Phytochemical Screening and GCMS Analysis of the Methanolic Extract of Plectranthus Ambonicus

Saisha Vinjamuri
Department of Biotechnology
BMSCE,Bangalore,Karnataka,India

Abstract- This paper reports the phytochemical screening and GCMS analysis of the methanolic extract from leaves of Plectranthus *ambonicus* which is used extensively in traditional medicine. The present investigation was carried out to screen for and identify potent bioactive compounds, which could lead to extraction of these bioactive compounds and evaluation of their therapeutic potential. Preliminary phytochemical screening was carried out using four solvents (viz., water, methanol, chloroform and petroleum ether) as per the procedure described by Harborne (1973) and Doss (2009). The methanolic extract which showed the presence of abundant phytochemical constituents was subjected for GCMS analysis. Seventeen compounds were identified by GCMS analysis, of which davanone was found to be the predominant phytochemical.

Keywords – *Plectranthus*, methanolic extract, GCMS, Davonone

I. INTRODUCTION

Plectranthus ambonicus is a tender, fleshy perennial herb, belonging to the family *Lamiaceae* and is a commonly used medicinal herb in India. The herb has green, thick, succulent, heart shaped and juicy leaves with scalloped edges. The raw leaves emanate an oregano-like flavor and odor when cut or crushed.

The leaves have been traditionally used for the treatment of respiratory problems like chronic coughs, cold, nasal congestion, bronchitis, asthma as well as other conditions like diarrhea, flatulence infections, and rheumatism. It is also reported to have anti-tumor and cytotoxic activities in addition to antioxidant, antimicrobial and antiepileptic properties.[1,2] .Though the plant has been used extensively in traditional medicine, significant literature on the composition of phytochemical constituents (bioactive components) present and their mode of therapeutic action is not available. So, the present study was aimed to investigate the phytochemical components present in the leaves of *Plectranthus ambonicus* by carrying out preliminary phytochemical screening in four solvents (viz., water, methanol, chloroform and petroleum ether) followed by GC MS analysis for the identification and quantification of phytochemical constituents. GCMS analysis of the methanolic extract which showed abundant phytochemical constituents was carried out.

.

II. METHODOLOGY

A. Collection of plant material: –

The plants were purchased from the local nursery and identified by a taxonomist. The leaves of the plant were collected, washed thoroughly and air dried for three days at room temperature. The air dried leaves were then ground to powder and passed through a fine sieve. The powder obtained was weighed and stored in air tight container at 40C

B. Preparation of solvent extract –

Solvent extracts (40 mL) of the powdered sample, each containing two grams were prepared using four different solvents viz., water, methanol, chloroform and petroleum ether and used for qualitative identification of the phytochemicals as per the procedure described by Harborne (1973) and Doss (2009)[3,4].

C. Phytochemical screening tests –

Phytochemical screening tests of the four solvent extracts was carried out as shown in **Table 1** as per the procedure described by Harborne (1973) and Doss (2009). [3, 4].

Table 1: Phytochemical Screening Tests for Plant Extracts.

Constituents being tested	Name of the test	Test procedure	Identification of the constituents
Alkaloids	Hager Test	2mL extract + few drops of Hager's reagent	Yellow precipitate
Carbohydrates	Molisch Test	2mL extract + 10mL H_2O + 2 drops Ethanolic α-naphthol (20%) +2mL H_2SO_4 (conc.)	Reddish violet ring at the junction
Glycosides	Liebermann Test	2mL extract + 2mL $CHCl_3$ + 2mL CH_3COOH	Violet to Blue to Green coloration
Anthraquinones	Borntrager Test	3mL extract + 3mL Benzene + 5mL NH_3(10%)	Pink, Violet or Red coloration in ammonical layer
		(a) 5mL extract + 5mL H_2O + heat	Froth appears
Saponins	Foam Test	(b) 5mL extract + Olive oil (few drops)	Emulsion forms
Flavonoids	Lead acetate Test	1mL extract + 1mL $Pb(OAc)_4$ (10%)	Yellow coloration
Terpenoids		2mL extract + 2mL $(CH3CO)_2O$ + 2-3 drops conc. H_2SO_4	Deep red coloration
Steroids	Salkowski Test	2mL extract + 2mL $CHCl_3$ + 2mL H_2SO_4 (conc.)	Reddish brown ring at the junction
Phlobatannins	(Precipitate Test)	2mL extract + 2mL HCl (1%) + heat	Red precipitate
Coumarins		2mL extract + 3mL NaOH (10%)	Yellow coloration
Emodins		2mL extract + 2mL NH_4OH + 3mL Benzene	Red coloration
Anthocyanins		2mL extract + 2mL HCl + NH_3	Pinkish red to bluish violet coloration
Leucoanthocyanin		5mL extract + 5mL Isoamyl alcohol	Organic layer into Red
Tannins	*(Braymer's Test)*	2mL extract + 2mL H2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate
Proteins	*Xanthoproteic Test*	1mL extract + 1mL H_2SO_4(conc.)	White precipitate

D. GC MS analysis of the methanolic extracts –

GCMS analysis of the methanolic extract of *Plectranthus ambonicus* which showed the presence of considerable phytochemical constituents was carried out in a GC_MS Clarus 500 Perkin Elmer system employing the following

conditions : Restek RtxR– 5, (30 m X 0.25 mm)(5% diphenyl / 95% dimethyl polysiloxane), running in electron impact mode at 70 eV.

Helium (99.999%) was used as carrier gas at a constant flow of 1 mL/min and an injection volume of 1.0 µL was employed (split ratio of 10:1); injector temperature 280^0C. The oven temperature was programmed from 40°C (isothermal for 5 min.) with an increase of 6^0C / min to 280 ^{0}C, then ending with an isothermal for 15min at 280°C. Mass spectra were taken at 70 eV; at 0.5 seconds of scan interval and fragments from 40 to 550 Da. Total GC running time was 60 minutes**.**

Interpretation of the mass spectra obtained was conducted using the database of National Institute Standard and Technology (NIST). The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

III. RESULTS

a.)*Phytochemical screening tests*

Preliminary phytochemical screening of the leaves of *Plectranthus ambonicus* was carried out in four solvents (viz., water, methanol, chloroform and petroleum ether) as per the procedure described by Harborne (1973) and Doss(2009) (**Table 1**). It was observed that many of the phytochemicals could be extracted using methanol as solvent as shown in **Table 2.**

Table 2: Phytochemical Screening Analysis of the Plant Extracts

TESTS	Aqueous Extract	Methanol Extract	Chloroform Extract	Petroleum Ether Extract
Alkaloids	-	+	+	-
Carbohydrates & Glycosides	+	+	+	+
Phytosterols Salkowski test	+	+	+	+
Saponins	+	-	-	-
tannins	+	+	-	-
Proteins & Amino acids	-	-	-	-
Flavonoid's test	+	-	-	-
Terpenoid's test	-	+	+	-
Phlobatannins test (Precipitate test)	-	-	-	-
Coumarin's test	+	+	-	-
Emodin's test	-	-	-	-
Anthocyanin's test	-	-	-	-
Leucoanthocyanin's test	-	-	-	-

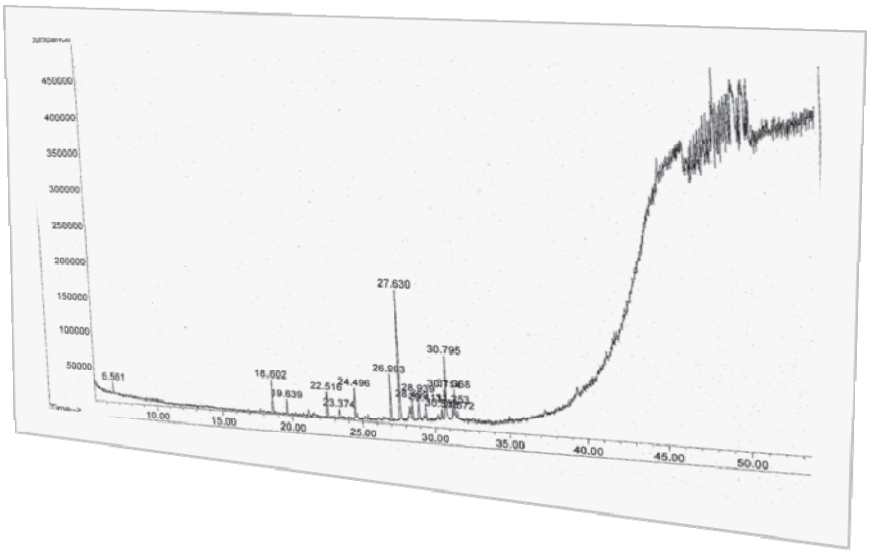
(+) = Presence, (-) = Absence

b.) *GC MS analysis* of the methanolic extracts

GCMS analysis of the methanolic extract of *Plectranthus* which showed the presence of abundant phytochemical constituents was carried out in a GC_MS Clarus 500 Perkin Elmer system as described earlier. The phytochemicals were identified and characterized by comparing the mass spectra obtained with the NIST library.

GC-MS chromatogram analysis of the methanolic extract showed seventeen peaks indicating the presence of seventeen phytochemical constituents (**Figure 1)**.Of the seventeen compounds identified, the most abundant phytochemical had a retention time of 27.635min (**Table 3**). On comparing with the NIST database, this compound was found to be 1,5- hepten-3-one,6 methyl-2 – (tetrahydro -5- methyl -5-vinyl-2furyl)-(+)- 2 commonly called as davanone.(**Figure 2.**)

Fig. 1.GCMS Peak of Methanolic Extracts of Leaves of Plectranthus



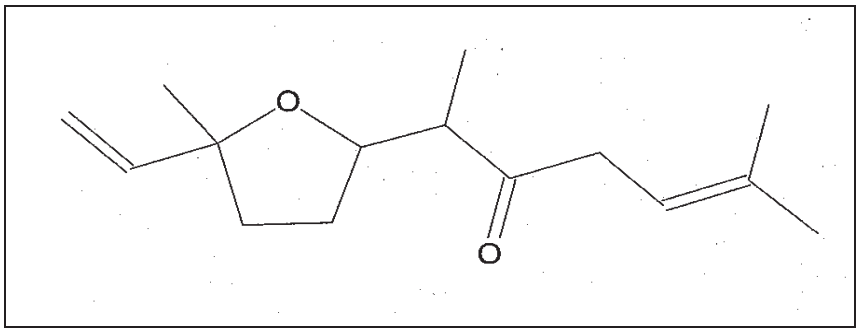
Source : GCMS chromatogram obtained for methanolic extracts of Plectranthus

Table 3: GCMS Analysis of Methanolic Extracts of Leaves of *Plectranthus.*

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.560	135	141	147	M9	15179	464578	7.34%	2.097%
2	18.608	1279	1286	1293	M4	47392	1654082	26.13%	7.467%
3	19.639	1379	1384	1390	M5	18398	566650	8.95%	2.558%
4	22.511	1652	1657	1663	M5	34444	1229732	19.43%	5.551%
5	23.374	1731	1739	1745	M8	12745	578596	9.14%	2.612%
6	24.500	1839	1846	1852	M4	40974	1515632	23.94%	6.842%
7	26.994	2075	2083	2095	M4	61509	2094680	33.09%	9.456%
8	27.635	2135	2144	2153	M2	173697	6330502	100.00%	28.576%
9	28.498	2221	2226	2234	M8	25845	910589	14.38%	4.110%
10	28.940	2261	2268	2275	M8	33733	1569673	24.80%	7.086%
11	29.414	2307	2313	2322	M8	21351	777105	12.28%	3.508%
12	30.508	2411	2417	2422	M7	11937	414059	6.54%	1.869%
13	30.718	2430	2437	2440	M6	21915	508933	8.04%	2.297%
14	30.792	2441	2444	2450	M	65399	1768613	27.94%	7.984%
15	31.255	2481	2488	2490	M6	13401	420767	6.65%	1.899%
16	31.371	2493	2499	2506	M5	33286	1063692	16.80%	4.802%
17	31.571	2515	2518	2524	M7	9787	285136	4.50%	1.287%

Source : GCMS chromatogram obtained for methanolic extracts of Plectranthus

Figure 2.Chemical structure of the compound with retention time of 27.635min.



Source : NIST database /library

Name: 5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2.alpha.(R*),5.alpha.]]-
Formula: $C_{15}H_{24}O_2$
MW: 236 CAS#: 20482-11-5 NIST#: 249599 ID#: 67112 DB: mainlib
Other DBs: None
Contributor: TNO Volatile Compounds in Food - Chemical Concepts
10 largest peaks:
111 999 | 93 542 | 69 398 | 41 376 | 55 349 | 97 204 | 43 202 | 67 109 | 125 106 | 81 99 |
Synonyms:
1.5-Hepten-3-one, 6-methyl-2-(tetrahydro-5-methyl-5-vinyl-2-furyl)-, (+)-
2.Davanone
3.5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-
4.5-Hepten-3-one, 6-methyl-2-(tetrahydro-5-methyl-5-vinyl-2-furyl)-
5.6-Methyl-2-(5-methyl-5-vinyltetrahydro-2-furanyl)-5-hepten-3-one #

Estimated Kovats RI:
Value: 1627 iu
Confidence interval (Diverse functional groups): 89(50%) 382(95%) iu

IV.CONCLUSION

The GCMS analysis of the methanolic fraction of the leaves of *Plectranthus* indicates the abundance of Davanone. Further work needs to be carried out to evaluate the bioactivity of this compound and its mode of action to assess its potential as a therapeutic agent.

REFERENCES

[1] B Arunkumar , Sathasivam , Kthikeyan and Elangovan, Evaluation of phytochemical and antibacterial activity of *Plectranthus ambonicus*,*IJRAP*,**2011**,2(1): 292-294
[2] Thaniarasu, Senthil Kumar and Abubacker, Rao M, Preliminary Phytochemical Screening And Evaluation of Antibacterial Activity of Different Solvent Extracts of *Plectranthus Bourneae Gamble* (*Lamiaceae)*,*Asian J Pharm Clin Res*, **2015**,8(1): 79-82
[3] Harborne JB. Phytochemical Methods. Chapman and Hall Ltd.,London: U.K., **1973,** 49-188.
[4] Doss A. Preliminary phytochemical screening of some Indian medicinal plants. *Anc Sci Life* **2009**, 29:12-16.