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Research Article

GAS CHROMATOGRAPHY AND MASS SPECTRUM ANALYSIS OF *CATHARANTHUS PUSILLUS* MURRAY G. DON (APOCYANACEAE)

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Abstract: In the present study the bioactive compounds of *Catharanthus pusillus*, aerial parts and root extract were used to evaluate by Gas chromatography and mass spectrum (GC-MS) method. 50g of fine powder was packed with No.1 Whatman filter paper and placed in soxhlet apparatus along with methanol. The crude extract were collected and dried at room temperature, 30˚C after which yield was weighed and then performed to GC-MS analysis. Nine components from the aerial part and root of the above said plant were identified. The major compounds are identified in aerial parts such as Pregn-16-en-20-one, 11,18-bis (acetyloxy)-3,9-epoxy-3-methoxy-, (3à,5á,11à)-(15.60%),9-Octadecenoic acid (14.03%), 3-Methyl-trans-2,3-epoxycyclohexan-1-ol(8.53%), 1H-Purin-6-amine, [(2-fluorophenyl)methyl], (7.47%), Octadecanoic acid (6%). In roots, the major compounds are stigmasterol (13.26%), ç-Sitosterol (11.39%), 2, 5-Dimethoxy-4-ethylamphetamine (4.68%). This study helps to explore the potential compounds responsible for the biological activities of antimicrobial, antioxidant, antidiabetic and anticancer for application of drug formation in pharmaceutical fields.

Key words: *Catharanthus pusillus*, Bioactive compounds, GC-MS, stigmasterol and Octadecanoic acid

INTRODUCTION

Natural remedies from medicinal plants are found to be safe and effective. Many plants species have been used in folkloric medicine to treat various ailments. Even today plant derived bioactive constituents to play a major role in primary health care as therapeutic remedies in many developing countries [1]. Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food. They also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes [2-3]. The use of plants in ethnomedicine is increasing around the World. The World Health Organization (WHO) has reported that approximately 80% of the World's population currently used as herbal medicine. The herbal drugs with easily accessible liquid such as water, milk to safe and reliable for human beings [4-5].

In recent years, gas chromatography and mass spectrum (GC–MS) has been applied unambiguously to identify the structures of different phytoconstituents from plant extracts and biological samples with great success, [6-7]. Gas chromatography and mass spectrum is a reliable technique to identify the phytoconstituents of volatile matter, long-chain branched hydrocarbons, alcohols, acids and esters [8]. The family Apocynaceae consists of several important medicinal plants with wide range of biological activities and interesting phytochemical constituents. *Catharanthus pusillus* is one of important medicinal plant belongs to the family Apocynaceae, which is used for hypoglycemic and anti-diabetic activity [9]. The leaf powder of *C. pusillus* were mixed with coconut oil and used for treat the antidandruff activity and also used to kill the lice [10]. In this present study to analysis the bioactive compounds from methanol extract of *Catharanthus pusillus* by Gas chromatography and mass spectrum.

MATERIAL AND METHODS

Plant material

Catharanthus pusillus healthy and matured plants were collected from the field at foothills of Madukarai hills, part of Western Ghats of Coimbatore district, Southern India. The plants were identified self and binomially by botanical Survey of India (Southern part Coimbatore, Tamilnadu, India).

Extraction of plant material

Plant materials thoroughly washed and shade dried at room temperature after that grind into powder was packed with No.1 Whatman filter paper and placed in soxhlet apparatus along with methanol. The crude extract were collected and dried at room temperature, 30˚C after which yield was weighed and then performed.

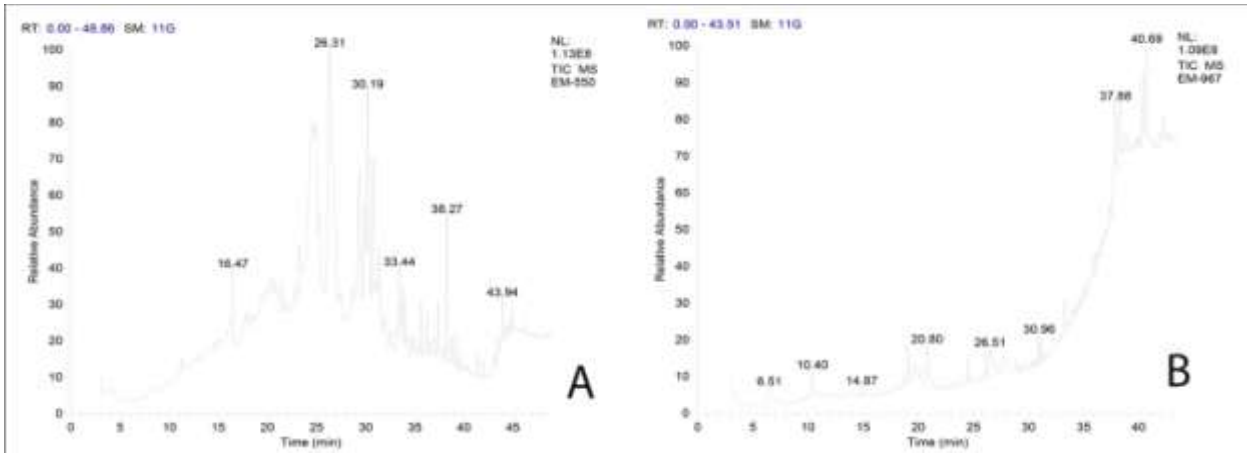
Gas chromatography and mass spectrum analysis

Gas chromatography (GC) analysis was carried out at South Indian Textile Research Institute (SITRA), Coimbatore. It is one of the key techniques generally used for screening/ identification of many groups of plant phytochemicals. The high attainable separation power in combination with wide range of the detectors that are

employed in various detection principles can be coupled. Gas chromatography is an important, often irreplaceable tool in the phytochemical analysis even at trace level of plant chemical compounds. Gas chromatography study includes the important optimization process such as i) introduction of sample extract onto the GC column, ii) separation of its components on an analytical column and iii) detection of target analysis by using mass spectrometric (MS) detector. Five ml of methanol extract was evaporated to dryness and reconstituted in 2 µl methanol. The extracts were then subjected into GC-MS analysis. Chromatographic separation was carried out with CE GC 8000 top MSMD 8000 Fyson instrument with Db 35 mr column (10 m x 0.5 mm, 0.25 µm film thicknesses). Heating programs were executed from 100 - 250 ^{0}C at 3 minutes by using the helium was used as a carrier gas with a flow rate of 1 ml/minute in the split mode (1:50). An aliquot (2 µl) of oil was injected into the column with the injector heater at 250 ^{0}C. Injection temperature at 250°C, interface temperature at 200°C, quadruple temperature at 150°C and ion source temperature at 230°C were maintained. Injection was performed in split less mode. The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV and the detector operated in scan mode from 20 to 600 atomic mass units (amu). Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST.

RESULT

GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitrogen compound. The GC-MS analysis of *Catharanthus pusillus*, aerial parts contain 9 compounds and also roots revealed the presence of 9 phytoconstituents that could contribute the medicinal quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Table 1, 2 and Fig 1 A and B. In aerial parts, major compounds are Pregn-16-en-20-one, 11,18-bis(acetyloxy)-3,9-epoxy-3-methoxy-, (3a,5a,11a)-(15.60%), 9-Octadecenoic acid (Z) (14.03%), 3-Methyl-trans-2,3-epoxycyclohexan-1-ol(8.53%), 1H-Purin-6-amine, [(2-fluorophenyl)methyl] (7.47%),Octadecanoic acid (6.00%). In roots the major compounds are Stigmasterol (13.26%), ç-sitosterol (11.39%) and 2, 5-Dimethoxy-4-ethylamphetamine (4.68%).



A- Aerial parts, B- Root.

Figure 1 A-B. Showing the GC-MS chromatogram of the aerial and root part extract of *C. pusillus*.

DISCUSSION

Octadecanoic acid, and stigmasterol compounds have the property of antioxidant, antimicrobial, hypocholesterolemic, antiarthritic, anti-inflammatory [11]. Anticancer and Antiarthritic, Hepatoprotective are shown by ç-sitosterol [12]. Tin-Wa et al., [13] was carried out the isolation of Locherinine from *Catharanthus pusillus* which was used for one of the cytotoxic activity against human cancer. Shashi *et al*., [14] also reported, the leaves and flowers of *Catharanthus roseus* were analyzed by GC-MS. The major identified compounds are hexdecanic acid, palmitic acid, tricosane, tetracosane were used for antimicrobial activity, antioxidant, antidiabetic and anticancer activity.

CONCLUSION

It was concluded that the methanol extract of aerial and root parts of *Cathranthus pusillus* possess various potent bioactive compounds. It is recommended as a drug formulation in pharmaceutical industries. Further studies are needed to explore the potential bioactive compounds responsible for the biological activities of *Cathranthus pusillus.*

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Table 1: Detection of bio-active compounds and their pharmacological properties of aerial part extract of *Catharanthus pusillus* by GC-MS.

S.No	Retention time(RT)	% of Peak Area	Compound Name	Molecular formula (MF)	Molecular weight (MW)	Nature of the compound	Biologial activity
1	24.95	15.60	Pregn-16-en-20-one, 11,18-bis(acetyloxy)-3,9-epoxy-3-methoxy-, (3à,5á,11à)-	$C_{26}H_{36}O_7$	460	Aldehyde	Antimicrobial activity
2	26.31	14.03	9-Octadecenoic acid (Z)	$C_{18}H_{34}O_2$	282	Fatty acid	Hypocholesterolemic, antiarthritic, nematicide, 5-alpha reductase inhibitor.
3	29.33	8.53	3-Methyl-trans-2,3-epoxycyclohexan-1-ol	$C_7H_{12}O_2$	128	Fatty acid	Antiacne, hepatoprotective
4	30.19	7.47	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	$C_{12}H_{10}FN_5$	243	Alkaloid	Antitumor activity
5	30.72	6.00	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Palmitic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic, flavor,hemolytic 5-alpha reductase inhibitor
6	38.27	5.69	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	Ester	Anti-fouling Antimicrobial
7	29.87	4.07	(1R*,1'R*)-1,1'-(2,2,3,3-Tetramethylcyclopropane-1,1- diyl)bis(2,2-dimethyl-1-propanol)	$C_{17}H_{34}O_2$	270	Fatty acid	Nematicide, Pesticide, Antioxidant, Hypercholesteroiemic
8	16.47	3.91	Tridecanal	$C_{13}H_{26}O$	198	Tridecane aldehyde	Antimicrobial activity
9	33.44	3.03	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	$C_{12}H_{10}FN_5$	243	Alkaloid	Antitumor activity

Table 2: Detection of bio-active compounds and their pharmacological properties of root part extract of *Catharanthus pusillus* by GC-MS.

S.No	Retention time (RT)	Peak Area (%)	Compound Name	Molecular formula (MF)	Molecular weight (MW)	Nature of the compound	Biological activity
1	37.88	13.26	Stigmasterol	$C_{29}H_{48}O$	412	Steroid	Antimicrobial, antioxidant, anti-inflammatory and diuretic.
2	40.69	11.39	ç-Sitosterol	$C_{29}H_{50}O$	414	Steroid	Hepatoprotective, antimicrobial, antiasthma and body pain.
3	40.33	7.04	Vitamin E	$C_{29}H_{50}O_2$	430	Vitamin	Anticoronary, anticariogenic, antioxidant, antileukemic,
4	38.30	4.81	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	Ester	Anti-fouling, antimicrobial activity.
5	20.80	4.68	2,5-Dimethoxy-4-ethylamphetamine	$C_{13}H_{21}NO_2$	223	Alkaloid	Anticancer activity
6	19.06	4.57	9Oxabicyclo[4.2.1]nonan-2-ol	$C_8H_{14}O_2$	142	Cypionic acid	It's used for Pharmaceutical and drugs formulation.
7	6.48	4.20	Pyridine, 3-ethyl-	C_7H_9N	107	Alkaloid	Anticancer and rheumatisum
8	33.25	3.89	3Phenylbicyclo(3.2.2)nona-3,6-dien-2-one	$C_{15}H_{14}O$	210	Flaonoids	Antioxidant and antitumor activity.
9	38.88	3.18	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-,octadecyl ester	$C_{35}H_{62}O_3$	530	Ester	Antioxidant activity