

Chemical Composition and Antibacterial Activity of the Essential Oil from the Seeds of *Plectranthus hadiensis*

Raju Sripathi, Subban Ravi*

Department of Chemistry, Karpagam University, Karpagam Academy of Higher Education, Coimbatore-641021, Tamilnadu, India.

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ABSTRACT

Plectranthus is a large and widespread genus of Lamiaceae family with a diversity of ethnobotanical uses. In traditional medicine, the juice of stem and leaves of *Plectranthus hadiensis* which is mixed with honey is taken as a remedy for diarrhea. The aim of the present study is to determine the chemical composition of the essential oil from the seed of *P. hadiensis* and to evaluate antimicrobial efficacy of the oil. The essential oil of the seeds from *P. hadiensis* is obtained by hydro-distillation and analyzed by gas chromatography coupled with mass spectrometry (GC/MS). It results in the identification of 25 compounds representing 99.3% of the total oil. The main compound is Piperitone oxide (33.33%). Antibacterial activity of the essential oil of *P. hadiensis* is tested against two Gram-positive and two Gram-negative bacteria, using zone of inhibition method. The essential oils inhibit the organisms and shows the zone of inhibition in the range of 20-35mm. The essential oil can serve as an antibacterial agent.

Keywords: Lamiaceae, *Plectranthus hadiensis*, essential oil, Piperitone oxide.

INTRODUCTION

Essential oils are natural, complex volatile compound mixtures characterized by a strong odour. Essential oils are composed mainly of terpenoids, including monoterpenes and sesquiterpenes, their oxygenated derivatives and a variety of molecules such as aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones, and exceptionally nitrogen- and sulphur-containing compounds and coumarins. Known for their antimicrobial medicinal properties and their fragrance, they are invariably used in preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and local anesthetic remedies¹.

The genus *Plectranthus* consists of 300 species, distributed from Africa to Asia and Australia³. In India, about 30 *Plectranthus* species are known⁴, of which *P. amboinicus*, *P. vettiveroides*, *P. barbatus*, *P. mollis*, *P. coetsa*, and *P. incanus* are the most common species used in the traditional Indian Ayurvedic medicine since ancient times to cure many disorders and diseases^{5,6}. Phytochemical studies of the genus reveals that Indian *Plectranthus* species are rich in essential oil. The essential oil is obtained only from very few species *P. amboinicus* Lour., *P. barbatus* Andrews, *P. fruticosus* L He, *P. incanus* Link, *P. japonicus* Burm. F., *P. melissoides* Benth. *P. rugosus* Wall and their composition have been reviewed and reported⁷.

Plectranthus hadiensis is reportedly cultivated in Tamil Nadu on river banks and sandy loams. The root and stem of this plant has a quite distinct and specific aroma. The herb accepts as the source of Hribera (Iruveli) in Kerala is

Coleus Zeylanicus (Benth.) Cramer (syn. *Plectranthus zeylanicus* Benth). This species is reportedly an endemic taxon of Sri Lanka, where it is known by the Sinhalese name Iruveriya, the juice of stem and leaves of which is mixed with honey is taken as a remedy for diarrhea. This plant belongs to the Lamiaceae family and is used in Ayurveda. So far only one report on its phytochemical analysis⁸ was available and that to no reports are available with respect to its essential oil. Recently in our laboratory we studied the essential oil composition from the aerial parts of *P. hadiensis*. This prompts us to carry out the present work to study the essential oil from the seeds of *P. hadiensis*.

MATERIAL AND METHODS

Plant material

The seed from the plant *P. hadiensis* was collected from the Nilgiri district during the summer season (April 2016) and authenticated by Dr. G.V.S.Murthy, Botanical Survey of India, Southern Regional Centre, Coimbatore.

Extraction of essential oil

Fresh seeds of *P. hadiensis* was cut down into small parts and exposed to hydro distillation for 3 hours using a Clevenger type apparatus. The essential oil obtained was dried over anhydrous sodium sulphate to absorb the traces of water present along the essential oil. The essential oil was then stored at 4°C until use.

GC-MS Analysis

GC-MS along with an ESI system with the ionization energy of 70 eV was utilized for analysis. Helium (99.99%) was used as carrier gas, with the flow rate of

*Author for Correspondence: ravisubban@rediffmail.com

Table 1: Essential oil composition from the seeds of *P. hadiensis*.

S.No	Compounds	RI (iu)	% composition of the essential oil
1.	1,5,5,5-Trimethyl-6-methylene-cyclohexene	992	0.94
2.	L-Fenchone	1121	4.24
3.	Copaene		8.82
4.	8,11,15-Eicosatrienoic acid	2390	0.90
5.	beta-Cubebene	1339	2.45
6.	111,11-Dimethyl-spiro[2, 9] dodeca-3,7-dien	1452	1.21
7.	4,4'-Dimethylbicyclohexyl-3, 3'-dine,2,2'-diene		1.82
8.	Beta-Farnesene	1440	7.40
9.	Alpha- Caryophyllene	1579	6.95
10.	2-Isopropenyl-5-methylhex-4-enal	1092	1.02
11.	Germacrene D	1515	0.47
12.	Benzocycloheptene,2,4a,5, 6, 7 ,8,9,9a-octahydro-3 ,5,5-trimethyl	1494	1.37
13.	Piperitone oxide	1171	33.33
14.	delta - Cadinene	1469	4.66
15.	Disophenol	1288	0.80
16.	p-Cymen-8-ol	1197	2.16
17.	Isolongifolan-8-ol	1593	0.54
18.	3,5-Heptadienal,2-ethylidene-6-methyl	1182	5.98
19.	2-Oxabicyclo[2.2.2]octan-6-one,1,3, 3-trimethyl	1230	0.92
20.	delta-Cadinol	1580	2.70
21.	2 (3H)-Naphthalene ,4,4a,5, 6, 7,8-hexahydro- 1-methoxy	1449	2.25
22.	15,15'-Bi-1,4,7,10,13-pentaoxacyclohexadecane	3628	0.37
23.	alpha-Hydroxymyristic acid	1932	1.09
24.	P-Cymen-3-ol	1262	5.45
25.	Octaethyleme glycol monododecyl ether	3654	2.11

1ml/min. The injection port temperature was set at 250°C, initial column temperature was kept at 40°C for 1 min, and then gradually increased to 240°C at the flow rate of 3°C /min. The components were identified by comparing their mass spectra with those in the GC-MS library and literature and by comparing their relative retention times by those of authentic samples on the HP-5 MS capillary column.

Antibacterial activity

The antibacterial activity of the essential oil from *P. hadiensis* was determined by disc diffusion method. The inoculums for the experiment were prepared in fresh nutrient broth from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture was adjusted by the addition of sterile saline or broth. This method depends on the diffusion of the essential oil from a cavity through the petri dish, to an extent such that growth of the added microorganism was prevented entirely in circular area or zone around the cavity containing the essential oil. The standardized inoculums were inoculated in the plates by dipping a sterile in the inoculums, removing excess of inoculums by pressing and rotating the swab firmly against the side of the culture tube before the plates are seeded above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60° after each application. Finally pass the swab round the edge of the agar surface. Leave

the inoculums to dry at room temperature with the lid closed^{9,10}. Tetracyclin is used as a standard substance. Then petri dishes were placed in the refrigerator at 4°C or at room temperature for 1 hour for diffusion. After the incubation period (at 37°C for 24 hours), diameter of zone of inhibition in mm obtained around the well was measured. The diameter obtained for the essential oils were compared with that of the diameter produced by tetracycline. The diameter of zone of inhibition was proportional to the antibacterial activity of the essential oil.

RESULT AND DISCUSSION

Pharmaceutical properties of aromatic plants are in the part attributed to essential oils that are known as flavoring additives to cosmetics, disinfection agents and medicinal means for a long time. It is well-known that the same taxon growing in different areas and in different seasons may have widely differing chemical components and hence differing biological properties.

Essential oil obtained from the seed of *P. hadiensis* by hydrodistillation was a yellowish liquid with a specific herbal scent and sweet smell. The plant yielded 1.86%, of the essential oils from the seed. The yield of the oil from the seed is high when compared with the oil from the whole plant material irrespective of the season. The chemical composition of the obtained oil was determined by GC/FID and GC/MS. Many compounds are found to be belonging to the classes of aldehydes, alcohols,

Table 2: Antibacterial activity of the essential oil from the seed of *P. hadiensis* expressed as the diameter of the inhibition zone in mm in the disk sensitivity assay.

Sample	<i>Staphylo</i> <i>aureus</i>	<i>coccus</i> (mm)	<i>Stepto</i> <i>coccus</i> (mm)	<i>Escherichia</i> <i>coli</i> (mm)	<i>Pseudomonas</i> <i>aeruginosa</i> (mm)
Essential oil (Seeds)	29		31	32	35
Tetracyclin	28		36	35	37

terpenes, esters, acids and hydrocarbons. The oil analysis resulted in the identification of 25 compounds representing 99.3%. The essential oil consists of Oxygenated monoterpenes (53.92%) predominantly followed by sesquiterpenes (28.85%) and Oxygenated sesquiterpenes (3.2%). It doesn't contains monoterpenes. When this result is compared with the essential oils obtained from the aerial part of *P. hadiensis*, the rainy season oil consists of sesquiterpenes (37.08%), oxygenated monoterpenes (31.11%) and monoterpenes (9.7%). Oxygenated monoterpenes (39.23%) dominates in the summer season oil, followed by sesquiterpenes (37.48%) and monoterpenes (9.14%).

The major constituents (Table 1) from the seed essential oil are Piperitone oxide (33.33%), copaene (8.82%), β -Farnesene (7.4%), α -caryophyllene(6.95%), 3,5-Heptadienal,2-ethylidene-6-methyl(5.99%), P-Cymen-3-ol (5.46%) and L-Fenchone (4.24%). This is quite distinct from the essential oil obtained from the aerial part of *P. hadiensis* where the dominant component is L-Fenchone (30.42 to 31.55%). Piperitone oxide and beta-farnesene is reported as the major compounds from *P. mollis* collected from the Western Ghats and from *P. incanus* link^{11,12}.

Anti-bacterial activity

Many microorganisms, which cause damage to human health, exhibit drug resistance due to inadequate use of antibiotics. Thus, there is a need for the discovery of new substances from natural sources, including plants. The essential oil from the seed exhibited good antibacterial activity against *S.aureus* and *P.aeruginosa* and moderate activity against *S.* and *E.coli* (Table 2). The antibacterial activity of the essential oil from the seed is almost on par with the standard compound tetracycline. The maximum antibacterial activity is shown in terms of zone of inhibition to *Paeruginosa* (35mm) and *S. aureus* (29 mm), but least sensitive micro-organisms were *E. coli* and *S.* (32 and 31 mm respectively).

CONCLUSION

The GC-MS analysis of the essential oil from seeds of *P. hadiensis* resulted in the identification of 25 compounds representing 99.3% of the total oil. The major compound, is Piperitone oxide (33.33%).

Antibacterial activity of the essential oil was tested against two Gram-positive and two Gram-negative bacteria by using zone of inhibition method and it showed the zone of inhibition in the range of 20-35mm.

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