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Original Article

Composition, Antifungal and Cytotoxic activities of Essential oils of *Piper barberi* fruits

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Abstract

In the present study, antifungal activity of essential oils of *P. barberi* fruits was assessed by disc diffusion method and brine shrimp lethality test (BST) was carried out to determine 24hr mortality of *Artemia salina*. In total, 13 compounds were identified by GC/MS method accounting for 99.99% of the constituents. 1,8 ceneole (39.65%), *α*-pinene (11.87%) and eugenol isomer (9.40%) and camphor (7.49%) were the main constituents. Maximum percentage of inhibition of essential oils activity against *Aspergillus foetidus, A. fumigates, A. ochraceus, A. flavus* and *Penicillium notatum*. Brine shrimp lethality test was carried out to determine LC_{50} value of 88µl/ml was observed the toxic level at 24hr.

Keywords: Piperaceae, *Piper barberi*, fruits, essential oil, 1,8 ceneole, α-pinene, antifungal activity, cytotoxic activity

Introduction

Piperaceae comprising of 12 genera and about 1400 species, mainly to be found in tropical regions (Barroso,1978). The genus *Piper* (L.) contains more than 700species, they are growing in tropical and subtropical rain forest forests (Raju and Maridass, 2011). Piperaceae species are mostly pioneer shrubs with several medicinal uses likes are gynecological maladies, vaginitis, intestinal disorders, psychotropic, antimicrobial, antioxidant and cytotoxic effects, cough, bronchitis, intestinal diseases, rheumatism, vertigo, asthma, chronic indigestion, colon toxins, obesity, sinusitis, congestion, fever, paralytic, arthritic disorders, diarrhoea and cholera (Sumathykutty *et al*., 1999; Sashidhar, 2002; Ravindran, 2000; Andreia de Araujo Morandim-Giannetti *et al*., 2010).

Fig.1: Natural habitat of *Piper barberi* (L.)

Literature of essential oils of *Piper* species have been reported earlier authors (Gopalakrishnan *et al*., 1993; Martins *et al*., 1998; Sumathykutty *et al*., 1999, Jirovetz *et al*., 2002; Orav *et al*., 2004; Oyedeji *et al*., 2005; Andreia de Araujo Morandim-Giannetti *et al*., 2010; Rahman *et al*., 2011). The volatile oil of pepper has been shown to have antimicrobial activity (Dorman and Deans, 2000; Raju and Maridass, 2011). The antifungal and cytotoxic activities of *Piper barberi* (L.) fruits were carried out through hydrodistillation method obtained the essential oils and its constituents were identified by GC-MS methods.

Materials and Methods

Plant materials

Fresh fruits of *Piber barberi* were collected in Kodaiyar region, Southern Western Ghats Tamil Nadu. Specimens were identified and authenticated by plant taxonomist.

Isolation of Essential oils

The essential oils were obtained from 50gms of fresh fruits of *P. barberi* by hydrodistillation using a Clevenger-type system for 5hr. The aqueous phase was extracted three times with 50 ml hexane. The pooled organic phases were dried with sodium sulphate, filtered and the solvent evaporated until dryness. The oil was obtained and stored at 4°C for further use.

GC-MS analysis

The identification of essential oil constituents was performed using a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a HP-5971 mass selective detector and capillary column HP-5 (25m x 0.2mm x 0.33mm diam.). GC-MS was done by following program, injection temperature program conditions set at 220°C, column set at 60°C, with heating temp of 4 °C/ min and final temperature 240°C for 8min, and the FID detector set at 250°C. Helium was used as carrier gas at 1mL/min. The GC-MS electron ionization system was set at 70 eV. *Piper barberi* essential oil was solubilized in pentane for the analyses. The oil constituents were identified by comparison of retention time, Wiley 138 and Nist 98 libraries and literature (Adams,2001).

Antifungal activity assay

The antifungal activity of essential oil of *Piper barberi* fruits was tested against five pathogenic fungi, *Aspergillus fumigates, A. flavus, A.foetidus, A. ochraceus*, and *Penicillium notatum* by the poison plate method (Boue *et al*., 2005). Essential oils were dissolved in 1ml dimethyl sulfoxide before mixing with 90ml potato dextrose agar (PDA). The essential oils were tested at a concentration of 50µl/ml. All kinds of fungi were incubated in PDA at 27±1°C for 4 days to get new mycelium for antifungal assay. Then mycelia dishes of approximately 4 mm diameter were cut from culture medium and one of them was picked up with a sterilized inoculation needle and inoculated in the center of PDA plate aseptically. The inoculated plates were incubated at 27±1°C for 5 days. Acetone with sterile distilled water served as control, while positive control of nystatin was prepared by dissolving in dimethyl sulfoxide (DMSO) and diluting with 0.9% saline to give a final concentration of 1mg of nystatin/ml in a 5% DMSO solution. The radial growth of the fungal colonies was measured and the data were statistically analyzed. The inhibiting effects of essential oil against on these fungi were calculated by the formula: I(%) = [(*C*-*T*)/(C-0.4)]x100, where *C* represents the diameter of fungi growth on untreated PDA, and *T* represents the diameter of fungi on treated PDA while I means the inhibition rate.

Brine shrimp lethality test

The brine shrimp lethality test (BST) was used to predict the cytoxicity of the essential oils and was conducted according to the methods (Meyer *et al*., 1982; Mclaughlin, 1990; Oloyede *et al*.,2010). Brine shrimp eggs obtained from Bay of Bengal at Tuticorin, Tamil Nadu, South India. The shrimp's eggs were hatched in sea water for 48h at room temperature. The nauplii (harvested shrimps) were attracted to one side of the vials with a light source. Solutions of the oils were made in DMSO, at varying concentrations (25, 50, 100, 200 and 400µl/ml) and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the triplicate vials. Control brine shrimp larvae were placed in a mixture of sea water and DMSO only. After 24h, the vials were examined against a lighted background and the average number of larvae that survived in each vial was determined. The concentration-mortality data were analyzed statistically by using Probit analysis for the determination of LC_{50} values and linear regression for the each concentration essential oils of *P. barberi* (Meyer *et al*.,1982; Raju and Maridass,2011; Mclaughlin,1990).

Results and Discussion

The hydrodistillation of the fruits of *P. barberi* yield 0.72% (v/w) yellowish oil. About 99.99% of the total oils found to be 13 chemical constituents identified by GC-MS methods (Table-1; Fig.2). The major constituents detected were 1,8 ceneole (39.65%), *α*- pinene (11.87%), eugenol isomer (9.40%) and camphor (7.49%). In previous studies, minor oil contents detected as 1, 8 ceneole, *α*- pinene of *Piper chaba* leaves were reported (Rahman *et al*., 2011). Earlier studies, *α*- pinene was minor content detected as leaves of several *Piper* species such as *Piper aduncum*, *Piper amalago*, *Piper cernuum*, *Piper diospyrifolium*, *Piper crassinervium*, *Piper gaudichaudianum*, *Piper solmsianum*, *Piper regnellii*, *Piper tuberculatum*, and *Piper umbelata* (Andreia de Araujo Morandim-Giannetti *et al*.,2010). The minor constituents detected were camphene (5.46%), *β*-phellandrene (4.51), borneol (4.91%), *β*-pinene (4.13%), *α*-terpineol (3.87%), myrcene (3.64%), *p*-cymene (2.31%) and bornyl acetate (1.98%). Gopalakrishnan *et al*., (1993), reported that the most relevant compounds of detected were pinene (5.07±6.18%), *β*-pinene (9.16±11.08%), myrcene (2.20±2.30%) and *p*-cymene (0.0±0.18%). Our study, *β*- caryophyllene (0.77%) was detected as trace constituent of *Piber barberi*. According to Martins *et al*., (1998) reported *β*-caryophyllene was detected as major constituents of *P.nigrum* (15.4%), *Piper capense* (12.6%) and *P. umbellatum* (9.8%). To

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our knowledge, this is the first reported that a detailed composition of the essential oil obtained from *P. barberi* fruits.

Antifungal activity

The results of the antifungal activity of the essential oils of *P. barberi* showed in the table -2. Maximum percentage of oils of *P. barberi* showed active against *Aspergillus foetidus, A. fumigates, A. ochraceus, A. flavus* and *Penicillium notatum*. Aspergillosis is caused due to inhalation of *Aspergillus fumigatus* spores. *Aspergillus fumigatus* is an opportunistic pathogen which usually affects lung diseases. In the lungs, *Aspergillus fumigatus* forms tangled mass of fungus fibers, blood clots. The fungus mass gradually enlarges, destroying to lung tissue in the process, but usually does not spread to other areas (Sunita Bansod and Mahendra Rai, 2008). Various publications have documented the antimicrobial activity of essential oils and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and fennel (Morris *et al*.,1979; Ross *et al*.,1980; Yousef and Tawil,1980; Hili *et al*.,1997; Lis-Balchin and Deans, 1997).

Table-1: Essential oils composition of *Piper barberi* fruits

Sl. No.	Retention time (Rt)	Composition	% of yields	Identification methods
1	2.34	α- pinene	11.87	Rt, GC-MS
2	2.37	camphene	5.46	Rt, GC-MS
3	3.11	β-phellandrene	4.51	Rt, GC-MS
4	3.45	β-pinene	4.13	Rt, GC-MS
5	3.56	myrcene	3.64	Rt, GC-MS
6	4.56	p-cymene	2.31	Rt, GC-MS
7	6.78	1,8 ceneole	39.65	Rt, GC-MS
8	6.79	camphor	7.49	Rt, GC-MS
9	7.11	eugenol isomer	9.40	Rt, GC-MS
10	7.34	borneol	4.91	Rt, GC-MS
11	7.81	caryophyllene	0.77	Rt, GC-MS
12	8.01	α- terpineol	3.87	Rt, GC-MS
13	8.07	bornyl acetate	1.98	Rt, GC-MS
Total			99.99	

Table - 2: Antifungal activity of essential oils of *P. barberi* fruits

Fungi	Growth of Mycelium (Untreated)		Growth of Mycelium (Treated)		% of Inhibition	
	Control (96hr)	Nyasin (96hr)	EO (96hr)	Nyasin (96hr)	EO	Nyasin
Aspergillus fumigates	15	7	7	7	88.89	100
A. flavus	13	7	8	7	71.43	100
A.foetidus	17	9	7	9	90.91	100
A. ochraceus	16	8	8	8	80.00	100
Penicillium notatum	14	8	9	8	62.50	100

Disc size: 6mm; EO: Essential oils; Formula: I(%) = [(*C*-*T*)/(C- 6)]x100

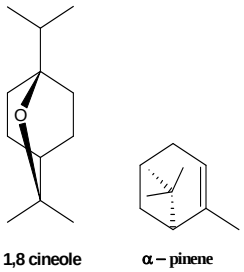

Fig.2: Structure of Major constituents

Brine shrimp lethality activity

In brine shrimp lethality bioassay (Table -3), the essential oils showed lethality against the brine shrimp nauplii. It showed different mortality rate (%) at different concentrations in 24hr. From the plot of percent mortality versus log concentration, LC_{50} value 88(µl/ml) was observed in the table-3,4 and fig.3. This result confirmed the previous reports about the toxicity of *P. barberi* on human being and lower animals

as reported (Walter *et al.,* 1971, and Jirí *et al.,*2005, Maridass,2008; Aiyelaagbe *et al*., 2010; Oloyede *et al*., 2010; Oloyede, 2011). The toxicity was determined using brine shrimp larvae (*Artermia silina*) nauplii which are living organisms with no advanced nervous system. The dominance of hydrocarbons in the essential oil accounted for the toxicity level.

Table-3: Brine shrimp lethality (24hr) of various concentrations of essential oils of *P. barberi* fruits

Concentrations (µl)	No. of Animal	No. of mortality	% Mortality	Log dose	Probit Mortality
Control	50	0	0	-	-
Gallic acid 50	50	50	100	-	7.51
EOPb 25	50	5	10	1.39	3.72
EOPb 50	50	18	36	1.69	4.64
EOPb 100	50	26	52	2.0	5.05
EOPb 200	50	39	78	2.3	5.77
EOPb 400	50	43	86	2.69	6.08

Table-4: Statistical analysis of Brine shrimp lethality (24hr) of various concentrations of essential oils of *P. barberi* fruits

Regression Statistics					
Multiple R	0.861841793				
R Square	0.742771277				
Adjusted R Square	0.614156915				
Standard Error	14.36305803				
Observations	4				
ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1191.405	1191.405	5.775181	0.138158
Residual	2	412.5949	206.2974		
Total	3	1604			

	Coefficients	*Std Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	41.98974359	11.31416	3.711256	0.065546	-6.69116	90.67064
25	0.098871795	0.041142	2.403161	0.138158	-0.07815	0.275893

RESIDUAL OUTPUT				PROBABILITY OUTPUT	
Observation	*Predicted 10*	*Residuals*	*Standard Residuals*	*Percentile*	*10*
1	46.93333333	-10.9333	-0.93229	12.5	36
2	51.87692308	0.123077	0.010495	37.5	52
3	61.76410256	16.2359	1.384443	62.5	78
4	91.42564103	-5.42564	-0.46265	87.5	86

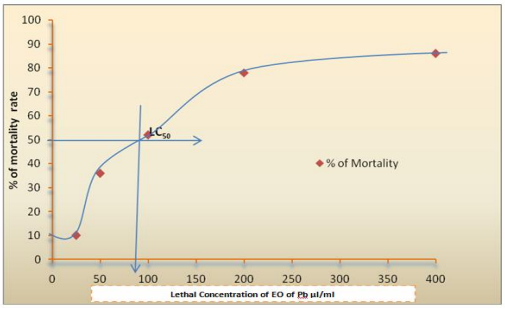

Fig.3: Graphical representation of LC_{50} value

Conclusion

The conclusion of the our results indicated that the essential oils mainly had about 1,8 ceneole, α-pinene, eugenol isomer and camphor may be acted as antifungal and cytotoxic activities.

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