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IN VITRO ANTIBACTERIAL ACTIVITY OF CRUDE LEAF EXTRACTS FROM *TECOMA STANS (L)* JUSS. ET KUNTH, *COLEUS FORSKOHLII* AND *POGOSTEMON PATCHOULI* AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT: Medicinal plants are the wealthy source of antibacterial agents and curatives. *Tecoma stans, Coleus forskohlii* and *Pogostemon patchouli* are commonly practiced medicinal plants in the villages of Salem District, Tamilnadu (India). Plants grown in this region are not systematically tested for their biological activities in general and antimicrobial activity in particular. Hence, *In vitro* antibacterial activity of crude leaf extracts of these 3 plants was tested by disc diffusion method against 5 human pathogenic bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis, Salmonella typhi, Klebsiella pneumoniae* and *Vibrio parahemolyticus*. Gram-negative bacterial strains were more susceptible to the crude extracts as compare to gram-positive. However, this study revealed maximum growth inhibition and effectiveness was remarkably observed in the extracts of *Coleus forskohlii, Pogostemon patchouli* and then in *Tecoma stans*. These results indicate that leaves have a potential broad spectrum antibacterial activity.
Keywords: *Tecoma stans, Coleus forskohlii*, *Pogostemon patchouli*, Antibacterial activity, Human pathogens, Disc diffusion

INTRODUCTION

Scientific interest in phytomedicine has burgeoned due to increased efficacy of new plant-derived drugs, emerging interest in natural products and increasing concerns about the side effects of conventional medicine. Even many literatures describes that modern medicine has emerged from the origin of traditional herbal medicine. Ancient peoples used medicinal plants as herbal remedies to cure all mankind ailments. The ancient indigenous practice of combining and concentrating several plants as decoction (extracting together in boiling water) to treat the whole person and focused different organ system along with the presenting complaint[1]. Many herbs comprise remarkable properties and functions on multiple biochemical pathways capable to control several organ systems simultaneously. No doubt, many medicinal herbs still holds valuable active compounds of medicinal value which have yet to be discovered. The need of the hour is to screen enormous medicinal plants for its potential biological activity. On the basis of traditional use, Herbs are selected and combined for their ability to inhibit microbial growth in various part of the body and support organ systems responsible for detoxification and immune function. Herbal medicine is also renowned as Phytomedicine – the use of whole plants or part of plants such as seeds, berries, roots, leaves, barks and flowers to prevent or treat illness. A survey of World Health Organization (WHO) indicates that about 70-80% of the world population in the developing countries depends on herbal sources as their primary healthcare system[2, 3]. Phytoconstituents such as flavonoids, alkaloids, tannins and triterpenoids are rich source of many medicinal plants challenges the modern medicine and stimulating opportunity for the expansion of modern chemotherapies against wide range of microorganisms[4, 5]. Due to the increasing failure of chemotherapeutics and rapid development of multiresistant bacterial strains of clinically important medical pathogens acquired the interest of scientist to

develop newer broad spectrum antimicrobial agents[6, 7]. 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[8, 9]. *Tecoma stans, Coleus forskohlii* and *Pogostemon patchouli* are commonly practiced in managing some ailments of microbial origin in the villages of Salem District, Tamilnadu (India). Plants grown in this region are not systematically tested for their biological activities in general and antimicrobial activity in particular. Hence considering the aforesaid, this study is aimed to explore the antibacterial potential of three traditionally used medicinal plants belonging to two different families to substantiate the folklore claims. *Tecoma stans* (L). Juss. et Kunth (Family: Bignoniaceae) is a growing evergreen shrub noted for its bell shaped yellow flowers. It is commonly referred as Ginger - Thomas and Yellow bells. It has naturalized in much of tropical and subtropical Africa, Asia, the Pacific islands, and Australia[10]. The plant has ethno medicinal importance for the treatment of arterial hypotension, hypoglycemia and diabetes[11, 12]. The leaves contain active alkaloids such as Tecomine and Tecostanine[13, 14]. *Coleus forskohlii* (Family: Lamiaceae) is a perennial herb belongs to the part of the mint family of plants and has long been cultivated in India, Thailand and parts of South east Asia as a spice and as a condiment for heart ailments and stomach cramps. Mainly it is used in the treatment of eczema and psoriasis. The roots of the plant are a natural source of forskolin – the only plant-derived compound presently known to directly stimulate the enzyme adenylate cyclase, and subsequently cyclic AMP. Many other diterpenoids as deacetyl forskolin, 9-deoxyforskolin, 1,9-deoxyforskolin, 1,9-dideoxy-7-deacetylforskolin have been isolated. Other minor phytochemicals are Allylroyleanone, Barbatusin, Plectrin, Plectirinon A, Acetoxycoleosol, Coleol, Coleonone, Coleosol, Deoxycoleonol, Crocetin dialehyde, Napthopyrones[15]. *Pogostemon patchouli* (Family: Lamiaceae) is a tender perennial herb that hails from Indonesia, Malaysia, the Philippines, and southern China. It is widely grown for Patchouli oil that is used in perfumery. The main constituents are ß-patchoulene, Caryophyllene, Seychellene, Norpatchoulenol, Patchouli alcohol and Pogostol. The therapeutic properties of this 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[16]. The objective of this research was to authenticate the antibacterial activity of the crude extract of crude extract obtained form the leaves of *Tecoma stans, Coleus forskohlii* and *Pogostemon patchouli* against some selected pathogenic bacteria to extend the list of antimicrobial herbs.

MATERIALS & METHODS

Plant materials

Fresh explants were directly obtained and authenticated from the Horticultural Research Centre, Yercaud, Salem, India. Leaves and roots of *Tecoma stans (L)* Juss. et Kunth, *Coleus forskohlii* and *Pogostemon patchouli* were taken for investigation of antibacterial property. Fresh plant materials were washed under running tap water, air dried in shade and then homogenized to make fine powder.

Preparation of Crude extracts

10g of air dried plant powder of each plant was mixed with distilled water and boiled on slow heat for 2 hours. The boiled decoction was then filtered through 8 layered muslin cloth and centrifuged at 5000g for 10 min and collected the supernatant. The above procedure was repeated twice. After 6 hours, the supernatant collected at an interval of every 2 hours, was pooled together and concentrated to make the final volume[17]. Then the extracts were filter sterilized and stored at 4ºC for future use.

Bacterial strains

Medically important bacterial strains used in this study were *Staphylococcus aureus*, *Staphylococcus epidermidis, Salmonella typhi, Klebsiella pneumoniae* and *Vibrio parahemolyticus* procured previously by our department from MTCC (IMTECH), Chandigarh, India. The bacterial strains were maintained in nutrient agar, routinely sub-cultured. These bacteria served as test pathogens for antibacterial activity assay.

Screening for antibacterial activity

The antibacterial assay performed by agar disc diffusion method[18]. 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 35°C with shaking. Inocula were prepared by diluting overnight bacterial cultures approximately 10^8 cells per ml in sterile saline. The suspension of tested bacterial strains (0.1 ml of 10^8 cells per ml) was spread on the Muller-Hinton agar plates[19] and nutrient agar plates. Filter paper discs (6 mm in diameter) were impregnated in 20 µl of the plant extracts and dried aseptically. The discs are placed on the bacterial lawn of agar plates and incubated at 37ºC for 24 h. The diameters of the inhibition zones were measured using a scale in millimeters (mm) from the size of clear zone larger than 0.6 mm. Experiments were performed in

triplicates to obtain a standard results and the maximum zone of inhibition (ZOI) against the pathogens were noted.

RESULTS

The results of the antibacterial activity of *Tecoma stans, Coleus forskohlii* and *Pogostemon patchouli* crude extracts, assayed *in vitro* by the disc diffusion method. The growth inhibitory effect of *Staphylococcus aureus*, *Staphylococcus epidermidis, Salmonella typhi, Klebsiella pneumoniae* and *Vibrio parahemolyticus* are presented in Table – 1. All the tested pathogens are highly susceptible to the crude extracts. However, our study revealed a remarkable antibacterial activity against gram-negative bacterial strains than gram-positive. The most effective activity was proven by *Coleus forskohlii* with maximum zone of inhibition ranging from 15mm against *Salmonella typhi* and 14mm with *Staphylococcus aureus. Pogostemon patchouli* inhibited the growth of *Staphylococcus epidermidis* with 15mm. All the 3 extracts inhibited *Klebsiella pneumoniae* and *Vibrio parahemolyticus* with 8mm and 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 extracts of *Coleus forskohlii, Pogostemon patchouli* and then in *Tecoma stans.* These results indicate that leaves 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.

DISCUSSION

In the recent years many research work reported on the antibacterial activity of plant extracts on human pathogenic bacteria[19, 20]. The present study also revealed the antibacterial potential and ethno medicinal claims for *Tecoma stans, Coleus forskohlii* and *Pogostemon patchouli*. The traditional practice of crude extracts of these plants holds active constituents with antimicrobial properties and suggested as antimicrobial therapeutic agents against infectious diseases caused by the tested pathogens of this study. The growth media also seem to plays a vital role in the evaluation of the antibacterial activity. Visible zone formation, bacterial growth inhibition and bacterial lawn or growth pattern of cells with equally distributed characteristics is the major reason to define a good culture media. Our study included two different media explicitly nutrient agar media and Muller-Hinton agar. All these plant extracts showed bacterial growth inhibition in both the media with maximum inhibition values. Besides, the ZOI obtained in Muller-Hinton agar by the extracts was not observed in nutrient agar. Consequently, the ZOI noted in nutrient agar lacked in Muller-Hinton agar. These differences in susceptibility patterns may be due to the less diffusibility of the crude extracts in the agar. In comparison, Muller-Hinton agar is the best medium showed visible zone formation and growth inhibition, to be used to determine the antibacterial potential. A previous study[21] also reported that Muller-Hinton agar appears to be the best medium to describe the antibacterial activity. Not only crude extracts, use of different solvent extracts is also a matter of concern 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 extracts can be subjected to isolation of the therapeutic compounds and antimicrobials for further Pharmacological studies[22]. Ethno botanical approach is one of the universal practices applied in choosing the plants for pharmacological study[23]. Although, these plants declared the antibacterial activity against 5 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. This study is extendable with other major pathogenic bacteria to develop a novel broad spectrum antibacterial formulation in future. Now, our research will be focused to develop a broad spectrum antibacterial combined herbal formulation with these plants.

Table. No.1: Inhibitory effect of leaf crude extracts of *Tecoma stans, Coleus forskohlii* and *Pogostemon patchouli* against various medical pathogens

Microorganisms	*Tecoma stans*		*Coleus forskohlii*		*Pogostemon patchouli*	
	Zone of inhibition (mm)*					
	NAM	MHA	NAM	MHA	NAM	MHA
Staphylococcus aureus (G +ve)	7	-	-	14	-	12
Staphylococcus epidermidis (G+ve)	-	7	-	-	-	15
Salmonella typhi (G –ve)	-	10	15	-	11	-
Klebsiella pneumoniae (G –ve)	-	8	8	-	8	-
Vibrio parahemolyticus (G –ve)	10	7	10	-	10	-

G +ve: gram-positive, G –ve: gram-negative, *Values are mean of three replicates,
NA: Nutrient agar media, MHA: Muller-Hinton agar, - : No inhibition zone

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