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PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOLIC LEAF EXTRACT OF *PLECTRANTHUS AMBOINICUS* (LOUR) SPRENG

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ABSTRACT

The leaf of *Plectranthus amboinicus* is an important medicinal plant was subjected to phytochemical screening and GC-MS analysis methanolic extracts. The methanolic leaves extracts were screened for their preliminary phytochemical components. The solvent extract with higher phytochemical yield was subjected to quantitative analysis using the gas chromatography-mass spectrometry (GC-MS) technique. The methanolic leaves extract showed the highest phytochemical content. GC-MS analysis of the methanolic extract showed a total of thirty phytocompounds among which most of the compounds were medicinally important. The major phytoconstituents were methyl 2-oxononanoate (12.80), phenol,2-methoxy(2-propenyl)- (10.14), nonadecanoic acid (7.56), cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-, pivalate (7.50), perilla acetate (6.65), eliminoxy (6.44), methylsulfonylmethane (6.20) and minor phytoconstituents was stigmasterol (5.44), 9-hexadecenal (3.76), octadecane-1,2-diol, bis (trimethylsilyl) ether (3.42), caryophyllene (3.32), squalene (3.03), n-hexadecanoic acid (2.87) in the methanolic extract of *Plectranthus amboinicus*. These findings support the traditional use of *P. amboinicus* in various disorders.

Keywords **:** GC-MS analysis; *P. amboinicus;* leaf extract; phytochemical; solvent extraction.

Introduction

The subcontinent is rich in medicinal plants and is one of the richest countries in terms of genetic diversity of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Mitchell and Cotran, 2000). Several plants have been used in folklore medicine. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. *Plectranthus amboinicus* is a succulent herb and has the typical fourcornered stem of the Lamiaceae family.

The leaves are very thick and succulent, grayish green and hairy. The plant grows to a length of around 50 cm. The leaves are highly aromatic with a strong flavor of mixed herbs. The herb grows easily in a well drained, semishaded position. It is frost tender and grows well in sub tropical and tropical locations, but will also do well in cooler climates if grown in a pot and brought indoors, or moved to a warm sheltered position in winter.

The leaves have also had many traditional medicinal uses, especially for the treatment of coughs, sore throats and nasal congestion, and also for a range of other problems such as infections, rheumatism and flatulence. In Indonesia *Plectranthus amboinicus* is a traditional food used in soup to stimulate lactation for the month or so following childbirth. The herb is also used as a substitute for oregano in the food trade and food labeled "oregano flavored" may well contain this herb. The multidrug resistant strain of many microorganisms has revealed exploration of alternative antimicrobial agent. Medicinal plants have become the focus of intense study in terms of validation of their traditional uses through the determination of their actual pharmacological effects. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often adulterated and possess side effects. Inflammation is a local response of living

mammalian tissues to the injury. It is a body defense mechanism reacting in order to eliminate or limit the spread of injurious agents.

Plant metabolism has been able to separate phytochemicals in two categories, namely, primary or secondary. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, and chlorophylls. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids, and glucosides (Saxena *et al.,* 2013).

Leaves are used in the treatment of menorrhagia, rheumatism, dyspepsia, indigestion, dysmenorrhea, diabetes, hypertension, menstrual disorders, antiallergic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective, antihelminthic, hypolipidemic, skin diseases, bleeding diarrhea, and antiviral properties. Currently, herbal research has been mainly focusing on isolation, characterization, identification, and quantification of bioactive constituents and secondary metabolites (Das and Sharangi 2017). Gas chromatography-mass spectrometry (GC-MS) is one such sophisticated analytical technique used in identification, detection, and analysis of the constituents. It comprises GC coupled to a MS, by which complex mixtures of plant-related compounds may be separated, identified, and quantified (Chaskar *et al.,* 2017). Hence, the present study is attempted to evaluate the phytochemical constituents, GC-MS analysis and antimicrobial activity of the leaf extracts of *P. amboinicus*.

Material and Methods

Collection and identification of the plant material

The fresh leaves of *Plectranthus amboinicus* was collected from Gudiyatham, Tamil Nadu, India (Fig. 1). The sample was authentically identified from Department of Botany, K.K. Government Arts College, Thiruvannamalai. The leaves were thoroughly rinsed with tap water and ensured that it was devoid of contaminants. The leaves were air dried at room temperature and pulverized to fine powder and stored in air tight container at room temperature for further analysis.

Preparation of leaf extracts

The extract was prepared using three solvents of increasing order of polarity *viz.,* acetone, methanol, chloroform and aqueous. For the preparation of aqueous extract, about 10g of the leaves were homogenised with 10ml of hot water using mortar and pestle, 90ml of hot water was mixed with the residue and stirred for 30 min. The finely pooled extract was centrifuged at 10,000 rpm for 15min at 4°C. The collected supernatant was concentrated using rotary evaporator and used for further analysis. While, for the acetone, methanol and chloroform extraction, 10g of the dried leaf powder was added to 100ml of the respective solvent and extraction was performed by cold maceration method for 72h. After extraction, it was filtered using Whatman filter paper and the solvent was evaporated to dryness under vacuum using a rotary evaporator. The crude extract was weighed and dissolved in a known volume of dimethyl sulphoxide (DMSO). The extraction yield was expressed as:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the dry extract (g)}}{\text{Weight of the sample used for the (g)}} \times 100$$

Phytochemical analysis

The acetone, methanol, chloroform and aqueous extracts of *P. amboinicus* leaves were subjected to preliminary phytochemical tests namely carbohydrates, saponins, flavonoids, proteins, alkaloids, tannins, phenols, glycosides, terpenoids and sterols as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1978).

Fig. 1: Photographs of *Plectranthus amboinicus* plant (aerial part) and leaves grown in Gudiyatham, Tamil Nadu

Gas Chromatography with Mass Spectrometry analysis

The identification of various compounds was determined by GC-MS analysis of the leaf methanolic extracts. The study was conducted at Periyar University's Department of Microbiology at Salem, Tamil Nadu. The GC-MS analysis of the methanolic leaf extract of *Plectranthus amboinicus* was performed using a Perkin Elmer GC-MS (Model: Shimadzu QP-2020) equipped with an Agilent column (30m×250µm×0.25µm). The oven temperature was programmed at 40°C for 2 min and then increased to

30°C for 6 min, at 10°C/min. Helium was used as the carrier gas at flow rate of 1.0 ml/min. The 1 µl of the methanolic leaf extract of *Plectranthus amboinicus* was injected with split ratio10:1 at injector temperature was 280°C. The characterization of compounds was determined based on the retention time. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS, National Institute of Standard Technology (GC-MS, NIST-2017) library.

Statistical analysis

The results of the phytochemical and proximate measurements were presented as the mean standard deviation. The values are ± SD for three samples in each group.

Result and Discussion

Phytochemical Analyses of Crude Extract

The leaves of *P. amboinicus* showed more phytochemicals such as flavonoids, alkaloids, phenols, glycosides, sterols, carbohydrates, saponins, proteins and quinones when compared to others. Methanol extracts contained more strong phytochemicals, flavonoids, alkaloids, phenols, glycosides, sterols and moderate amount of carbohydrates, saponins, and quinones were also present. This extract was free of tannins. On contrary, in the acetone and chloroform extract flavonoids, phenols, terpnoids and sterols were present and the rest of the secondary metabolites were absent. The fewer amounts of phytochemicals, flavonoids, phenols, terpenoids, and sterols were present in aqueous extracts. The aqueous extract included no proteins, alkaloids, tannins, glycosides, quinones, carbohydrates, or saponins (Table-1).

In the present study, phytochemical activities of acetone, methanol, chloroform and aqueous extracts of leaves of *P. amboinicus* was analysed and the results showed that leaves extracts exhibited the strong phytochemicals. Among the extracts, methanol extracts showed more strong phytochemicals. *P. amboinicus* leaves showed more phytochemicals such as flavonoids, alkaloids, phenols, glycosides, sterols and moderate amount of carbohydrates, saponins, and quinones when compared to other parts of the plant. Methanol extracts of leaves contained more strong phytochemicals, flavonoids, alkaloids, phenols, glycosides and sterols.

On the contrary, saponins and alkaloids were completely absent in all tested crude extracts. Generally, terpenoids had beenreported to display antioxidant, anticancer, antiinflammatory, antibacterial, antiviral and antimalarial properties (Yang *et al.,* 2020). Other compounds, i.e., flavonoids, phenolic compounds and tannins were existed in the ethylacetate and methanol extracts. Flavonoids are typicallyknown to display health promoting properties such asantioxidant and anti-allergic activities as well as effectiveagainst nerve diseases (Arumugam *et al.,* 2016). Phenolic compounds areconsidered as an important phytochemical which showedantioxidant properties and useful for the treatment of skin aging, wounds and burns (Działo *et al.,* 2016). Tannins are a type ofpolyphenol which have been reported to possessantimicrobial, antitumor and antiviral activities (Yang *et al.,* 2020). Moreover, glycosides commonly act as antioxidant, antimicrobial and antidiarrheal agents (Alam *et al.,* 2020), were discovered in methanol extract only.

Table 1: Preliminary phytochemical screening of *Plectranthus amboinicus* leaves with different solvents

S. No	Phytochemical constituents	Acetone	Methanol	Chloroform	Aqueous
1.	Carbohydrates	-	++	-	-
2.	Saponins	-	++	-	-
3.	Flavonoids	++	+++	++	+
4.	Proteins	-	+	-	-
5.	Alkaloids	-	+++	-	-
6.	Tannins	-	-	-	-
7.	Phenols	++	+++	++	+
8.	Glycosides	-	+++	-	-
9	Terpenoids	+	+	+	+
10	Sterols	++	+++	++	+
11	Quinones	-	+	-	-

Note: (+++) = more strong; (++) = strong; (+) = positive; (-) = negative

The gas chromatogram of *Plectranthus amboinicus* leaf extract depicted the presence of distinctive compounds which were clearly depicted as peaks (Fig. 2) and their retention time, molecular weight, area (%) and structure are tabulated in Table-2. The name of the compounds and their molecular formula are tabulated. The major compounds present in the leaves were methyl 2-oxononanoate (12.80), phenol,2-methoxy(2-propenyl)- (10.14), nonadecanoic acid (7.56), cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-, pivalate (7.50), perilla acetate (6.65), eliminoxy (6.44) and methylsulfonylmethane (6.20). In addition, the minor compounds such as stigmasterol (5.44), 9-hexadecenal (3.76), octadecane-1,2-diol, bis (trimethylsilyl) ether (3.42), caryophyllene (3.32), squalene (3.03) and n-hexadecanoic acid (2.87).

Similarly, Mamani and Alhaji (2019) reported that the twenty six were identified in *Coleus aromaticu*s by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) was analysed. The prevailing compounds were the GC-MS analysis revealed the presence of various compounds like 1,2-benzenedicarboxylic acid, diethyl ester, phytol, octadecenal, dibutyl phthalate, 2- hexadecen-1-ol, 3,7,11,15-tetramethyl, hexadecanoic acid, methyl ester, oleic acid, 9,12,15-octadecatrienoic acid, (z,z,z), 9,12,15-octadecatrienoic acid, ethyl ester, (z,z,z) and solanesol. The activity of phytocomponents identified in the methanolic extracts of the *Coleus aromaticus* leaves by GC-MS.

Table 2 : Phytochemical compounds identified in the methanolic leaf extract of *Plectranthus amboinicus* by GC–MS analysis

Peak#	R.T	Area %	Name of the compound	Molecular formula
1.	3.022	0.09	Campesterol	$C_{28}H_{48}O$
2.	4.245	0.08	Neophytadiene	$C_{20}H_{38}$
3.	13.788	1.06	Undecane	$C_{11}H_{24}$
4.	19.517	1.09	Phytol	$C_{20}H_{40}O$
5.	20.310	3.76	9-hexadecenal	$C_{16}H_{30}O$
6.	20.779	6.65	Perilla acetate	$C_{12}H_{18}O_2$
7.	22.234	3.32	Caryophyllene	$C_{15}H_{24}O$
8.	22.442	7.50	Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-, pivalate	$C_{33}H_{54}O_3$
9.	22.491	6.20	Methylsulfonylmethane	$C_2H_6O_2S$
10.	22.695	5.44	Stigmasterol	$C_{29}H_{48}O$
11.	23.308	6.44	Eliminoxy	$C_{13}H_{16}O_2$
12.	24.575	7.56	Nonadecanoic acid	$C_{19}H_{38}O_2$
13.	25.515	12.80	Methyl 2-oxononanoate	$C_{10}H_{18}O$
14.	21.511	2.52	1-nonadecene	$C_{19}H_{38}$
15.	21.965	2.87	n-hexadecanoic acid	$C_{16}H_{32}O_2$
16.	22.117	2.81	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$
17.	32.860	0.21	Tocopherols	$C_{29}H_{50}O_2$
18.	36.410	0.65	Methyl commate c	$C3_1H_{50}O_4$
19.	24.112	0.42	Undecyl trichloroacetate	$C_{13}H_{23}CL_3O_2$
20.	25.800	10.14	Phenol,2-methoxy(2-propenyl)-	$C_{10}H_{12}O_2$
21.	23.925	3.42	Octadecane-1,2-diol, bis (trimethylsilyl) ether	$C_{24}H_{54}O_2Sl_2$
22.	26.021	3.03	Squalene	$C_{30}H_{50}$
23.	26.478	1.87	1,3-diethoxy-1,1,3,3-tetramethyldisiloxane	$C_8H_{22}O_3Sl_2$
24.	26.700	1.90	2,6-di-tert-butylphenol	$C_{14}H_{22}O$
25.	27.016	2.03	1,2-benzenedicarboxylic acid	$C_{24}H_{38}O_4$
26.	27.205	0.33	Methane, chlorodifluoro	$CHCLF_2$
27.	27.736	1.45	4-ethyl-1,2-dimethoxybenzene	$C_{10}H_{14}O_2$
28.	27.767	1.87	Carvone	$C_{10}H_{14}O$
29.	27.767	1.84	Neophytadiene	$C_{20}H_{38}$
30.	28.396	0.65	Benzene, (ethenyloxy)-	C_8H_8O

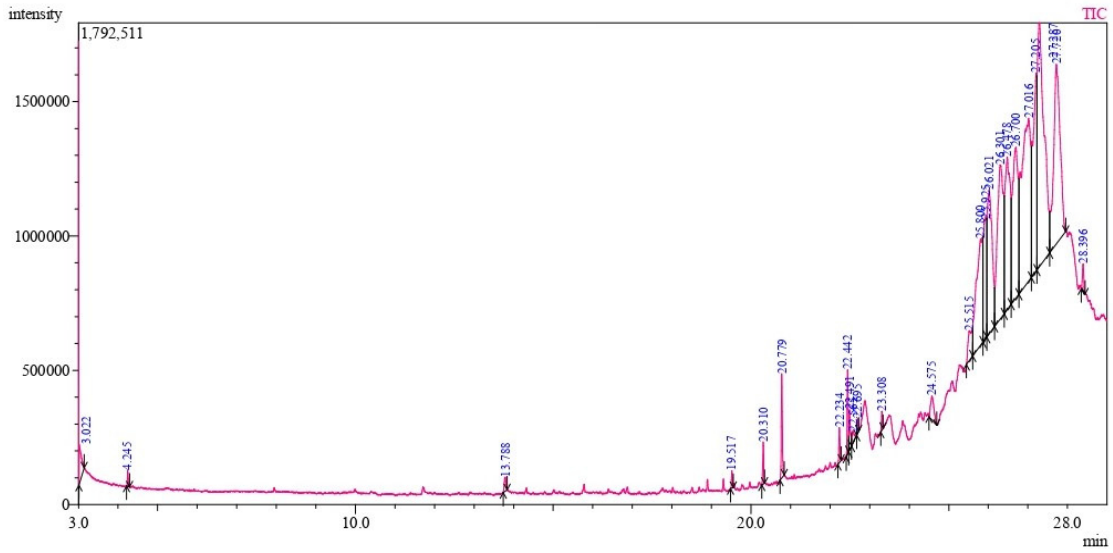

Fig. 2 : GC-MS chromatogram of leaf methanolic extract of *Plectranthus amboinicus*

Conclusion

From the results, the plant leaves has rich in phytochemicals and secondary metabolites such as saponins, flavonoids, alkaloids, phenols, sterols, glycosides and terpenoids which are probably responsible for its medicinal properties. The findings also demonstrate the inventive ways in which plants and their secondary metabolites may be used, as well as the significant reliance of certain individuals on traditional medicine. Furthermore, a comparative analysis of medicinal plants provides a comprehensive understanding of the nature of the plants and their therapeutic potential based on their historic uses.

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