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Anti-Bacterial Activity of *Coleus forskohlii* Extracts against Some Pathogenic Bacteria

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ABSTRACT

Medicinal plants are rich source of antibacterial agents and curatives. Coleus forskohlii (Lamiaceae), a traditional medicinal plant of South India, has the source of bio-reductant and stabilizers. In the present study, In vitro antibacterial activity of crude extracts of root, shoot and leaves of C. forskohlii were screened using ethanol, methanol, chloroform, petroleum ether, hexane and hot water. The plant extracts were tested by disc diffusion method against seven pathogenic bacteria i.e. Bacillus subtilis, Pseudomonas fluorescence, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus and Streptococcus pneumoniae. Gram-negative bacterial strains were more susceptible to the crude extracts as compared to gram-positive. The extracts of C. forskohlii exhibited maximum growth inhibition and remarkable effectiveness. These results indicate that the extracts of coleus root, shoot and leaves have a potential broad spectrum antibacterial activity.

Key words: *Coleus forskohlii*, Antibacterial activity, Pathogenic bacteria, Disc diffusion.

INTRODUCTION

Plants are the first medicines for mankind and hundreds of plant species are harvested for their medicinal properties all over the world. In spite of modern development of sophisticated pharmaceutical chemicals to treat illnesses, medicinal plants remain an important tool for treating illness. Medicinal plants are important for pharmacological research and drug development, not only when constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds [1]. In the recent past antimicrobial, antioxidant and anti-inflammatory activities of some plants were investigated [2,3,4].

Coleus forskohlii (wild) Briq [Syn. *C. barbatus* (Andr.) Benth.] is a plant of Indian origin [5] and belongs to the family Lamiaceae (previously Labiatae). It is the most important species of genus *Coleus* popularly known as Mainamool or Manganiberu or Makandi beru in Karnataka and Garmar in Maharashtra. It is distributed in sub-tropical Himalayas from Gharwal to Nepal up to an altitude of 2500m above mean sea level including Pakistan and Sri Lanka. Traditionally, the roots have been used for medicinal purposes by the Ayurvedic schools of medicines [6]. The therapeutic properties of the coleus volatile oil in skin care are anti-inflammatory, antiphlogistic, antiseptic, astringent, cicatrisant, cytophylactic, diuretic and tonic. The fresh leaves have medicinal value and are used as a decoction with other drugs to treat nausea, diarrhea, cold and headache [7].

In India the coleus crop is cultivated in several parts of Gujarat, Maharashtra, Rajasthan, Karnataka, Tamil Nadu and Andhra Pradesh. It is being grown in an area of more than 2500 hectares for its tuberous roots. Being the source of important constituents source of many medicinal plants challenges the modern medicine and throws open

stimulating opportunity for the expansion of modern chemotherapies against wide range of microorganisms [8,9]. Due to the increasing failure of chemotherapeutics and rapid development of multi resistant bacterial strains of clinically important medical pathogens acquired the interest of scientist to develop newer broad spectrum antimicrobial agents [10]. The less availability and unaffordable cost of new generation antibiotics initiated to look for alternative phytomedicine to discover plant derived constituents with claimed antimicrobial activity. The extractable bioactive compounds in medicinal plants are a significant alternative approach to synthetic antibiotics, which could be used as valuables in human disease management. Many herbs with significant antimicrobial activity have been reported in different traditional literatures [11,12]. The objective of this research is to authenticate the antibacterial activity of the extracts obtained from the various parts of *Coleus forskohlii.*

MATERIALS AND METHODS

Preparation of crude extracts

The *Coleus forskohlii* plant was collected from CIMAP centre, Uppal, Hyderabad, A.P., India. The root, shoot and leaf of the plant was removed and washed with fresh water and dried under shade at room temperature, cut into small pieces and powdered in a mixer grinder. The powdered root, shoot, leaves were stored in sterile containers for further use. Each of the powdered air-dried plant material was extracted with organic solvents *i.e*. ethanol, methanol, chloroform, petroleum ether, hexane and hot water. 25g of each powdered sample was mixed in a conical flask with 100ml of organic solvent, plugged, then shaken at 120rpm for 30min and kept for 24h. After 24h, each of the extracts was filtered rapidly through four layers of gauge and then through Whatman No.1 filter paper. The crude samples were assayed for antibacterial activity.

Bacterial strains

Medically important bacterial strains of *Bacillus subtilis, Pseudomonas fluorescence, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae* were procured from MTCC (IMTECH), Chandigarh, India. The bacterial strains were maintained in nutrient agar, routinely sub-cultured. These bacteria were used as test pathogens for antibacterial activity assay.

Screening for antibacterial activity

The antibacterial assay was performed by agar disc diffusion method [13]. All the microbiological media used in this experiment were obtained from Hi-media Laboratories, Mumbai. Overnight cultures were prepared by inoculating approximately in 2ml nutrient broth with 2–3 colonies of each organism taken from nutrient agar. Broths were incubated overnight at 30°C with shaking. The suspension of tested bacterial strains (0.1ml) was spread on the nutrient agar plates. Filter paper discs (6mm in diameter) were impregnated in 20µl of the plant extracts and dried aseptically. The discs were placed on the bacterial lawn of agar plates and incubated at 30ºC for 24h. The diameters of the inhibition zones were measured using a scale in millimeters (mm). Experiments were performed in triplicates to obtain standard results and the maximum zone of inhibition (ZOI) against the pathogens were noted.

RESULTS AND DISCUSSION

In the recent years there are many research works reported on the antibacterial activity of plant extracts on human pathogenic bacteria [14,15]. The present study also revealed the antibacterial potential and ethno medicinal claims for *Coleus forskohlii.* The results of the antibacterial activity of *C. forskohlii* crude extracts, assayed *in vitro* by the disc diffusion method on *Bacillus subtilis, Pseudomonas fluorescence, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae* are presented in Table 1. All the tested pathogens were highly susceptible to the crude extracts. However, the antibacterial activity was more pronounced observed against gram-negative bacterial strains than gram-positive strains.

Use of different solvent extracts is also an important approach to isolate higher active compounds from the plants. Many studies suggested that different solvent extracts of various plants has tremendous biological activity. Such an effective extract can be subjected to isolation of the therapeutic compounds and antimicrobials for further pharmacological studies [16]. In the present study, the different plant extracts i.e. root, shoot, leaf with different organic solvents (ethanol, methanol, chloroform, petroleum ether, hexane and hot water) were found to have maximum antibacterial activity. The results obtained different zone of inhibition in the evaluation of the antibacterial activity of the different extracts against the tested bacteria. The most effective activity was by root extract (hexane) of *C. forskohlii* with maximum zone of inhibition ranging from 15mm against *Streptococcus*

pneumoniae and12mm with *Pseudomonas aeruginosa.*10mm and 12mm zone of inhibition was found in the ethanol leaf extract on *Klebsiella pneumoniae* and *Staphylococcus aureus.* 12mm and 10mm zone of inhibition was found in the ethanol, methanol shoot extract on *Klebsiella pneumoniae and Pseudomonas aeruginosa.* All the 3 methanol extracts inhibited *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with 10mm respectively. Though all the 3 extracts were found effective, the highest zone of inhibition and the effectiveness is the major consideration in the case of antibacterial activity.

In comparison, the maximum growth inhibition was observed in the root extracts of *C. forskohlii.* Ethno botanical approach is one of the universal practices applied in choosing the plants for pharmacological study [17]. Although, these plant extracts declared the antibacterial activity against 7 medically important human pathogens, to support this claim on the basis of scientific origin, the rate and extent of bacterial killing (kill kinetics) - Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentrations (MBCs) are the matters under study. These results indicate that root, shoot and leaves of *C. forskohlii* have a potential broad spectrum antibacterial activity. In future, these extracts can be combined as a formulation to treat the infectious diseases caused by the test organisms. The results are in accordance with the previous studies [15,18].

Table 1. Inhibitory effect of leaf, shoot, root crude extracts of *Coleus forskohlii* against various pathogenic bacteria

Solvent	Zone of inhibition (mm)																				
	Bacillus subtilis			*Pseudomonas fluorescence*			*Pseudomonas aeroginosa*			*Klebsiella pneumoniae*			*Escherichia coli*			*Staphylococcus aureus*			*Streptococcus pneumoniae*		
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L
Methanol	8	8	2	4	6	4	5	10	6	7	10	7	4	7	3	3	4	4	3	4	4
Ethanol	-	5	7	-	4	7	13	7	7	8	12	12	7	-	7	-	-	10	3	4	4
Hexane	-	-	-	5	-	2	12	4	5	6	6	5	-	7	-	6	-	3	15	6	-
Chloroform	2	1	4	5	5	-	7	5	6	5	4	4	-	-	4	3	3	3	3	4	4
Petroleum ether	-	-	1	4	-	6	6	5	5	5	6	6	-	-	5	7	3	-	5	3	2
Hot water	-	-	-	2	-	-	-	-	4	6	-	-	5	-	-	2	-	-	-	-	-
control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

R= root; S= shoot; L= leaf

CONCLUSION

In the present study crude extracts of the plant material obtained in polar and less polar organic solvent were tested against seven standard bacteria (*Bacillus subtilis, Pseudomonas fluorescence, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae).* The observations showed a clear-cut view about the antibacterial activity of different extracts. All extracts were effective against all of the tested bacteria. Ethanol extract was showing very good inhibitory effect against these bacteria. From the present study, it is evident that *Coleus forskohlii* could play an important role in finding new clinically effective antibacterial drug and could be useful in understanding the relationship between traditional cures and current medicines.

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