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Original Article

Evaluation of antimicrobial Potential of *Piper* (L.) species

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Abstract

In this present study, antibacterial and antifungal activities of *Piper* species was investigated against *Bacillus subtilis* (gram +ve), *Staphylococcus aureus* (gram +ve), *Escherichia coli* (gram -ve), *Salmonella typhi* (gram -ve), *Aspergillus niger,* and *Candida albicans* using the disc diffusion method. Crude essential oil of *Piper* species obtained through hydrodistillation also screened for its strong antibacterial activity against *Staphylococcus aureus* (gram +ve), and *Escherichia coli* (gram -ve). The maximum inhibitory activity of essential oils of *Piper* species was exhibited against *Bacillus subtilis* (7-24mm), and it was followed by *S. aureus* (6-26mm), *Escherichia coli* (7-26mm), *Salmonella typhi* (7-25mm), *C. albicans*(7-16mm) and *Aspergillus niger* (7-22mm).
Keywords: Piperaceae, *Piper* species, essential oils, antimicrobial activity

Introduction

Plants and plant parts are being a rich source of effective medicine and have no side effect. About 80% of world population are still dependent on traditional medicines or herbal medicines. Plants are invaluable sources of pharmaceutical products that have drawn the attention of many scientists (Olalde Rangel, 2005). According to Loi *et al*., (2005) the plants and plant products are possessing several medicinal properties such as anti-biotic, anti-infective, anti-cancer and anti-aging. The valuable medicinal properties of different plants are due to the presence of several constituents i.e. saponines, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters (Tiwari and Singh,2004).

The family Piperaceae comprises 12genera and about 1400 species of mainly found in tropical region (Barroso,1978). The genus *Piper* (L.) contains more than 700 species, they grow in tropical and subtropical rain forest forests. *Piper* species exist in South India are economically important, as they are closely related to the cultivated black pepper. *P. betle* L., another economically important species which is mainly used in the paan industry. Several species of *Piper such as P.longum*, *P. cubeba*, *P. retrtofractum*, etc., are used in indigenous system of medicine in India (Parthasarathy *et al*.,2006). Piper species are used in the treatment for several diseases such as veneral diseases, intestine disorders (Addae-Mensah *et al*.,1977), geneto-urinary maladies, epilepsy and to prevent conception (Atal *et al*.,1975). The aim of this present study is to screen the bioactive effect of crude essential oil of seven *Piper* species from South India.

Material and Methods

Plant materials

The plant materials of Piper species were collected from the Kalakad-Mundanthurai Tiger Reserve (KMTR). The Kalakad-Mundanthurai Tiger Reserve (KMTR) Forest lies between 8° 20' and 8° 55' North latitude and between 77° 10' and 77° 35' East longitudes in Southern Western Ghats of Tirunelveli and Kanyakumari districts, Tamil Nadu, India.

Extraction and determination of essential oils

100gms of fresh fruits of seven *Piper* species were harvested (Table-1), weighed and essential oil extracted by hydrodistillation in a Clevenger apparatus, using a 500ml flask during 2hr. Distillation was repeated to obtain the oils in concentrated form for the experiment. The oils thus obtained were separated from water in the condenser and stored in airtight containers under refrigeration (4^oC).

Tested pathogens

Bacillus subtilis (gram +ve), *Staphylococcus aureus* (gram+ve), *Escherichia coli* (gram -ve), *Salmonella typhi* (gram -ve), *Aspergillus niger* and *Candida albicans.*

Preparation of test Samples

A required number of sterile filter paper discs were placed into sterile Petri dishes. Isolated essential oils of Piper species were dissolved in Dimethyl Sulfoxide (DMSO) to prepare the couple of the concentrations of

25µl/ml, and 100µl/ml. Each disc was then loaded with 25µl/ml, and 100µl/ml essential oils and dried under laminar flow for 10min.

Determination of Antimicrobial Activity

Screening of the essential oil for biological activity was performed through agar diffusion disc impregnated method (Smith *et al*.,2002), Inoculum containing bacterial or mould cells was applied onto nutrient agar plates. On each plate, a reference antibiotic disc was also applied (Kanamycin for the bacteria and nystatin for the fungus). The reference antibiotic disc contained 200mg of antibiotic/ml. A control disc with DMSO was also placed in each plates. For test samples the filter paper discs were made by cutting discs (6mm) with a perforator, placing 5 of these discs in a vial and adding 25µl and 100µl of each essential oil and the discs were left dry. Each disc was impregnated with the chosen concentration of 25µl and 100 µl essential oils using sterile syringe. Plates were incubated at 37ºC for 24 hr and 48 hr for the bacteria and moulds respectively. Triplicate set of plates were maintained for all samples. After incubation, the plates were examined for areas of no growth around the disc (zone of inhibition). The radius of the inhibition zone was measured from the edge of the disc to the edge of the zone. Larger the inhibition zone diameter, greater is the antimicrobial activities. Measurements were recorded, average values were calculated and tabvulated (Table-2). Both strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains grow up to the edge of the disc.

Results and Discussion

A wider utilization of Piper species in different systems of traditional medicine were being attested by so many authors (Smith *et al.,* 2002; Holetz *et al.,* 2002). From the results of this present study, it is evident that the essential oil of Piper species obtained through hydro distillation method were more inhibitory against the tested pathogens. The maximum yield of the essential oils of *Pipers* species such as *P. longum* L.(0.063%), *P. cubeba* L.f. (0.063%), *P. wightii* (0.056%), *P. betle* L.(0.046%), *P. attenuatum* (0.042%), *P. nigrum* L.(0.034%), and *P. barberi* (0.024%).

Table-1: Vernacular name, medicinal uses and yield (%) of the essential oils of *Piper* species

Species Name	Vernacular	Mode of Administration	Fresh wt	Yield of Volume (ml)	% of essential oils
P. cubeba L.f.	Vazhmilagu	The fruits extracts are drunk to reduce fever.	100	0.63	0.063
P. longum L.	Kattuthupli	The leaf and the fruit juice are drunk to treat Cough & cold.	100	0.65	0.065
P. betle L.	Kattu Vettrilai	Leaves are heated on fire and bound on the affected part for relief from swelling and inflammation.	100	0.46	0.046
P. nigrum L.	Nallamilagu	The dried seed power is used to treat cut and wounds.	100	0.34	0.034
P. attenuatum Buch.Ham	Kattumilagu	The seed power is used to treat cough and wounds.	100	0.42	0.042
P. barberi Gamble	Thulimilagu	The seed power is used to treat cough and wounds.	100	0.24	0.024
P. wightii Miq	Nallimilagu	The seed power mixed with one teaspoon honey orally is used to treat cough.	100	0.56	0.056

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Table -2: Antimicrobial of essential oils of *Piper* species

Piper sps	Tested oils Disc/µl	Diameter of zone of inhibition (mm)*					
		Bacteria				Molds	
		B.subtilis	*S. aureus*	*E.coli*	*S. typhi*	*A.niger*	*C. albicans*
P.cubeba	Control	-	-	-	-	-	-
	25	16	6	12	16	15	11
	100	23	17	21	25	16	12
	Ref. drug	22	23	24	25	22	14
P. longum	Control	-	-	-	-	-	-
	25	8	11	15	11	12	8
	100	19	27	19	16	16	13
	Ref. drug	22	23	24	25	22	14
P. betle	Control	-	-	-	-	-	-
	25	7	12	12	15	16	11
	100	23	26	16	19	18	16
	Ref. drug	22	23	24	25	22	14
P. nigrum	Control	-	-	-	-	-	-
	25	8	11	7	12	11	11
	100	24	16	18	24	17	16
	Ref. drug	22	23	24	25	22	14
P. attenuatum	Control	-	-	-	-	-	-
	25	8	10	9	8	7	11
	100	14	15	16	12	12	13
	Ref. drug	22	23	24	25	22	14
P. barberi	Control	-	-	-	-	-	-
	25	9	11	13	7	11	7
	100	17	18	22	23	16	9
	Ref. drug	22	23	24	25	22	14
P. wightii	Control	-	-	-	-	-	-
	25	11	7	11	12	11	7
	100	18	19	26	14	13	11
	Ref. drug	22	23	24	25	22	14

Control: DMSO; Ref. drug: Kanamycin/ Nystatin *All values are average of triplicates

All the tested essential oils of *Piper* species were exhibited a variable spectrum of antimicrobial activity. table-2. The essential oils of *Piper* species were active against gram positive, gram negative bacteria and moulds (25, and 100µl/mL, respectively). The maximum antibacterial activity was exhibited by *P.betle* and *P. wightii* active against *S. aureus* (gram+ve) and *E.coli* (gram - ve) respectively. Lopez *et al.,* (2002) reported the antimicrobial properties of *P. lanceafolium* and also evaluated the antifungal activity *Candida albicans*, A similar kind of screening was done by Holetz et al (2002) with *P.regnellii.* The results of the present study are being correlative with the earlier reports. Antifungal activity of essential oils extracted from *P. multiplinervium* was found to be maximum. In case bacterial pathogens, it was maximum against *Staphylococcus aureus, Escherichia coli (*Ruegg, 2006). High level antibacterial and antifungal potential of the Piper species extract reported in this present study may be due to the principal active constituents in the essential oil of *Piper nigram* are sabinene (3.9-18.8%), *β*-pinene (3.9-10.9%), limonene (8.3-19.8%) and *β*- caryophyllene (28.4-32.9%) (Uni-Graz, 2005; Pruthi,1962). From this result, it is necessitated that to investigate the specific constituent which is responsible for this activity and such characterization studies are underway.

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