Original Research Paper

Volume-9 | Issue-8 | August - 2019 | PRINT ISSN No. 2249 - 555X

Pharmacology

EVALUATION OF ANTIMICROBIAL ACTIVITY IN THE LEAVES EXTRACT OF *OCIMUM TENUIFLORUM L.*

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ABSTRACT The present study was aimed to evaluate the antibacterial and antifungal activity of the ethanolic leaves extract of *Ocimum tenuiflorum* L. The antimicrobial activities were determined using various species of bacteria and fungi. Disc diffusion method of ethanolic leaves extract was used for the antimicrobial activity and the zone of the inhibition was analyzed. The ethanolic leaves extracts of *O. tenuiflorum* L. was active against all the tested bacteria and fungi. The highest antibacterial activity against *E.coli* (20mm) followed by *Staphylococcus aureus* (19mm) and *Enterococcus aerogenes* (17mm). The ethanolic leaves extract showed the highest antifungal activity against *Candida albicans* (10mm) followed by *Candida vulgaris* (9mm) and *Aspergillus niger* (8mm).Our results suggested that the ethanolic leaves extract of *O. tenuiflorum* L. exhibits the wide range of antimicrobial activity.

KEYWORDS : *Ocimum tenuiflorum*, ethanolic extract, antibacterial, antifungal activity.

INTRODUCTION

Plant extracts has been used to treat various microbial disease from ancient time in traditional medicinal systems. Ability of using most of the medicinal plants for the treatments of various diseases may lie in the antioxidant and antimicrobial effect of phytochemicals [1]. The primary benefits of using plants derived medicines are that they are relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment [2].

O. tenuiflorum L.(Lamiaceae), the most sacred herb (Holy Basil) of India is well known for its enormous therapeutic activities and prevention against diseases. It is a rich source of secondary metabolites and has an outstanding role in medicine. Secondary metabolites carryout a number of protective functions in the human body which includes anti- arthritic activity, anti- hyperlipidemic activity [3], anti-pyretic activity [4], anti- ulcer activity [5], anti- asthmatic activity [6], anti- cataleptic activity [7], anti- cataract activity [8], anti- coagulant activity [9], anti- emetic activity [10], anti- helminthic activity [11], anti- oxidant activity [12], anti- plasmodial activity [13], anti-spasmodic activity [14], anti- stress activity[15] and anti- thyroidic activity [16].

Due to the development of resistance in pathogenic microorganisms to antibiotics used in modern medical science, there is a growing attention towards plant extracts as a source of new antimicrobial drug discoveries [17]. The antimicrobial activities of the plant extract can be attributed to the presence of secondary metabolites including glycosides, alkaloids, terpenoids, flavonoids and saponins. These active components usually interfere with the growth or metabolism of microorganisms in a negative manner resulting in cell death [18].

MATERIALS AND METHODS

Collection of plant material

The leaves of *O. tenuiflorum* L. were collected from Allithurai, Tiruchirappalli District. The leaves were washed with sterile water and dried in shades. Then the leaves samples were powered in mechanical grinder.

Preparation of ethanolic extracts

The ethanolic leaves extract was prepared by drenched 40gm of the dried leaves powder in 1litre of ethanol by using a hot percolation extractor for 24 hrs continuously. The leaves extract were filtered through Whatmann filter paper No.1 (125mm). The filtered sample extract was concentrated and dried by using a rotatory evaporator under reduced pressure.

Antibacterial and Antifungal activity

The purpose of this study was to examine the antibacterial and antifungal activity of the crude ethanolic extracts towards the selected microorganisms using disc diffusion method.

Collection of test organisms

To examine the antibacterial activity of plant extract, three strains such as *Escherichia coli* (MTCC 25922), *Enterococcus aerogenes* (MTCC 29212), *Staphylococcus aureus* (MTCC 25923) were used as test organisms. All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Bacterial strains were cultivated at 37ºC and maintained on nutrient agar (Difco, USA) slant at 4ºC.

Screening of antibacterial activity (Disc Diffusion method)

Antibacterial activity of crude ethanolic extract was determined using the disc diffusion method.The petridishes (diameter 60 mm) was prepared with Muller Hinton Agar and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of crude ethanolic extract at various concentrations of 20-100µg/ml respectively. Prepared discs were placed on the top layer of the agar plates and left for 30 minutes at room temperature for compound diffusion. Negative control was prepared using the ethanolic solvent. The petriplates were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters and the experiment was repeated twice.

Screening of Antifungal Activities:

Culture Media: The medium used for antifungal activity was Sabouraud's dextrose agar Hi media Pvt. Bombay, India.

Inoculum: The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 hours and the suspensions were checked to provide approximately 10^5CFU/ml.

Fungal strains used: The clinical fungal test organisms used for study are *Candida albicans* (MTCC 282), *Candida vulgaris* (MTCC No.184) *and Aspergillus niger (*MTCC 227*)* were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

Screening of antifungal activity

Antifungal activity of crude extracts was determined using the disc diffusion method. The petriplates (diameter 60 mm) was prepared with Sabouraud's dextrose agar (SDA) and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of crude extract at various concentrations of 20-10µg/ml respectively. Prepared discs were placed on the top layer of the agar plates and left for 30 minutes at room temperature for compound diffusion. The petriplates were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters.

RESULT

Antibacterial activity of ethanolic leaves extract of *O. tenuiflorum* L. by disc diffusion assay method

The results of the antibacterial activity of ethanolic leaves extract of *O. tenuiflorum* L. against different microorganisms by disc diffusion method are showed in Table 1. The extract showed inhibitory activity against *Escherichia coli* (20 mm), *Staphylococcus aureus* (19 mm), *Enterococcus aerogenes* (17mm) at the concentration of 100µg/ml. At 80 µg/ml concentration, the ethanolic leaves extracts exhibited the

antibacterial activity against all the tested bacteria, but it was more susceptible against *Escherichia coli* (18 mm). However, the extracts showed better inhibitory actions against pathogens at a concentration of 60, 80 and 100 µg/ml than the lower concentration. As the concentration of extracts increased from 20- 100 µg/ml, the inhibitory actions of the plant extracts increased towards all the strains used in this study.

Table 1: Antibacterial activity of ethanolic extract of *Ocimum tenuiflorum* L. leaves

Plant extracts	Concentrations (µg/ml)	Organisms/Zone of inhibition (mm)		
		Ethanolic extract of O. tenuiflorum L. leaves		
		Escherichia coli	*Staphylococcus aureus*	*Enterococcus aerogenes*
Ethanolic Extracts	20	10	11	10
	40	12	12	11
	60	13	13	13
	80	18	15	15
	100	20	19	17
Control	10 µl/disc	0	0	0

Antifungal activity of ethanolic leaves extract of *O. tenuiflorum* L.

The antifungal susceptibility test of the different concentration of ethanolic leaves extract of *O. tenuiflorum* L. against the tested organisms showed in Table 2. From the results of ethanolic leaves extract the highest activity was demonstrated against *Candida albicans* (10 mm zone of inhibition) at 100 µg/ml, followed by *Candida vulgaris* (9 mm zone of inhibition), *Aspergillus niger* (8 mm zone of inhibition) at 100 µg/ml. At the concentration of 80µg/ml, the extracts exhibited the antifungal activity against all the tested fungi, but it was more susceptible against *Candida albicans, Candida vulgaris* (9 mm). However, the ethanolic leaves extract showed better inhibitory activity against the tested organisms at a concentration of 60, 80 and 100 µg/ml than the lower concentration. As the concentration of extracts increased from 20-100 µg/ml, the inhibitory actions of samples increased towards all the strains used in this study.

Table 2 Antifungal activity of ethanolic extract of *Ocimum tenuiflorum* L. leaves

Plant extracts	Concentrations (µg/ml)	Organisms / Zone of inhibition (mm)		
		Ethanolic extract of O. tenuiflorum L. leaves		
		Candida albicans	*Candida vulgaris*	*Aspergillus niger*
Ethanolic Extracts	20	5	6	6
	40	6	7	7
	60	8	8	7
	80	9	9	8
	100	10	9	8
Control	10 µl/disc	0	0	0

DISCUSSION

The antimicrobial activity of ethanolic leaves extract of *O. tenuiflorum* L. was evaluated against the microorganisms by the disc diffusion method (zone of inhibition and zone diameter). In the antibacterial assay among the three bacteria tested *E.coli* and *S.aureus* were the most sensitive organism for *O. tenuiflorum* L. leaves extract and showed minimum inhibitory effect against *E. aerogenes*. Our results are similar to the results of Londhe *et al*.,

[19] and they found out antibacterial activity with maximum zone of inhibition against *E.coli* (17mm) and minimum inhibitory effect on *P.aeruginosa* (16mm).The antifungal activity was performed against *A. niger* with largest zone of inhibition of 20mm was obtained in *O.tenuiflorum.*

Gomathinayagam Subramanian *et al*., [20] studied the antimicrobial activity of different extract of *O. tenuiflorum* L. leaves and they determined maximum inhibition against *E. Coli* (19.56 nm) and *S.aureus* (13.45nm) for 900 (µ)L.

CONCLUSION

Tulsi is used as a traditional herb and it has diverse pharmacological activities such as anticancer, antidiabetic, antistress, analgesic, expectorant, hepatoprotective and insect repellent.In conclusion, the present study suggested that Tulsi leaves has potential to resist against the microorganisms and may serve as good source for new antimicrobial agent with therapeutic potentials.

ACKNOWLEDGMENT

The authors express sincere thanks to The Principal, Holy Cross College (Autonomous), Trichy District, Tamil Nadu, India, for providing necessary facilities and encouragement

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