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Phytochemical and GCMS Analysis of *Canthium coromandelicum* Leaves Extract

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Abstract: The identification of components presents in the plants is very important to the pharmaceutical, cosmetic, fragrance and food industries. This study report the phyto constituents present in ethanolic extract of *Canthium coromandelicum* leaves.The present work was designed to investigate the preliminary phytochemical and GCMS analysis of ethanol extract of *Canthium coromandelicum*. Phytochemical screening of leaves extract revealed the presence of tannins, steroids, saponins, flavanoids, glycosides and phenolic compounds. The extract was analyzed by Gas Chromatography- Mass Spectrometry (GC-MS) and 21 different compounds were identified.

Keywords : Canthium coromandelicum, phytochemicals,GC-MS.

Introduction

In order to overcome health problems, the tribes of developing countries primarily rely on herbal medicines which are giving beneficial effect to humans [1]. The herbs are constantly being screened for their biological and pharmacological activities such as anti-diabetic, anti-oxidant, anti-microbial, laxative, and anti-cancer activities [2-6]. Gas Chromotography-Mass Spectrometry (GC-MS) is a valuable for tool for reliable identification of phyto compounds [7,8]. This plant is reported to have a antioxidant activity[9,10] and various phytochemical, antimicrobial and wound healing studies have already been carried out with *canthium coromandelicum* leaf extract[11]. In the present study, the ethanol extract of *canthium coromandelicum* were evaluated for GCMS analysis.

Material and Methods

Plant material and extraction procedure

10gm powdered leaves of *Canthium coromandelicum* plant material was soaked in 20ml of absolute alcohol overnight and then filtered through a Whatman No.41 filter paper along with 2gm Sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulfate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1ml. The extract contains both polar and non-polar phytocomponents.

Gas Chromatography–Mass Spectrometry (GC/MS) analysis:

GC/MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with a Elite-1 fused silica capillary column (30 m × 0.25 mm ID. ×1 μMdf, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the

carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 µl was employed (split ratio of 10:1). Injector temperature 250℃; Ion-source temperature 280℃. The oven temperature was programmed from 110℃ (isothermal for 2 min.), with an increase of 10℃/min, to 200℃, then 5℃/min to 280℃, ending with a 9 min. isothermal at 280℃. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0.

Test for Alkaloids (Meyer's Test)

The extract of *Canthium coromandelicum* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent [12]. The samples were then observed for the presence of turbidity or yellow precipitation [13].

Test for Tannins

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins [14].

Test for Terpenoids and Steroids

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform.Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids [12].

Test for Glycoside

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and Concentrated sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer [12].

Test for saponins

The extract (50mg) was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins[15].

Test for Flavonoids

To 1ml of the extract, a few drops of dilutesodium hydroxide was added. An intense yellow colour was produced in the plant extract, which become colourless on addition of a few drops of dilute acid indicates the presence of flavonoids[16].

Test for Phenolic compounds

The extract is dissolved in distilled water and to this few drops of 1% lead acetate were added a bulky white precipitate was formed, which indicates the presence of phenolic compounds[16].

Results and Discussions

In the present study the phytochemical screening (Table1) and GC-MS study(Table2) were evaluated.

Table 1. Phytochemical screening of *Canthium coromandelicum*

Phytochemicals	Ethanolic Extract
Alkaloids	Absent
Tannins	Present
Terpenoids and Steroids	Present
Glycosides	Present
Saponins	Present
Flavanoids	Present
Phenolic compounds	Present

Identification of Components

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. The Name, Molecular weight and Structure of the components of the test materials were ascertained(Figure 1).21 components were identified, among the 21 components Squalene ($C_{30}H_{50}$) is the major component available at RT 23.18 and 27.62% peak area. Squalene has several beneficial properties. It is a natural antioxidant[17] it has a preventive effect on breast cancer, possesses tumor-protective, and cardio-protective properties[18,19].

Table.2. Phyto components identified in the ethanolic extract of *Canthium coromandelicum*

[GC MS study]

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	2.66	Glycerin	$C_3H_8O_3$	92	17.43
2.	9.28	Lactose	$C_{12}H_{22}O_{11}$	342	13.55
3.	10.70	10-Methyl-E-11-tridecen-1-ol propionate	$C_{17}H_{32}O_2$	268	7.07
4.	11.14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.23
5.	12.30	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.68
6.	13.79	Phytol	$C_{20}H_{40}O$	296	3.65
7.	14.47	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.97
8.	15.78	1,3-Dioxolane, 4-[[(2-methoxy-4-octadecenyl)oxy]methyl]-2,2-dimethyl-	$C_{25}H_{48}O_4$	412	0.49
9.	16.86	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	$C_{26}H_{50}O_2$	394	0.45
10.	19.42	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	5.70
11.	20.16	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	0.53
12.	21.53	Octadecane, 6-methyl-	$C_{19}H_{40}$	268	0.46
13.	23.18	Squalene	$C_{30}H_{50}$	410	27.62
14.	24.27	tert-Hexadecanethiol	$C_{16}H_{34}S$	258	0.31
15.	26.37	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	$C_{27}H_{44}O_3$	416	0.45
16.	27.35	Vitamin E	$C_{29}H_{50}O_2$	430	0.79
17.	29.02	Cholestan-3-ol, 2-methylene-, (3á,5à)-	$C_{28}H_{48}O$	400	0.68
18.	30.07	1-Heptatriacotanol	$C_{37}H_{76}O$	536	1.90
19.	30.89	Pregn-4-ene-1,20-dione, 12-hydroxy-16,17-dimethyl-	$C_{23}H_{34}O_3$	358	3.89
20.	31.41	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	1.73
21.	32.70	Cholest-4-en-3-one	$C_{27}H_{44}O$	384	9.43

*Parameters tested are not covered under the scope of NABL accreditation

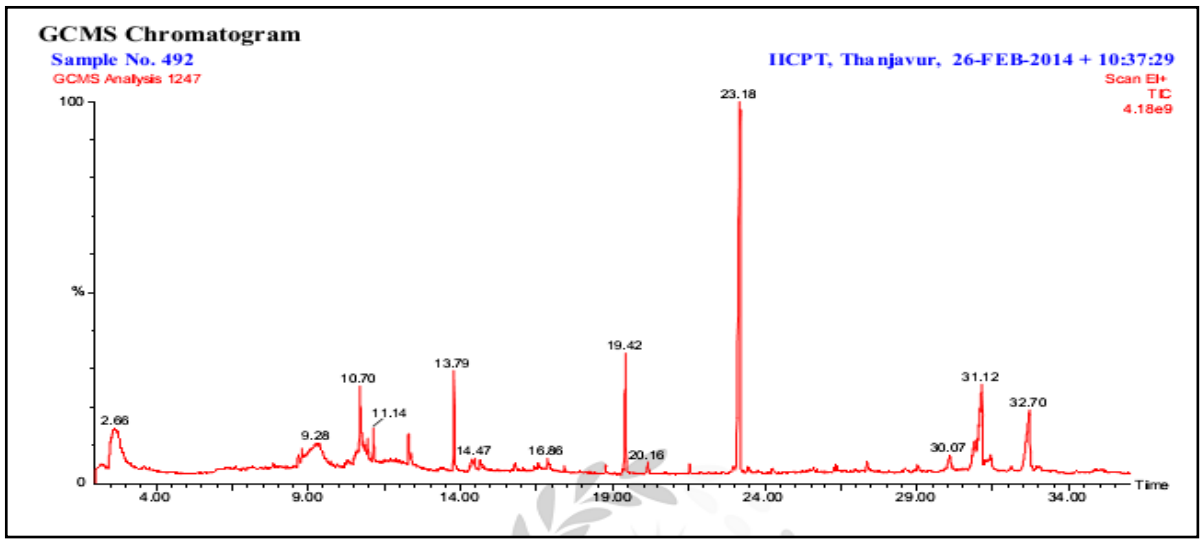

Fig.1.GC-MS Chromatogram of Ethanolic Extract of *Canthium coromandelicum*

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