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Original Article

TOTAL PHENOLIC, FLAVANOID CONTENTS AND GC-MS ANALYSIS OF *CANTHIUM COROMANDELICUM* LEAVES EXTRACT

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ABSTRACT

Objective: In this study methanol extract of leaves of *canthium coromandelicum* (Family: Rubiaceae) was screened for the presence of phytochemical components by GC-MS analysis. In addition total phenolics and flavanoids were also estimated.

Methods: GC-MS analysis of plant extract was performed using a Perkin-Elmer GC Clarus 500 system and Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST).

Result: 20 chemical compounds are identified from the plant extract. Squalene ($C_{30}H_{50}$) is the major component available at RT 23.10 and 15.02% peak area. Other most prevailing phytocomponds are Phytol, 25-desacetoxy- Cucurbitacin b, n-Hexadecanoic acid, Sitosterol etc., *Canthium coromandelicum* contained the total phenolic of 25.49±0.09 mg/g and the total flavonoids of 12.37±0.15mg/g which are equivalent to gallic acid and quercetin, respectively.

Conclusion: The presence of various bioactive compounds justifies the use of *canthium coromandelicum* for various ailments by traditional practitioners.

Keywords: *Canthium coromandelicum,* GC-MS, Phytochemical screening, Methanol extract.

INTRODUCTION

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties [1]. The screening of medicinal plants for active compounds has become very significant, it has been shown that in vitro screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [2]. Antioxidant activity [3,4], various phytochemical, antimicrobial and wound healing studies have already been carried out with *canthium coromandelicum* leaf extract[5]. In this study, the methanol extract of *Canthium coromandelicum* is analyzed using GC-MS for the identification of the chemical constituents.

MATERIALS AND METHODS

Determination of Total Phenols and Flavanoids

Phenolics: Total phenolic content (TPC) were quantified in each test sample, following the protocol of Bray and Thorpe[6], which included the preparation of a regression curve of standard phenol (Gallic acid). Samples were diluted with distill water to give concentration of 0.4mg/ml. A 0.5 ml of each sample was added with 0.5 ml of Folin- Ciocalteu reagent and 1.0 ml of distill water. After a period of 2-5 minutes, the tubes were added with 0.5 ml of 10% Na_2CO_3. After 1 hr incubation at room temperature the absorbance was measured on a spectrophotometer UV-VIS spectrophotometer at 760 nm using distill water as a blank. Gallic acid (0-100 mg/L) dissolved in distill water was used to prepare standard curve concentration and values were expressed as microgram of gallic acid equivalents (mg Gallic acid/g extract). Three replicates were taken for each concentration and the average optical absorbance was plotted against the respective concentration to compute a regression curve which followed the Beer's law.

Flavanoids: 0.5 ml of different solvent extract of *Canthium coromandelicum* was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 minutes, the absorbance was measured at 415 nm along with standard quercetin and blank. The concentration obtained by comparing with the calibration curve prepared from a reference solution containing quercetin [7].

Plant material and extraction procedure

The leaves of *Canthium coromandelicum* was shade dried at room temperature. The dried material was then homogenized to obtain coarse powder and stored in air-tight bottles for further analysis. 10 gm powdered plant material was soaked in 20 ml of methanol overnight and then filtered through a Whatman No. 41 filter paper along with 2 gm Sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with Sodium sulphate was wetted with methanol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1 ml. The extract contains both polar and non-polar phytocomponents. This methanol extract is used for GC-MS analysis.

GC Programme

Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 x 0.25 mm x 0.25 mm df**,** Equipment: GC Clarus 500 Perkin Elmer, Carrier gas: 1 ml per min, Split: 10:1, Detector: Mass detector Turbo mass gold-Perkin Elmer**,** Software: Turbomass 5.2**,** Sample injected: 2 ml

Oven temperature Programme

110° C -2 min hold, Up to 200° C at the rate of 10 ° C/min-No hold, Up to 280 ° C at the rate of 5° C / min-9 min hold, Injector temperature 250° C, Total GC running time 36 min

MS Programme

Library used NIST Version-Year 2005,Inlet line tem1perature 200° C, Source temperature 200 ° C, Electron energy: 70 eV, Mass scan (m/z): 45-450, Solvent Delay: 0-2 min, Total MS running time: 36 min

RESULTS AND DISCUSSIONS

Table 1 indicates the results of quantitative estimation of Phenolics and Flavonoids.

Table 1: Results of quantitative estimation of total phenolics and flavanoids

Phytochemicals	Result
Totoal Phenolics	25.49±0.09 gallic acid equivalents mg/g extract
Flavanoids	12.37±0.15 quercetin equivalents mg/g extract

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. In the GC-MS analysis, 20 bio active phytochemical compounds were identified in the methanol extract of *Canthium coromandelicum* (Table-2 & Figure1).

Table 2: Major Phyto-components obtained through the GC/MS Study of *Canthium coromandelicum*

S.No.	RT	Name of the compound	Molecular Formula	MW	Peak Area%
1	8.70	l-Gala-l-ido-octose	$C_8H_{16}O_8$	240	4.71
2	8.80	2-Octen-1-ol, 3,7-dimethyl-,	$C_{14}H_{22}O_3$	226	5.73
3	10.00	isobutyrate, (Z)-Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216	13.95
4	10.75	3,7,11,15-Tetramethyl-2- hexadecen-1-ol	$C_{20}H_{40}O$	296	9.85
5	11.00	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	3.36
6	11.18	8-Dodecen-1-ol, acetate, (Z)-	$C_{14}H_{26}O_2$	226	2.54
7	12.34	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.63
8	13.84	Phytol	$C_{20}H_{40}O$	296	8.85
9	14.46	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	6.07
10	19.45	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280	0.88
11	23.10	Squalene	$C_{30}H_{50}$	410	15.02
12	23.46	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	1.01
13	26.35	Cholestan-3-ol, 2-methylene-, (3á,5à)-	$C_{28}H_{48}O$	400	1.50
14	27.24	Cucurbitacin b, 25-desacetoxy-	$C_{30}H_{44}O_6$	500	1.17
15	28.55	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	1.40
16	2.95	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)-	$C_{30}H_{48}O_2$	440	1.24
17	29.93	á-Sitosterol	$C_{29}H_{50}O$	414	5.54
18	30.88	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	1.96
19	31.28	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3á,4à,5à)-	$C_{32}H_{52}O_2$	468	4.55
20	32.48	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	$C_{27}H_{44}O_3$	416	2.14

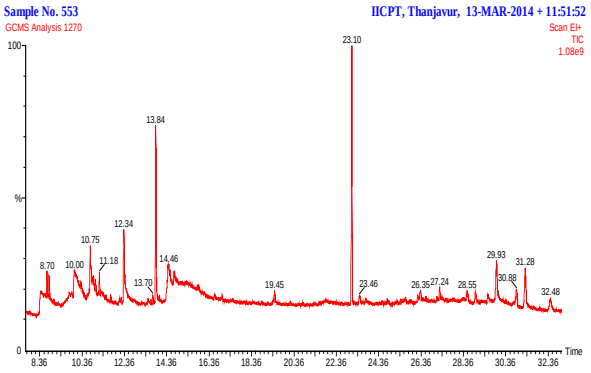

Fig. 1: Chromatogram obtained from the GC/MS with the methanol extract of *Canthium coromandelicum*

The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula. Squalene ($C_{30}H_{50}$) is the major component available at RT 23.10 and 15.02% peak area. Squalene has several beneficial properties. It is a natural antioxidant[8], serves in skin hydration[9] and has been used as emollient in adjuvant for vaccines[10]. As a compound of olive oil, it also has a preventive effect on breast cancer, possesses tumor-protective, and cardio-protective properties [11,12] and decreases the serum cholesterol level[13]. It has been reported that squalene emulsions given simultaneously with anti-cancer drugs provide favorable effects either directly or indirectly by enhancing efficacy of anti-cancer drugs [14-16]. Corresponding to the peak at RT 13.84 and peak area 8.85% is Phytol.

The molecular formula and molecular weight of this compound is $C_{20}H_{40}O$ and 296, respectively. Phytol is known to be antimicrobial, anti cancer, anti-inflammatory, hepatoprotective and anti –androgenic [17].The peak at R T 27.24 with a peak area of 1.17 % corresponds to 25-desacetoxy- Cucurbitacin b. It has the m.f. $C_{30}H_{44}O_6$ and m.w. of 500. Cucurbitacins have strong cyto-toxic activity [18] and antitumour[19] action. Cucurbitacins also possess antimicrobia l [20], antihepatoxic[21], anti-inflammatory[22] and antihelminthic activities. n-Hexadecanoic acid is found to be responsible for the peak at RT 12.34 with a peak area 8.63 %. This fatty acid has the m.f. $C_{16}H_{32}O_2$. It has a m.w. of 256. This compound has various activities like antioxidant, hypocholestrolemic,nematicide, pesticide, lubricant and inhibitory activity such as 5-α reductase inhibition[23]. The peak at RT 28.55 and peak area 1.30% is ethyl isoallocholate. Ethyl isoallocholate is suggested to be a sterol compound and it may use as an antibacterial, antioxidant, anti-tumor, cancer preventive, pesticide and chemo preventive agent [24]. This study has revealed the presence of many secondary metabolites and bioactive compounds in the leaf of *Canthium coromandelicum* which may be of a very important medicinal value and further study including isolation and purification of active phyto compounds.

CONCLUSION

Flavonoid and phenolic compounds have widely been reported as antioxidant agents positively correlated in the treatment of cardiovascular diseases[25-27]. In this study *canthium coromandelicum* contained the total phenolic of 25.49±0.09 gallic acid equivalents mg/g and the total flavonoids of 12.37±0.15 quercetin equivalents mg/g extract. From the present study, it is concluded that the phytochemicals was obsereved in methanol extract which reveals that *Canthium coromandelicum* is highly valuable in medicinal usage for the treatment of various human aliments.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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