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Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(3):792-798

GC-MS Analysis of bioactive components from the ethanolic leaf extract of *Canthium dicoccum* (Gaertn.) Teijsm & Binn.

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ABSTRACT

Medicinal plants are sources of important therapeutic aids for alleviating human ailments. Canthium dicoccum is one of the medicinally important plants belonging to the family Rubiaceae, commonly known as "nallamandharam" in Tamil. The major chemical constituents are Spathulenol (20.76 %), Caryophyllene oxide (19.25 %), Cedren-13-ol (10.62 %), Ledene oxide (5.24 %), m-mentho-4, 8-diene (6.41 %) and 2-furancarboxaldehyde (4.51 %). Thus the extract of Canthium dicoccum was characterized by substantial levels of sesquiterpenoids (55.87 %), nitrogenous compounds (12.93 %), aldehydes (8.7 %), terpinolene (6.41 %) and phenols (4.26 %). The presence of some of these constituents in the plant extract provides the scientific evidences for the antimicrobial, anti-tumor, Immuno modulatory and antioxidant properties of the plant.

Keywords: *Canthium dicoccum*, phytochemicals, GC-MS analysis, Spathulenol, Caryophyllene oxide, Cedren-13-ol, ledene oxide-(II).

INTRODUCTION

Canthium dicoccum is found in Western Ghats of India. The common names in Tamil are Nanjul, Nallamandharam and in Malayalam as Nanyul. It is a medium sized tree. Trees grow up to 10 m, leaves oblanceolate, glabrous, shortly acuminate at apex, margin entire, truncate at base; stipules triangular. Fruits are edible. The plants are common in dry-deciduous forests. Plant possesses antipyretic activity. In India, bark is used as febrifuge and also applied as plasters. The

decoction of roots is used internally for treating diarrhoea. Bark powder boiled with sesame oil is used externally for rheumatic pains [1].

A new flavonol glycoside, characterized as 7-*O*-(6-*O*-benzoyl-β-D -glucopyranosyl)-rutin, has been isolated from the leaves of *Canthium dicoccum* [2]. Though there are many works reported on various species of *Canthium*, a literature search revealed no references to previous work on *Canthium dicoccum* plant extract composition. Thus the objective of the study was to identify the active compounds from *Canthium dicoccum* leaf extract by GC-MS analysis.

EXPERIMENTAL SECTION

Collection of plant material

The leaves of *Canthium dicoccum* were collected from Puthupet, Pondicherry authenticated by the Director, Centre for Biodiversity and Forest Studies, Madurai Kamaraj University, and voucher specimens were deposited in the herbarium of Centre for Biodiversity and Forest Studies of our university (No.AM-06).

Preparation of powder and extract

Leaves (1KG) were shade dried, powdered and extracted with ethanol for 6-8 hours using soxhlet apparatus. The extract was then filtered through muslin, evaporated under reduced pressure and vacuum dried to get the viscous residue. The ethanolic extracts of the plant was used for GC-MS analysis.

GC –MS analysis

Preparation of extract

2 µl of the ethanolic extract of *Canthium dicoccum* was employed for GC/MS analysis.

Instruments and chromatographic conditions

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Identification of components

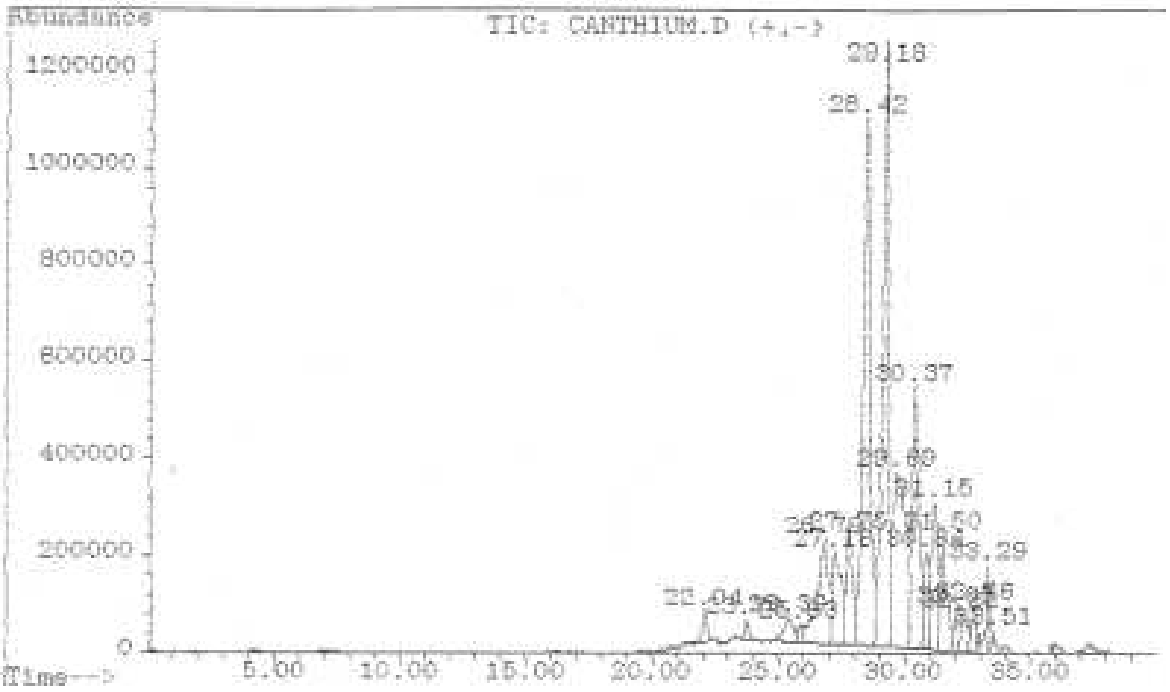
Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

GC-MS analysis

GC-MS chromatogram of the ethanolic extract of *Canthium dicoccum* is given in (Figure1).

Figure 1: GC-MS Chromatogram of ethanolic leaf extract of *Canthium dicoccum*



On comparison of the mass spectra of the constituents with the NIST library, nineteen peaks were obtained; all the phytoconstituents were characterized and identified (Table 1). The retention times (RT) are in minutes.

Table 1: Phytocomponents identified in the ethanolic leaf extract of *Canthium dicoccum* by GC-MS.

No	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area(%)
1.	22.04	Furfural	$C_5H_4O_2$	96.08	0.99
2.	23.69	Styrene	C_8H_8	104.14	0.49
3.	25.40	Benzofuran, 2,3-dihydro-	C_8H_8O	120.15	1.50
4.	25.83	Lactose	$C_{12}H_{22}O_{11}$	342.30	0.54
5.	26.79	m-Mentho-4,8-diene	$C_{10}H_{16}$	136.23	6.41
6.	27.18	2-Furancarboxaldehyde	$C_6H_6O_2$	110.11	4.91
7.	27.75	Phenol, 4-ethyl-2-methoxy	$C_9H_{12}O_2$	152.19	4.26
8.	28.42	(-)-Spathulenol	$C_{15}H_{24}O$	220.35	20.76
9.	29.19	Caryophllene oxide	$C_{15}H_{24}O$	220.35	19.25
10.	29.69	Urea,N'-(3,4-dichlorophenyl)-N-methoxy-N-methyl	$C_9H_{10}Cl_2N_2O_2$	249.11	12.93
11.	30.37	Cedren-13-ol	$C_{15}H_{24}O$	220.35	10.62
12.	30.84	2-Pentanethiol	$C_5H_{12}S$	104.21	2.66
13.	31.15	Ledene Oxide(II)	$C_{15}H_{24}O$	220.35	5.24
14.	31.50	Tetracyclo[6.3.2.0(2,5).0(1,8)] tridecan-9-ol, 4-4-dimethyl	$C_{15}H_{24}O$	220.35	4.20
15.	32.05	2,7-Dioxa-tricyclo[4.4.0.0(3,8)] deca-4,9-diene	$C_8H_8O_2$	136.15	0.97
16.	32.22	Formaldehyde, methyl (2-propynyl) hydrazone	$C_5H_8N_2$	96.13	1.45
17.	32.76	4-cyclopropylnorcarane	$C_{12}H_{19}NO_4$	241.28	1.19
18.	33.29	Benzaldehyde, 2-methyl	C_8H_8O	120.15	1.35
19.	33.51	1,5-heptadiene-3-yne	C_9H_{16}	124.22	0.43

The various phytochemicals which contribute to the medicinal activity of the plant are listed in (Table 2).

Table 2: Activity of phyto-components identified in the ethanolic leaf extract of *Canthium dicoccum* by GC-MS analysis

RT	Name of the compound	Compound Nature	**Activity
22.04	Furfural	Aromatic aldehyde	Nematicide, Fungicide
23.69	Styrene	Ethyl benzene	Antibacterial
25.40	Benzofuran, 2,3-dihydro-	Coumaran	Antihelminthic, Anti-inflammatory, Anti-diarrhoeal
25.83	Lactose	Sugar	Preservative, Nutritive
26.79	m-Mentho-4,8-diene	Terpinolene	Antimicrobial
27.18	2-Furancarboxaldehyde	Aldehyde	Antimicrobial, Preservative
27.75	Phenol, 4-ethyl-2-methoxy	Phenol	Antioxidant, Antifungal
28.42	(-)-Spathulenol	Sesquiterpene	Antimicrobial, Immunomodulatory and antitumor
29.19	Caryophllene oxide	Sesquiterpene	Antitumor, anesthetic, antibacterial, anti-inflammatory, analgesic, anti-inflammatory, antioxidant
29.69	Urea,N'-(3,4-dichlorophenyl)-N-methoxy-N-methyl	Nitrogenous compound	
30.37	Cedren-13-ol	Sesquiterpene	Antioxidant
30.84	2-Pentanethiol	Alkane	
31.15	Ledene Oxide (II)	Sesquiterpene	Antibacterial, Antioxidant
31.50	Tetracyclo[6.3.2.0(2,5).0(1,8)] tridecan-9-ol, 4-4-dimethyl	Alcohol	Anti-fungal
32.05	2,7-Dioxa-tricyclo[4.4.0.0(3,8)] deca-4,9-diene	Alkene	Antibacterial
32.22	Formaldehyde, methyl (2-propynyl) hydrazone	Aldehyde	Antimicrobial
32.76	4-cyclopropylnorcarane	Alkane	Antibacterial
33.29	Benzaldehyde, 2-methyl	Aldehyde	Antimicrobial
33.51	1,5-heptadiene-3-yne	Cycloalkane	Antibacterial

The components may be grouped in to main classes: sesquiterpenoids (55.87 %), alkene (2.76 %), sugar (0.54%), alkane (4.28 %), alcohols (4.20 %), nitrogenous compounds (12.93 %), aldehydes (8.7 %), terpinolene (6.41 %) and phenols (4.26 %). The major constituents was found to spathulenol (20.76 %), caryophyllene oxide (19.25 %), urea (12.93 %), cedren-13-ol (10.62 %), ledene oxide (5.24 %), m-mentha-4, 8-diene (6.41 %) whose retention time are found at 28.42, 29.19, 29.69, 30.37, 31.13 and 26.79 respectively. Lactose, a sugar molecule, preservative is found at RT 25.83.

Terpenoids are an important part of volatiles from plants. Caryophyllene oxide, an oxygenated terpenoid, well known as preservative in food, drugs and cosmetics, has been tested in vitro for antibacterial, antifungal activity [3, 4]. It is also suggested as potential anticarcinogenic agent [5] exhibit cytotoxic activity against several solid tumor cell lines [6]. The caryophyllene oxide in medicinal plants is reported to be potentially useful for diuretic activity [7], antioxidant activity [8].

Phenolic compounds such as Phenol, 4-ethyl-2-methoxy was found at retention time of 27.75. Alcohols are known to possess bactericidal rather than bacteriostatic activity against vegetative cells. The aldehydes such as Formaldehyde, methyl (2-propynyl) hydrazone and Benzaldehyde, 2-methyl were found at retention times of 32.22 and 33.29 respectively. Aldehydes, notably

formaldehyde and glutaraldehyde, are known to possess powerful antimicrobial activity. It has been proposed that an aldehyde group conjugated to a carbon to carbon double bond is a highly electronegative arrangement, which may explain their activity [9], suggesting an increase in electronegativity increases the antibacterial activity [10, 11]. Such electronegative compounds may interfere in biological processes involving electron transfer and react with vital nitrogen components, e.g. proteins and nucleic acids and therefore inhibit the growth of the microorganisms.

The major phytochemical constituent's present in ethanolic extract of *Canthium dicoccum* are presented as mass spectra and compound structures are in (Figure 2 to Figure 5). They were identified as spathulenol (20.76 %), caryophyllene oxide (19.25 %), cedren-13-ol (10.62 %) and ledene oxide (5.24 %) respectively.

Figure 2: The mass spectrum analysis and structure of Spathulenol

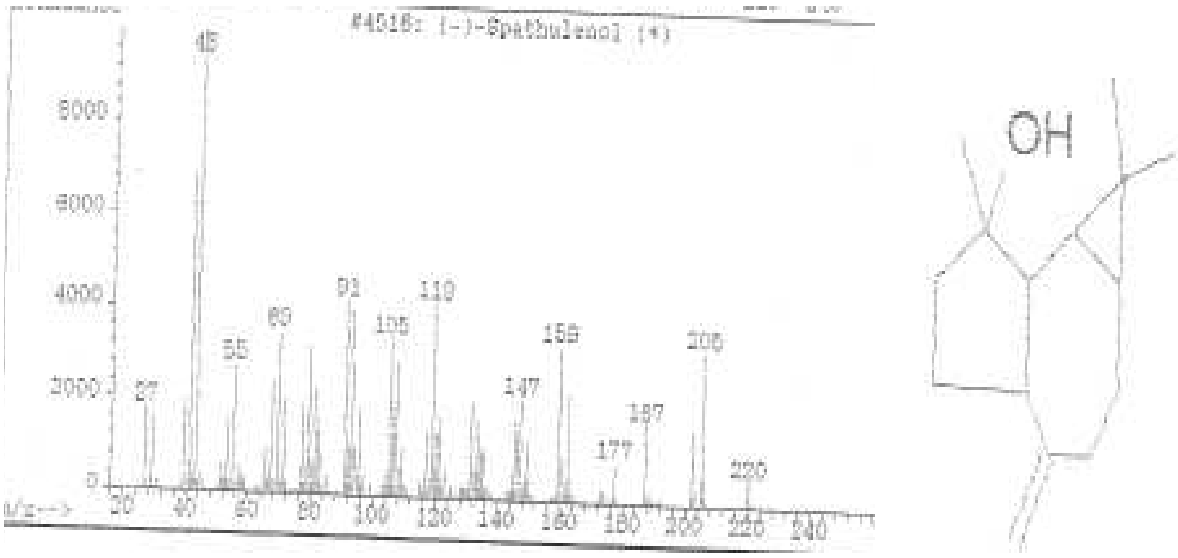


Figure 3: The mass spectrum analysis and structure of Caryophyllene oxide

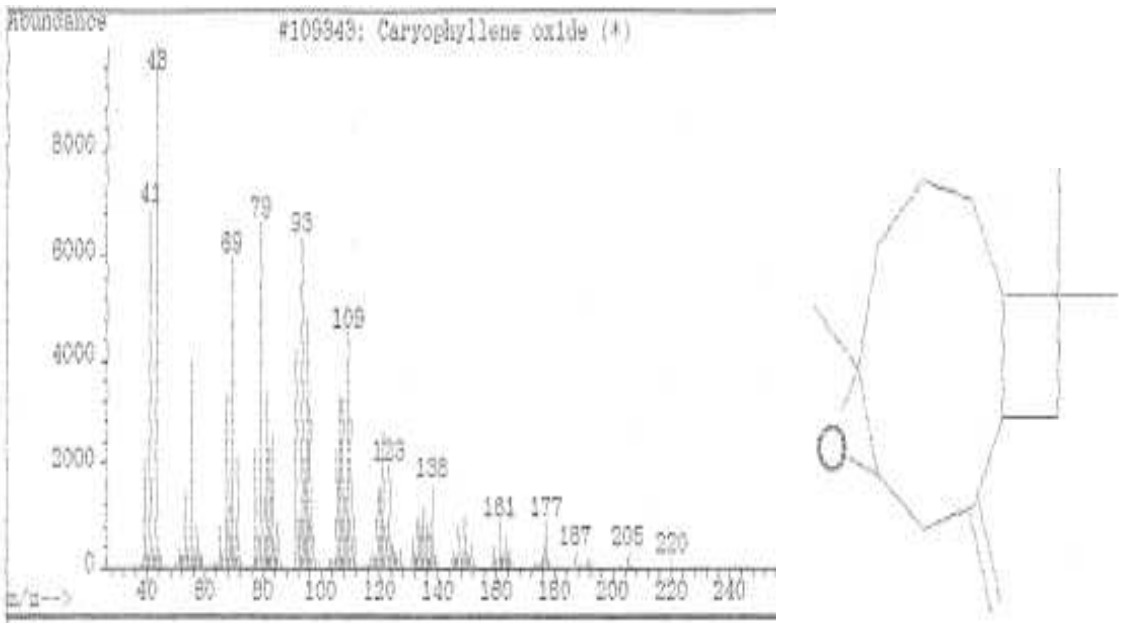


Figure 4: The mass spectrum analysis and structure of Cedren-13-ol

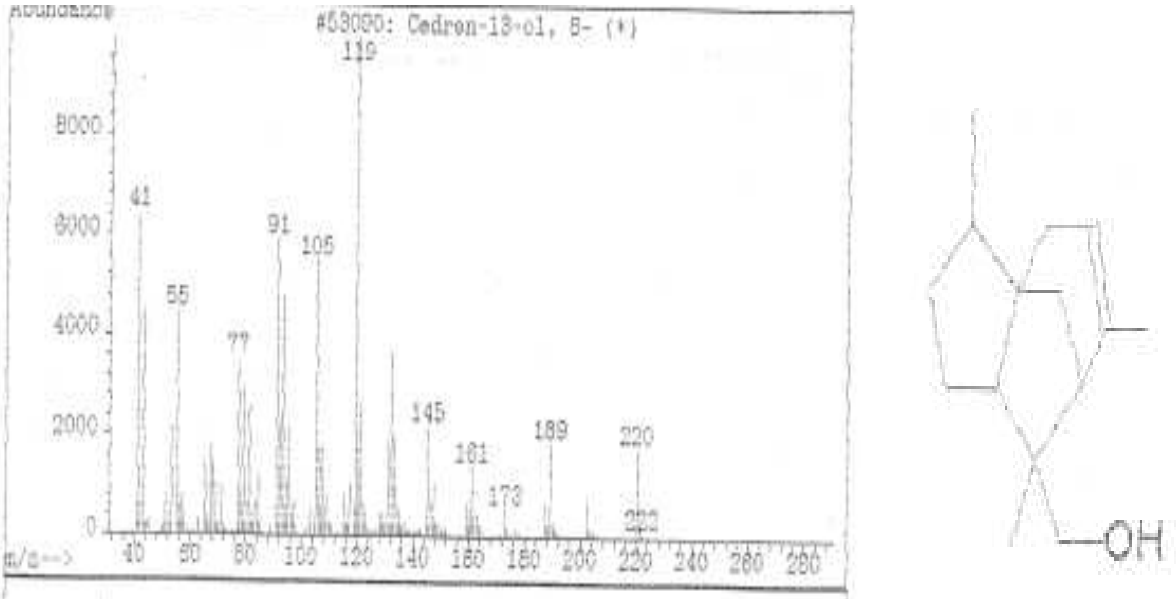
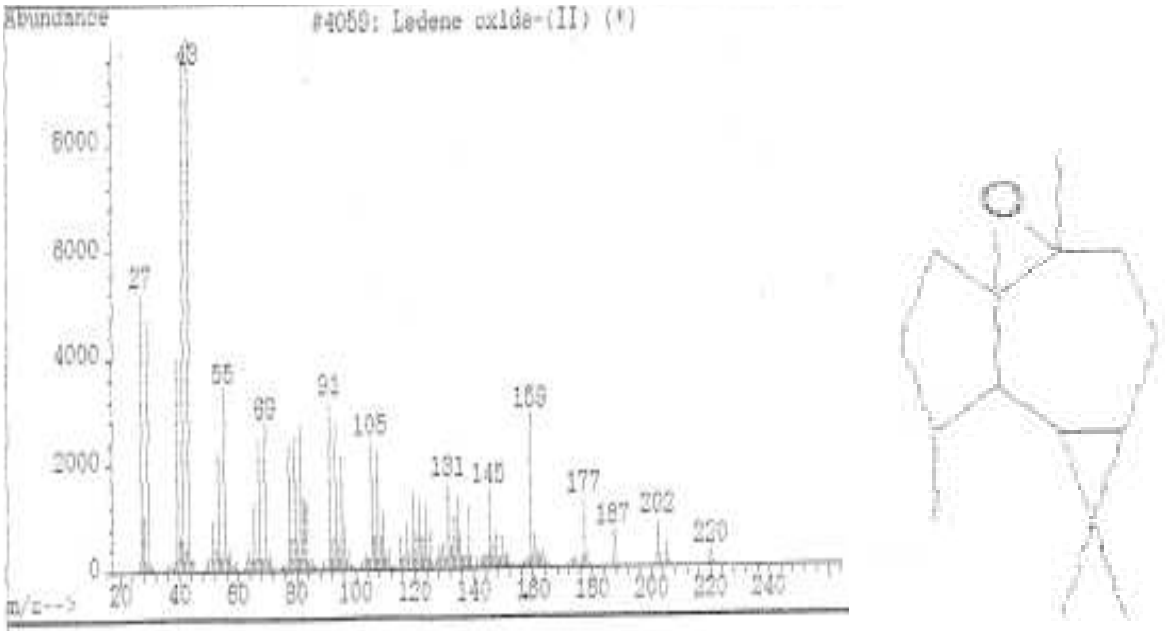


Figure 5: The mass spectrum analysis and structure of ledene oxide-(II).



CONCLUSION

The present study has been found useful in the identification of several constituents present in the ethanolic extract of the leaves of *Canthium dicoccum.* The presence of various bioactive compounds (identified as sesquiterpenoids, aldehydes, alcohols, terpinolene) justifies the use of the whole plant for various ailments by traditional practitioners.

It could be concluded that *Canthium dicoccum* plant is of phytopharmaceutical importance. Studies with these compounds may yield nature friendly strong anti-fungal agents of agricultural importance.

Acknowledgements
The authors thank the University Grants Commission of India for the grant of UPE (University with Potential for Excellence) project.

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