



ESSENTIAL OIL, PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITY OF *PLECTRANTHUS WIGHTII BENTH.*

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Abstract:

Varieties of plants are used for the treatment of some incurable disease like asthma, paralysis, hypertension and hysteria. The species *Plectranthus wightii* is used for the remedy of hypertension, hysteria fever, dysentery and paralysis and to sooth the active nerves during anxiety by the tribes in Nilgiris. This plant is used in aromatherapy to elevate these disorders by spreading fresh leaves over the bed of the patient every day. This prompted us to undertake the chemical screening of the volatile constituents and the whole plant through GC-MS analysis and also to evaluate the biological activity.

Key words: *Plectranthus wightii*, paralysis, folk medicine, GC-MS analysis.

1. Introduction:

The Western Ghats endemic plant kingdom is known for its chemical diversity¹. High percentage of medicinal application contributed by the genus *Plectranthus* is well established². The various species of *Plectranthus* is known for their medicinal properties such as digestive disturbances, skin infections, respiratory infections, general infections and fever, urinary infections, pain and musculoskeletal conditions.^{2,3} Medicinal plants are believed to be a potential source for the discovery of new drugs candidates⁴⁻⁷. *Plectranthus* species are used also to treat blood and circulation conditions and the nervous system disturbances².

Genus *Plectranthus* (Lamiaceae) comprises about 300 species of herbs and shrubs native to tropical regions¹. Four species belonging to this genus, *Plectranthus bournea*.Gamble, *Plectranthus coleoides* (or) *wightii* Benth, *Plectranthus mollis* (Alt) Spreng and *Plectranthus stecksit*. Hook are available in and around Nilgiris, Tamil Nadu. Active principles in the plant and their biological importance are well established in the literature⁸⁻¹². The increasing importance of the essential oil in the pharmaceutical and aromatherapy besides

their traditional role in cosmetics, perfumery and in preservatives is the subject of several investigations¹³⁻¹⁵. The essential oil contains mainly monoterpenes¹⁶⁻¹⁸, sesquiterpenes, poly acetylenes, Phenolic compounds¹⁹⁻²¹, frequently fatty acids, alcohol and esters²²⁻²⁹ infrequently nitrogen and sulphur containing compounds³⁰. As it is mentioned in the abstract the species *wightii* Benth is used for the treatment of incurable disorders. Such type of treatment indicates the presence of bioactive constituents in *Plectranthus wightii* Benth. So that this plant is subjected to present investigation and a specimen is kept in the Botany department (File No. 1090, collected on September 1996), Govt. Arts College, Udhagamandalam. The Nilgiris.

2. Materials and methods

2.1 Extraction of Essential oil constituents of *Plectranthus wightii* Benth.

Plectranthus wightii is a tall herb with white flowers speckled with red points, growing up to an altitude of 8000 feet and chiefly at higher levels. The volatile oil was extracted once from 500 g of green leaves and branches of *Plectranthus* species by water-distillation (2.5 h), utilizing a Clevenger apparatus. Finally, the obtained oil was desiccated using anhydrous Na_2SO_4 and kept at low temperatures ($+4^\circ\text{C}$) for further experiments. It was observed that the presence of piperitone oxide and piperitenone oxides presence in this plant and also these compounds distribution is not limited only with *Saturaja* and *Calametha* species as it is in the literature GC-MS details presented in table

2.2 Extraction of Phytoconstituents excluding essential oil of *Plectranthus wightii* Benth.

The dried plant (2Kg) material was subjected to n-hexane extraction to exclude the essential oil and other wax material, then the marc is subjected to ethanol extraction. The residue left after distillation of ethanol extract was used for further analysis.

Table 1: Phytochemical Analysis of *Plectranthus wightii* Benth ethanol extract

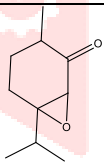
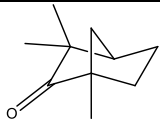
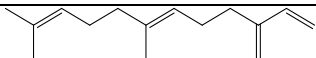
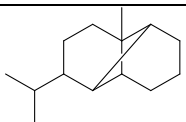
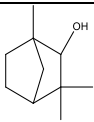
Detection	Test	Response	Result
Alkaloids	Mayer's test	No characteristic colour	-
Carbohydrates	Fehling's test	Red precipitate color	+
Phenolic compounds and Tannins	Ferric chloride test	No characteristic colour	-
Flavonoids	Alkaline Reagent Test	No characteristic colour	-
Terpenoids	Dinitrophenyl hydrazine test	Yellow interface layer	+

2.3 GC-MS analysis of essential oil and ethanol extract of *Plectranthus wightii* Benth

Gas chromatographic studies were carried out in a Hewlett-packard gas chromatography, model 5890, Shimadzu, Japan, equipped with electronic integrators 50 m x 0.2mm (film thickness 0.17 μ m), carbo wax and methyl silicone capillary column were used for the analysis. The programming temperature was maintained from 80 to 200°C. FID and injector temperature were 300°C and 250°C respectively. Nitrogen was used as the carrier gas at a flow rate of 1ml/minute with a split ratio of 1.7.5 and the constituents were identified and confirmed by comparison with those of the stored data in computer library (WILEY 229, NIST 107, NIST 21 libraries).

The chemical composition and content of the essential oil of *Plectranthus* identified by GC-MS analysis are given Table 2. The oil contains oxygenated monoterpenes (68.65%) and sesquiterpenes (2.16%) and sesquiterpene hydrocarbons (17.3%). Out of 28 constituents present in the oil ten compounds constitute 97%. The major components of the essential oil are monoterpene oxides, piperitone oxide (38.12%), fenchone (23.51%) and sesquiterpene hydrocarbon, α -copaene (6.49%), β -farnesene (6.76%) and the ethanol extract contains mono, sesquiterpenes, amino and amide compounds, some simple organic molecules are presented in Table.3

Table 2. GC-MS result of *Plectranthus wightii* Benth essential oil

Compounds	Structure	Retention Time	Percentage Composition
Piperitone oxide		14.94	38.12
Fenchone		9.59	23.51
β Farnescene		13.47	6.76
α Copaene		10.91	6.49
Fenchol		12.26	1.51

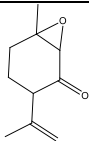
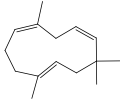
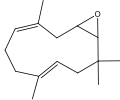
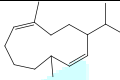
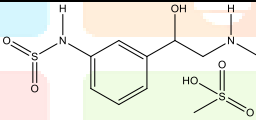
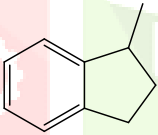
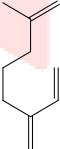
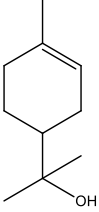
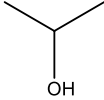
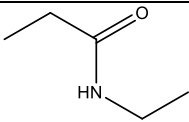
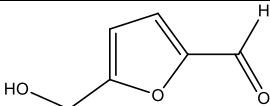
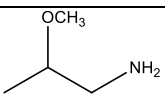
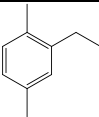
Piperitenone oxide		19.77	3.15
Hmulene		13.79	2.70
Humulene oxide		21.73	2.16
Germa crene		11.58	1.35

Table 3.GC-MS result of Plectranthus wightii Benth ethanol extract

Compounds	Structure	Retention Time	Percentage Composition
Amidephrine mesylate		18.90	21.63
1-methyl-Indan		12.58	19.33
myrcene		13.47	1.20
α -terpeneol		8.11	1.78
Isopropyl alcohol		21.25	11.50

N-ethyl-propanamide		23.27	10.81
5-(hydroxymethyl)-2-furancarboxaldehyde		18.39	12.15%
		22.03	7.15
2-ethenyl-1,4- dimethyl-benzene		19.51	0.23

2.4 Antioxidant activities

Presence of diverse nature of compounds and various folk medicinal ailments offered by the plant *Plectranthus wightii* Benth. The following systematic analysis against 2,2-diphenyl-2-picryl-hydrazyl (DPPH), hydroxyl (OH) and nitric oxide (NO) radical scavenging assays have been carried out.

2.4.1 Hydroxyl radical

Among all the free radicals, hydroxyl radical is by far the most potent and therefore the most dangerous oxygen metabolic and hence the elimination of this radical is one of the major aims of antioxidant administration³¹. It attacks proteins, DNA, polyunsaturated fatty acid in membranes and most biological molecules³². Hydroxyl radical is known to be capable of abstracting hydrogen atoms from membrane lipids and brings about peroxide reaction of lipids. Scavenging activity of the essential oil and ethanol extract of the plant *Plectranthus wightii* Benth on hydroxyl radical has been investigated and compared with the standard ascorbic acid. The IC₅₀ values indicates that the oil and the extract have shown moderate activity compared to the standard and the values are presented in the Table.

2.4.2 DPPH radical

The 2, 2-Diphenyl-2-picryl-hydrazyl (DPPH) assay is widely used for assessing the ability of radical scavenging activity and it is measured in terms of IC₅₀ values. Because of the presence of odd electron, DPPH shows a strong absorption band at 517 nm in the visible spectrum³³. As this electron becomes paired off in the presence of a free radical scavenger, this absorption vanishes, and the resulting decolourization is in stoichiometric proportion with respect to the number of electrons taken up.

2.4.3 Nitric oxide radical

Nitric oxide (NO) is a diffusible free radical which plays a role in damaging the cells and hence the metabolic path in diverse biological system. Nitric oxide inhibitors were shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases³⁴. These compounds are responsible for altering both the structural and functional behavior of many cellular components. With an expectation that the essential oil and the ethanol extract of *Plectranthus wightii* Benth may counteract the effect of NO formation and in turn possess considerable interest in preventing the ill effects of excessive NO generation in the human body. As it is expected the plant have shown considerable scavenging ability compared to the standard and the results are presented in the Table.4

Table. 4. Antioxidant activity of essential oil and ethanol extract of *Plectranthus wightii* Benth

Compounds	Essential Oil(IC ₅₀ μM)			Ethanol extract (IC ₅₀ μM)		
	DPPH	OH	NO	DPPH	OH	NO
1	61.40	58.87	31.73	53.28	52.29	33.12
2	64.21	60.43	45.32	47.41	55.30	41.63
3	68.35	65.64	38.76	50.34	48.64	39.27
Ascorbic acid (AA)	96.43	90.27	87.00	96.43	90.27	87.00

Table 5: Antibacterial activity of essential oil and ethanol extract of *Plectranthus wightii* Benth

Name of the species	Standard used	Essential oil	Ethanol extract	Standard
<i>Escherichia coli</i>	Chloromphenical	17	20	24
<i>Pseudomonas fluorescense</i>	Tetracycline	18	19	24
<i>Pseudomonas putida</i>	Chloromphenical	21	21	32
<i>Pseudomonas beteli</i>	Chloromphenical	-	12	20
<i>Salmonella paratyphi</i>	Tetracycline	15	09	19
<i>Staphylococcus aureus</i>	Tetracycline	-	13	20
<i>Bacillus subtilis</i>	Tetracycline	16	12	21
<i>Candida albicans</i>	Chloromphenical	11	09	20
<i>Bacillus cereus</i>	Tetracycline		10	25
<i>Pseudomonas aeruginosa</i>	Chloromphenical		07	19

3. Result and discussion

The Phytochemical screening of essential oil and ethanol extract of the plant *Plectranthus wightii* Benth was analysed using GC-MS analysis and the presence of piperitone oxide and piperitenone oxides presence was identified. Early these compounds were reported only from Calametha and saturaje species of Arabian countries. Antioxidant activity of ethanolic extract and the essential oil extract were analysed separately using α, α -Diphenyl- β -picryl-hydrazyl radical scavenging (DPPH) assay. The OH radical and NO radical scavenging activity were also performed for both the ethanol and the essential oil of *Plectranthus wightii* Benth.. The radical scavenging activity of the essential oil and the ethanol extract of the plant have showed proportional increase with respect to the concentration of the plant extract. The antibacterial activity was examined for both essential oil and ethanol extract against the bacterial strains of *Escherichia coli*, *Pseudomonas beteli*, *Pseudomonas fluorescens*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*. After the incubation period, a comparison on the activity in terms of zone of inhibition of the extracts and the commercial standard antibiotics revealed the moderate activity showed by the plant extracts against the pathogen.

4. References

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