared, the pet ether extract had lowest MIC of 1.562 mg/ml against two organisms *V. cholerae*, *Curvularia sp* and chloroform extract had lowest MIC of 1.562 mg/ml against only *C. neoformans*. Methanol extract though it has showed a moderate zone of inhibition in agar well diffusion method, had a lowest MIC value of 1.562 mg/ml against three organisms namely *S. pyogenes*, *C. neoformans* and *C. albicans*.

Phytochemical constituents such as tannins, alkaloids, flavonoids, phenolic compounds and several other aromatic compounds are secondary metabolites of plants that serve as a defense mechanism against predation by many microorganisms [18]. The demonstration of antimicrobial activity against both Gram-positive and Gram-negative bacteria and on various fungal strains may be indicative of the presence of broad spectrum antibiotic compounds in the extracts [19].

CONCLUSION

The demonstration of a broad spectrum of the antibiotic property of *L. marrubioides* extracts may help to discover new chemical class of antibiotic compounds that could serve as selective agents for infectious diseases in chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption. The effect of this plant on more pathogenic organisms, toxicological investigation and further purification of effective extract, however, needs to be carried out.

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CONFLICT OF INTERESTS

Declared none

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