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Research Article

Phytochemical Composition and *In vitro* Antimicrobial Activity of Essential Oil of *Piper hymenophyllum* Miq.: A Rare Wild Betel

K. Venkata Ratnam[1], L. Md. Bhakshu[2], R.R. Venkata Raju[3]*

[1]*Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad – 500 046*
[2]*Lecturer, Department of Botany, Government College for Men, Kadapa, Andhra Pradesh, India 516 004*
[3]*Department of Botany, Srikrishnadevaraya University, Anantapur, Andhra Pradesh, India 515003*

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ABSTRACT
The present aim of the study is to evaluate chemical composition and antimicrobial activity of the essential oil of the fruits of *Piper hymenophyllum* Miq. GC-MS analysis of the hydro distilled oil resulted in the identification of 15 compounds in fruit constitutes 98.65% of the oil. (E) phytol (21.87%), dihydro terpineol (17.42), α – terpineol (13.93%), trans-piperitol (9.66%), endo-fenchol (4.09%), camphene (3.92%) and γ-terpinen (3.91%) were the major compounds of fruit oil. The fruit essential oil was tested against human pathogenic bacteria and yeast. Among the tested microorganisms, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Klebsiella pneuminiae* exhibited highest sensitivity and strongly inhibited at very low MIC indicating the efficacy of the fruit oil on the Gram negative bacteria. In conclusion, essential oil obtained from *P. hymenophyllum* fruit showed beneficial effects to inhibit tested human pathogenic organisms by *in vitro* methods.

Key words: *Piper hymenophyllum*, essential oil composition, antimicrobial activity, GC-MS.

INTRODUCTION

Medicinal and aromatic plants and their products are the main source for natural therapeutic drugs and its derivatives. Essential oils (Eos) of plant origin and their constituents have been known to exhibit different pharmacological activities. Since last decade, there is increasing trend to use herbs in health and dietary choices for human welfare[1]. Indians have been using herbs and spices as food additives in their daily food, which are well known for their multifarious pharmacological and to produce multidimensional flavors in food[2].

The Genus *Piper*, the largest in the family Piperaceae, consisting 1000-2000 species distributed in tropical and subtropical regions of the World[3]. These plants contain diverse group of secondary metabolites such as alkaloids/amides, propenyl phenols, lignans, neolignans, terpenes, steroids, kawapyrones, piperolides, flavones, chalcones and dihydro chalcones[3,4]. Several *Piper* species reported for different pharmacological properties like antifeedant, antibacterial, antifungal, anti-inflammatory, antiamoebic and antiplatelet activities[3]. Through the review of the available literature indicate that several *Piper* species were rich source of essential oil components[5-9] with pronounced biological properties[10].

Piper hymenophyllum Miq. (Piperaceae) is a rare, much branched twining shrub, growing in shade and swampy areas of Eastern Ghats of India. In local language it is known as Adavi tamalapaku (wild betel). The plant has been using in traditional medicine to cure mouth ulcers, indigestion and intestine disorders[11]. *P. hymenophyllum* was reported to possess antibacterial and antiinflammatory[12] and cholinesterase inhibition activity[13]. The critical review of literature on pharmacological properties of *P. hymenophyllum* indicates that very few reports were noticed on phytochemical and antimicrobial properties of *Piper hymenophyllum* leaves[9,12,13]. To the best of our knowledge no previous reports were noticed on phytochemical and antimicrobial property of *P. hymenophyllum* fruits. Hence, the present work is focused on to evaluate phytochemical, antibacterial and antifungal properties of *P. hymenophyllum* fruits.

MATERIALS AND METHODS

Plant material

Fruits of *Piper hymenophyllum* were collected from Nallamala forests, a part of Eastern Ghats, India. The plant was identified with the help of regional and local floras[14,15] and a voucher specimen was deposited in the herbarium (# 24200) department of Botany, Sri Krishnadevaraya University, Anantapur, India.

Essential oil extraction and GC-MS analysis

Hundred grams of fresh fruits of *Piper hymenophyllum* were subjected to hydro distillation for five hours using a Clevenger type apparatus[16]. Hydro distillation of the fruits resulted yellow color oil (0.4%) and was separated, dried over anhydrous Sodium sulphate and stored at 4^0 C. The GC-MS analysis was carried out on Shimadzu 17A coupled with Shimadzu QA 5050A (Quadruple) Mass-Spectrometer equipped with EI and a fused silica column

*Author for Correspondence

DB5 (35 X 0.25 mm i.d.) of 0.25 µm film thickness coated with polysilphenyl - siloxane. One micro liter of concentrated solvent fraction was injected and the GC oven temperature kept at 50^0 C- 280^0 C for 40 minutes. Helium was used as carrier gas at a flow rate of 2 ml min^{-1} with a split ratio of 1:30 and ionization voltage of Mass spectral analysis was run by EI technique at 70 eV. The components were identified by comparing their relative retention indices with those of standard reference compounds and available literature data[17].

Table 1. Essential oil composition of *P. hymenophyllum* fruit

S.No.	Retention Index	Compound Name	Composition
1	1117	Endo-Fenchol	4.09
2	-	Dihydro terpineol	17.42
3	954	Campene	3.92
4	1231	Citronellol	3.01
5	979	α-Pineneoxide	3.81
6	1063	α-Terpinene	3.91
7	1165	Isomenthone	3.05
8	2109	(E)- Phytol	21.87
9	1179	Terpin-4-ol	2.06
10	1649	Naphthalene	2.32
11	1190	α-Terpineol	13.93
12	1351	α-Cubebene	5.62
13	1101	Linalool	2.79
14	1205	Trans-Piperitol	9.66
15	1800	1-Octadecane	2.53

Microbial strains

The microbial strains viz., *Bacillus cereus* MTCC 1429, *Staphylococcus aureus* MTCC 737, *Escherichia coli* MTTC 1687, *Pseudomonas aeruginosa* MTTC1688, *Klebsiella pneumoniae* MTCC 109, *Salmonella typhimurium* MTCC 98 and yeast *Candida albicans* MTTC 183, obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India, were used in the study.

Antimicrobial screening

The agar disc diffusion method was used to determine the antimicrobial activity of the essential oil[18]. Sterilized Whatmann filter paper discs (6 mm diameter) were individually impregnated with 10µl of the diluted oil samples (100mg/ml) and placed on the surface of the petri plates containing 20 ml of the respective media were seeded with 0.1 ml of previously prepared microbial suspensions (5 x 10^5 CFU/ml). Standard antibiotics viz., ampicillin, kanamycin and vancomycin (30 µg/ disc) obtained from Hi-media, Mumbai, were used as positive controls. The plates were incubated for 24 hours at 37^0 C for bacteria and at 30^0 C for 48 hours for yeasts. The inhibition zones formed around the discs were measured and expressed in millimeters. Three independent trials were conducted for each concentration. Model values were selected.

Determination of minimum inhibitory concentration (MIC) and minimum bacterial/ fungal concentrations (MBC/MFC)

The MIC and MBC/MFC were determined using a common broth micro dilution method[19]. Serial doubling dilutions of the oil was prepared in a 96-well micro titer plates ranging from 0.05 to 10 mg/ml. All the tests were conducted in nutrient broth for bacteria and Sabouraud Dextrose broth for yeasts. 10 µl of the previously prepared microbial suspensions (5 x 10^5 CFU/ml) were added to each well. The plates were incubated for 24 hours at 37^0 C for bacteria and at 30^0 C for 48 hours for yeasts. The MIC was defined as the lowest concentration of the oil showing no visible growth.

To determine MBC/MFC, 10 µl of the broth medium from each well of MIC tested plate was taken and incubated on Nutrient agar at 37^0 C for bacteria and at 30^0 C for 48 hours for yeasts. The least concentration showing no visible (except one or two colonies) growth on agar sub culture was taken as MBC/MFC value. This is the lowest concentration expressed in mg/ml. Each test was performed in three replicated and repeated twice. The results were tabulated.

Table 2 Antimicrobial activity of essential of *P. hymenophyllum* fruit

Organisms	Inhibition zone (mm)			
	1 mg/disc	MIC µg/ml	MBC mg/ml	Standards* µg/disc
Bacillus cereus	10	625	>5	22^A
Staphylococcus aureus	10	625	5	23^K
Escherichia coli	13	625	1.25	22^K
Pseudomonas aeruginosa	10	125	5	28^K
Klebsiella pneumonia	10	125	5	23^K
Salmonella typhimurium	13	125	1.25	17^K
Candida albicans	11	625	5	25^V

Note. * A: ampicillin; K: kanamycin; V: vancomycin

RESULTS AND DISCUSSION

Chemical composition of the essential oil

Piper hymenophyllum fruits on hydrodistillation yielded thick yellow colour essential oil with a characteristic odor. The chemical composition of the essential oil was listed in table-1. The fruit essential oil rich in monoterpenes (89.53%), of which (E) phytol (21.87%) was the most abundant component followed by dihydro terpineol (17.42), α – terpineol (13.93%), trans-piperitol (9.66%), endo-fenchol (4.09%), camphene (3.92%) and γ-terpinen (3.91%). Sesquiterpenes constitute 5.62% and others 10.47% only (Table 1). The essential oil composition of *P. hymenophyllum* leaf was reported (Utpala et al., 2014). Whereas to the best of our knowledge this is the first report on phytochemical profile and antimicrobial activity of *P. hymenophyllum* fruit essential oil.

Antimicrobial activity of essential oil

The antimicrobial activity of *P. hymenophyllum* essential oil exhibited a broad spectrum antimicrobial activity against two Gram positive, four Gram negative and yeast (Table 2). Previous report on antibacterial activity of methanol extract of *P. hymenophyllum* leaf showed positive effects against *K. pneumoniae* and no effect was reported on other test pathogens[12]. In the present study, gram negative bacteria *Escherichia coli* and *Salmonella typhimurium* were exhibited maximum inhibition to the fruit essential oil. However, *Salmonella typhimurium, Pseudomonas aeruginosa* and *Klebsiella pneuminiae* exhibited highest sensitivity and strongly inhibited at very low MIC indicating the efficacy of the fruit oil on the Gram negative bacteria especially found in contaminated drinking water, supports use of the plant seeds in intestinal disorders. Whereas *Escherichia coli* and *Salmonella typhimurium* strongly inhibited by expressing very less MBC values to the test oil sample.This may be due to having a thin lipopolysaccharide layer in their cell wall. In addition, oil also a rich source of anticancer phytochemical constituent such as (E) phytol[20]. The antimicrobial activity was also supported by the presence of α-terpineol at 13.09%, which is a potential antimicrobial compound in the *P. hymenophyllum* fruit essential oil supports the present investigation[21]. Linalool is an abundant component of a number of essential oils, known for its antiviral[22], antimicrobial[23] and anti-inflammatory activity[24]. Terpinen-4-ol, a minor component of the oil, was reported for bactioststic activity against several microorganisms[25]. More over the fruit essential oil also strongly inhibited the growth of *C. albicans* and *S. aureus*, indicates use of oil in the skin diseases.

In conclusion, the present observations clearly showed that the essential oil was active against both bacteria and yeast. The gram negative bacterium *Salmonella typhimurium* exhibited strongest inhibition to the fruit sample. The antimicrobial properties of essential oil of *P. hymenophyllum* might to be due to the synergetic or individual effect of the volatile components, Viz., (E) phytol, α – terpineol, linalool and terpinen-4-ol. Further attempts are being made to isolate and characterize the bio active components. *P. hymenophyllum* fruit showed beneficial effects to inhibit tested human pathogenic organisms by *in vitro* methods

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