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Evaluation Of Antimicrobial Activities On *Plectranthus Barbatus L.* Tuber

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ABSTRACT

The Lamiaceae family contains several medicinal plants, widespread tendency in folk medicine. This study presents antibacterial activities evaluation of *P. barbatus* in different extracts obtained were tested for their antimicrobial activity against three bacterias namely *B. cereus*, *E. coli* and *L. bacillus* . The highest antimicrobial activity was exhibited by the aqueous and acetonic extracts in almost all the pathogenic bacterias using the well diffusion method. In the chloroform root extracts observed the maximum inhibition were (26.33±1.45) were at 80 µl on *E.coli*, in the aqueous root extract maximum inhibition (25.5±1.38) were observed on at 80 µl on *E. coli.* In acetone root extracts (22.83±1.19) were at 80 µl on *B. cereus*, petroleum ether root shows the maximum inhibition (22.0±1.06) at 80 µl on *L. bacillus.* The aqueous extracts were much active against all the bacteria tested. The antibacterial activity shows that the zone of inhibition is increasing with the increasing concentrations in all the extracts.

Key words: Antimicrobial, Antifeedant, Diterpene and *Plectranthus.*

INTRODUCTION

Traditional herbalists in India use a variety of herbal preparations to treat different kinds of aliments including many microbial infections. In fact, the rural dwellers are virtually left with no alternative other than to patronize the herbal practitioners. Plants with medicinal properties have been commonly used as remedies for many infectious diseases, such as digestive and intestinal disorders, malaria and tuberculosis (Tekwu *et al.,* 2012). The natural plant products could be potential alternatives for controlling the pathogen associated with diseases. Natural products and their derivatives more than 50% of the drugs in clinical use in the world (Cowan, 1999). India represents by rich culture, tradition and natural biodiversity offers a unique opportunity for drug discovery researches. In recent years a number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants.

The genus *Plectranthus* it comprises about eighty species distributed worldwide. *Plectranthus* species contain many chemical compounds and exhibit several effects such as anti-inflammatory, antimicrobial and antifungal activities (Neusa *et al.,* 2014). In Northeast of Brazil *Plectranthus* is used in the treatment of digestive problems (Albuquerque *et al.,* 2007). In Europe, namely in Portugal, several *Plectranthus* species are cultivated as ornamentals.

Plectranthus barbatus L. (Sny: *Coleus barbatus*) is a perennial plant that grows to 45- 60 cm height. It has four angled stem and are often with hairy. The leaves are 7 to 12 cm in length, usually, its leaves are ovate and glandular hairy. Inflorescence is raceme, 15 -30 cm length. The flowers are purple or pale blue in colour. The root is typically golden brown, thick, fibrous and radially spreading. Roots are tuberous and fasciculate, conical fusiform straight orange red with strong aromatic odor. The flowering season is from August to September. In this medicinal plant *P. barbatus* is used as a remedy for stomachache and as a purgative, it is also resistant to insect attack, and an aphid antifeedant diterpene has been isolated from it (Kubo *et al.,* 1984). The stem root are to be used as a cure for gastric problems. They help in digestive system (Kiritkar and Basu, 1991). In East Africa *P. barbatus* is used as a remedy for stomach-ache (Abdel-Mogib *et al.,* 2002). Traditionally the roots have been used for preparation as condiment in pickles and preparation of pickles (Anonymous, 1950) and also for medicinal purposes by the Ayurvedic schools of

medicines. Root juice is given to children suffering from constipation. Roots are eaten for curing cough in Kumaon Himalayas and one to three teaspoonful of root decoction is recommended for treatment of asthma in Maharashtra. The therapeutic properties of this volatile oil in skin care are anti-inflammatory, antichloristic, antiseptic astringent, cicatrisant, cytophylactic, diuretic and tonic and the fresh leaves have medicinal value and are used as a decoction with other drugs to treat nausea, diarrhea, cold and headache (Arpana, 2008).

MATERIALS AND METHODS

Collection of Plant materials

The fresh Plant with tuber of *Plectranthus barbatus* were collected from January 2014 from China Salem, Salem District Tamilnadu and being identified by Dr. Karmegam, Department of Botany, Government Arts College Salem. Voucher specimen were deposited in Department of Botany, Government Arts College Salem.

Preparation of extracts for antimicrobial activity

Aqueous extract preparation

The *Plectranthus barbatus* tubers were washed with running tap water and dried for 30 minutes, then it was cut into small pieces, dried in room temperature for two weeks, grounded into powder with the help of hand mill and stored in room temperature, the 10gm of powdered tuber were macerated in water (50 ml) at 70°C for 120 min. This process was repeated twice. The extract obtained was filtered and lyophilized. To perform the assays the extracts were solubilised in distilled water and sterilized by filtration through a 0.2µ membrane filter (Microclar).

Preparation of different solvent extracts

The *P. barbatus* tubers were carefully washed with tap water, rinsed with distilled water, and air dried for one hour. Then it was cut into small pieces, dried in room temperature for two weeks, grounded into powder with the help of hand mill and stored in room temperature. The tuberous powder was macerated solvents including acetone, petroleum ether and chloroform extracts in a 1:3 proportion at room temperature, undergoing mechanical shaking for 4 hours followed by filtration. The extracts obtained were concentrated in a rotary evaporator at 40°C and the residue was extracted twice again analogously, thereby obtaining the crude solvent extracts. The concentrated extracts were weighed and preserved for further use (Vimalraj *et al.,* 2009)

Test Microorganisms

The microorganisms used in this study includes *Bacillus cereus, Escherichia coli* and *Lacto bacillus* obtained from the Department of Microbiology, Nandha Arts and Science College Erode. The bacterial strains were cultured on respective selective media and stored at 20°±2°C.

Preparation of inoculum

Exactly 18 hour broth culture of the test bacteria isolates was suspended into sterile nutrient broth and were standardized according to National Committee for Clinical Laboratory Standards (NCCLS) by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0×10^6 CFU/ml.

Antimicrobial assay – Well diffusion method

The modified agar well diffusion method was employed to determine the antibacterial activities**.** About 0.2 ml of the standardized 24 hour old broth culture of the test organisms were spread onto sterile Muller Hinton Agar plates. These were then allowed to set. With the aid of a sterile cork borer, wells of about 6 mm in diameter were bored on the plates. Different concentrations (40µl, 60µl and 80µl) of aqueous, acetone and chloroform and petroleum ether extracts were dispensed into the wells and then allowed to stand for about 15 minutes for pre diffusion of the extracts to occur. The plates were then incubated at 37^0C for 24 hours. At the end of the incubation period, inhibition zones formed on the agar plates were observed, measured and tabulated for various bacterial strains used.

RESULTS AND DISCUSSION

The results of the screening of crude *P. barbatus* tuber of aqueous, acetone and chloroform and petroleum ether extracts about antimicrobial activities are presented in Table 1.The inhibition of *B. cereus E. coli* and

S. NO	Root extract	Zone of Inhibition in diameter (mm)								
		Bacillus cereus			*Escherichia coli*			*Lacto bacillus*		
		40 µl	60 µl	80 µl	40 µl	60 µl	80 µl	40 µl	60 µl	80 µl
1	Aqueous root	13.50±0.76	15.83±0.94	21.33±1.33	18.83±1.40	20.33±1.14	25.5±1.38	21.8±1.13	23.16±1.01	23.66±1.14
2	Acetone root	10.10±0.96	14.16±0.79	22.83±1.19	9.66±0.66	13.66±0.88	19.83±1.74	10.0±1.09	13.5±1.15	17.00±0.96
3	Petroleum ether root	08.66±0.88	10.5±0.76	21.66±1.14	12.66±0.98	14.0±0.96	17.33±1.52	07.83±0.47	11.5±1.33	22.0±1.06
4	Chloroform root	13.50±1.05	16.33±1.45	22..83±1.47	10.5±0.76	13.5±0.88	26.33±1.45	9.16±1.01	16.83±1.01	20.66±1.11

L. bacillus were studied. The results showed that the aqueous root extracts showed much significant antibacterial activity, increasing in an extract concentration against all the bacteria strains were used. The maximum inhibition (25.5±1.38) were observed on aqueous root extract at 80 µl on *E. coli* (Plate 1B) followed by 23.16±1.01 at 80 µl of aqueous root extract against *L. bacillus*, (Plate 1C) and the same extract on *B. cereus* the inhibition were 21.33±1.33 at 80 µl table 1, (Plate 1A). Comparing with our results with aqueous leaves extracts of the *Plectranthus* species showed similar result on bacterias like *Streptococcus sobrinus* and *Streptococcus mutans* (Figueiredo *et al.,* 2010).

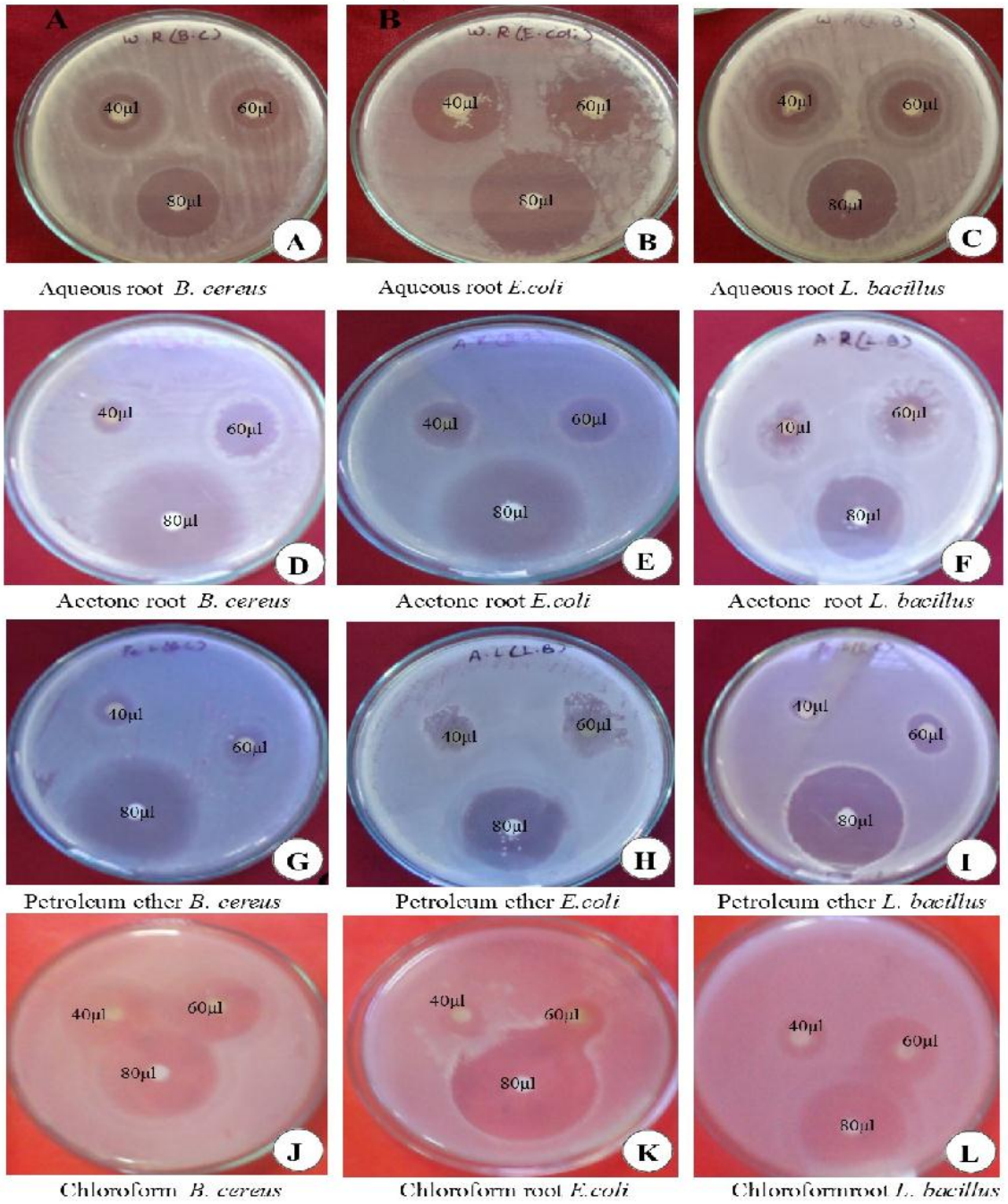
Table – 1 Antibacterial activity of aqueous acetone petroleum ether and chloroform extracts of *Plectranthus barbatus*.

In the maximum inhibition in acetone root extracts (22.83±1.19) were at 80 µl on *B. cereus* (Plate 1D) followed by 19.83±1.74 at 80 µl of acetone root extract against *E.coli*, (Plate 1E) and in the *L. bacillus* inhibition were 17±0.96 at 80 µl on the same extract. (Plate 1F). Diterpenes, a type of natural products widespread in the botanical Lamiaceae family, are either well represented in the *Plectranthus* genus. The diterpenes, owing to a great structural variability and a diverse range of oxidized patterns may be promising sources of antimicrobial prototype compounds (Simoes, 2010). *P. barbatus* petroleum ether root shows the maximum inhibition (22.0±1.06) were observed on aqueous root extract at 80 µl on *L. bacillus* (Plate 1I) followed by 21.66±1.14at 80 µl of aqueous root extract against *B. cereus,* (Plate 1G) and the same extract on inhibition on *E. coli* were 17.33±1.52 at the 80 µl table 1, (Plate 1H). Comparing with our report (Neusa *et al.,* 2014) reported the aqueous extract were responsible for the high antibacterial activity reported, methanol extracts from *P. barbatus* and *P. ecklonii* leaves showed high inhibitory effect on growth and viability of *Streptococcus sobrinus*, and *S. mutans* bacteria.

In the chloroform root extracts observed the maximum inhibition were (26.33±1.45) were at 80 µl on *E.coli* (Plate 1K) followed by 22..83±1.47at 80 µl of chloroform root extract against *B. cereus,* (Plate 1J) and in the inhibition were 20.66±1.11at 80 µl on the same extract against *L. bacillus*. (Plate 1L). (Stavri *et al.*, 2009), have investigated the chemistry and antibacterial activity of the extracts from *Plectranthus ernstii* and isolated three antimicrobial diterpenes. Another report to the ability of some *Plectranthus* species to produce antimicrobial metabolites led to several phytochemical studies (Gibbons, 2008). Supporting to our report in Brazil, the leaves of *P. ornatus* and *P. barbatus* were used for stomach and liver diseases used for their antibiotic and anti-inflammatory properties (El Masri *et al.*, 2007). In contrary to our report that the antibacterial activity of the extracts of five species of the *Plectranthus* genus on five bacteria was evaluated

among the studied extracts only the acetonic ones exhibited antibacterial activity and the remaining aqueous extracts were not active against the strains tested. The most pronounced activity, with inhibition zones higher than 20 mm, was shown by the acetonic extract of *P. madagascarensis* against all three Gram-positive bacteria tested. The *P. hadiensis* extract only exhibited activity against *B. subtilis* while the *P. verticillatus* extract showed antimicrobial activity against *S. aureus* and *B. subtilis* (Patrícia Rijo *et al.*, 2012).

PLATE – 1- Antibacterial activity of different extracts of *Plectranthus barbatus* root



CONCLUSIONS

In the present study, species of *P. barbatus* were analyzed to evaluate their antimicrobial activities. Different concentrations were considered for each extraction solvent to search for significant changes. It was revealed that the four extraction solvents studied, aqueous, acetone, petroleum ether and chloroform root extracts have different biological activities. The aqueous extracts were much active against all the bacteria tested.

The antibacterial activity shows that the zone of inhibition is increasing with the increasing concentrations in all the extracts.

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