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RESEARCH ARTICLE

Phytochemical Characterization Using Various Solvent Extracts and GC-MS Analysis of Methanolic Extract of *Canthium Coromandelicum (Burm. F)* Leaves

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ABSTRACT

The aim of the present study was to characterize the plant for the presence of biologically active phytochemicals using various solvent extracts of leaves *Canthium coromandelicum* and GC-MS analysis of methanolic leaf extract of the plant. In the present investigation, various extracts of the leaf of *Canthium coromandelicum* were screened for the presence of Steroids, Alkaloids, Flavonoids Saponins, Tannins, Terpenoids, Quinones, Glycosides, Phenol by standard qualitative test procedures and further this study was extended by analyzing the potent bioactive compounds in the methanolic extract of *Canthium coromandelicum* leaves using GC-MS analysis. In the qualitative phytochemical screening/characterization using various solvent extracts of plant, it was found that most of the biologically active phytochemicals were present in the methanolic extract of *Canthium coromandelicum l*eaves. The GC-MS analysis revealed the presence of thirteen compounds in the methanolic leaf extract of *Canthium coromandelicum*. The major constituents were Squalence, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Z-8-Methyl-9 tetradecenoicacid, Didodecyl phthalate, and2-Tridecen-l-ol(E)along with other minor constituents. Results confirmed the presence of therapeutically potent compounds in the leaves of extract *Canthium coromandelicum* predominantly Alkaloids, Resins, Saponins, Tannins, and Quinines.

KEYWORDS

Canthium coromandelicum, Biologically active, Phytochemicals, GC-MS

INTRODUCTION

A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phyto compounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies. Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action.

Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of chinese medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids and lipids[1].

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C*anthium coromandelicum (*Burm. F) Commonly referred to as (Kara Sinhala) are trees found in dry scrub and monsoon forests in Sri Lanka and Southern India. According to folklore the leaves made into a dry curry (mallum) have been used as a possible treatment for many Ailments including hyperglycaemia in generations past.

All the genus of the family are economically important. *Canthium* is a genus of about 230 species of shrubs or small trees. Plant pacifies vitiated kapha, diarrhea, fever, leucorrhea, worm infestation and general debility. In siddha system of medicine the plant was used in respiratory disorder, diuretic, diabetic, obesity. In Ayurvedha system of medicine the plant was used in cough, diuretic, tumor and as anthelmintic[2]. An antioxidant, wound healing activity and antitumor acitivity were reported. D-mannitol, phenolic acid, phenolic compounds, carbohydrates, proteins were found from *Canthium parviflorum*[3]. Pharmacological activities such as antimicrobial, antioxidant, antidiabetic, wound healing, diuretic, anti-inflammatory, antinociceptive, antitumor and antipyretic from various species of *Canthium* has been reported[4].

MATERIALS AND METHODS

Sample Collection

The plant samples of *Canthium coromandelicum (Burm. F)* were collected from Kolli Hills in Namakkal District, Tamilnadu.

Preparation of Extract

The leaves were washed thoroughly with tap water and in distilled water and then dried the leaves at room temperature. The dried leaves were ground to a fine powder in a mechanic grinder. About 25gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of different solvents separately. Solvents used were Aqeous, Methanol, Ethanol, Chloroform, Benzene, Hexane and Acetone. The process of extraction continues till the solvent in siphon tube of an extractor become colourless. Aqueous extract was prepared by the cold maceration method. The extracts were filtered through Whatmann No.1 filter paper and the solvent was removed by evaporating in a water bath, which gave rise to a solid mass of the extract.

Phytochemical Screening[5]

Alkaloids [Mayer's test (Potassium mercuric iodide)]

1.36gm of mercuric chloride was dissolved in 60ml distilled water and 5gm of potassium iodide and diluted to 100ml with distilled water. To 1.0ml of acidic aqueous solution of samples, few drops of reagent were added. Formation of white or pale precipitate showed the presence of Alkaloids.

Flavonoids

In a test tube containing 0.5ml of various extracts of the samples, 5-10 drops of dilute HCl and a small piece of Zn or Mg were added and then solution was boiled for few minutes. In the presence of flavonoids, the reddish pink or dirty brown colour was produced.

5.0 ml of dilute ammonia solution was added to the aqueous filtrate of the plant extract followed by the addition of concentrated H_2SO_4. A yellow coloration observed in the extract indicated the presence of flavonoids. The yellow colour disappeared on standing.

Phenols [Ferric chloride test]

To 1.0ml of alcoholic solution of samples, 2.0 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added and the formation of blue or green colour indicates the presence of phenols.

Tannins [Lead acetate test]

In a test tube containing about 5.0 ml of a various extract, a few drops of 1% solution of lead acetate was added. A yellow or red colour precipitate was formed, indicating the presence of tannins.

Saponins

In a test tube containing 5ml of various extract of sample, a few drops of sodium bicarbonate was added. The mixture was shaken vigorously

for 3mins. A honey comb like froth was formed and it showed the presence of saponins.

Glycosides

A small amount of various extract of sample was dissolved in 1ml of water and aqueous solution of sodium hydroxide was added. Formation of a yellow colour indicates the presence of glycosides.

Steroids

To 2.0ml of various extracts of samples, 1.0 ml of concentrated H_2SO_4 was added carefully along the sides of the test tube. Formation of red colour chloroform layer indicates the presence of steroids.

Quinones

Dilute NaOH was added to the 1 ml of crude extract. Blue green or red coloration indicates the presence of quinones.

Terpenoids (Salkowski test)

5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2S0_4 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Phlobatannins

When crude extract of each plant sample was boiled with 2 % aqueous HCl. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

Resins

Five milliliter of distilled water was added to the extract and observed for turbidity.

Gas Chromatography Mass Spectroscopy (GC-MS)

GLC and the combine operation provides to go for qualitative and quantitative identification of the many structural complex compounds that may be present together in a particular as plant extract. Interpretation of mass spectrum of GC-MS was done sing the database of national institute standard and technology having more than 62000 patterns. The mass spectrum of the unknown compounds with the spectrum of the known compounds stored in NIST library.

For identification of metabolities showing antimicrobial and antioxidant potential the samples was carried using a GC clarus 500 perkin elmer with thermal description system TD 20 coupled with mass spectrum Gas chromatography was conducted in the temperature programming mode with a Elite column [5% Diphenyl/ 95% Dimethyl poly siloxane], 30% 0.25mmX 0.25 . The initial column temperature was 110ºC for 2 minute hold followed by increased temperature upto 200ºC at the rate of 10ºC per minute. The injected temperature was 250 ºC and the GC/MS interface was maintained at 250ºC. The sample was injected via a glass injector working in the split made, with helium carrier gas flow rate was 5ºC per minute to 9 minute hold. The identification of components was accomplished by comparison of retention time 6 minute and fragment pattern as well as with mass spectra in the NIST spectral library stored in computer software. The relative percentage of methanolic extract was expressed as percentage with peak area normalization.

RESULTS

Phytochemical Characterization

The present study was carried out on the plant samples revealed the presence of medicinally important bioactive compounds. The results of the preliminary phytochemical screening was carried out on the varying solvents (mainly aqueous, methanol, ethanol, chloroform, benzene, hexane or respectively). The phytochemical characterization of *Canthium coromandelicum* were summarized in the table: 1. The result revealed the presence of medically active compounds in the *Canthium coromandelicum*. The leaf extracts revealed phytochemicals like saponins, steroids, terpenoids, tannins, quinones, alkaloids, flavonoids, glycosides, phenols. Supporting the reason for its wide range of biological activates. Results are presented in Table 1. In the qualitative phytochemical screening/ characterization using various solvent extracts

Table 1: Phytochemical screening of *Canthium parvifloram* plants using various extracts namely Aqueous, Methanol, Ethanol, Chloroform, Benzene and Hexane

S. No	Parameters	Aqueous	Methanol	Ethanol	Chloroform	Hexane	Benzene	Acetone
1	Alkaloids	+	++	++	+	++	++	+
2	Tannins	+	++	+	+	++	++	+
3	Cardiac glycosides	–	++	+	–	++	++	++
4	Steroids	+	++	++	+	–	–	–
5	Phenol	–	++	+	+	–	+	–
6	Flavonoids	+	+	+	+	–	+	–
7	Quinone	+	+	++	+	+	+	+
8	Terpenoids	+	+	++	-	++	++	++
9	Phtobatanins	+	+	++	+	+	+	+
10	Saponins	+	+	+	+	++	++	++
11	Resins	+	+	+	+	++	++	++

+: presence; -: Absence;

Table 2: Phyto-components Identified in *canthium coromandelicum*

***Components identified in the sample – II (C.P) (Code No. 602)**
[GC MS study]

No	RT	Name of the compound	Molecular Formula	M W	Peak Area %
1.	6.21	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	$C_{15}H_{24}$	204	3.15
2.	10.79	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	7.94
3.	11.04	2-Tridecen-1-ol, (E)-	$C_{13}H_{26}O$	198	1.58
4.	11.23	E-2-Tetradecen-1-ol	$C_{14}H_{28}O$	212	2.46
5.	13.91	Phytol	$C_{20}H_{40}O$	296	3.26
6.	17.49	2-Aminononadecane	$C_{19}H_{41}N$	283	0.48
7.	18.85	Octadecane, 6-methyl-	$C_{19}H_{40}$	268	0.91
8.	19.52	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	4.19
9.	20.23	1-Nonadecanol	$C_{19}H_{40}O$	284	1.24
10.	21.61	Valeric acid, 2-pentadecyl ester	$C_{20}H_{40}O_2$	312	1.58
11.	23.16	Squalene	$C_{30}H_{50}$	410	34.94
12.	29.75	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240	30.37
13.	30.77	Heptadecanoic acid, heptadecyl ester	$C_{34}H_{68}O_2$	508	7.90

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

Table 3: Shows activities of phyto-components identified in *canthium coromandelicum* leaf extract by GC-MS

S.No	RT	Name of the Compound	Compound Nature	Activity
1	6.21	1,6,10-odecatriene,7,11-dimethyl -3-methylene-,(E)	Not Found	Not Found
2	10.79	3,7,11`,15-Tetramethyl-2-Hexadecan-1-ol	Terpene alcohol	Antimicrobial, Anti-inflammatory
3	11.04	2-Tridecen-1-ol	Alcohol	No activity reported
4	11.23	E-2-Tetradecen-1-ol	Not Found	Not Found
5	13.91	Phytol	Diterpene	Antimicrobial, Anticancer, Cancer preventive, DiureticAntimicrobial, Anticancer, Cancer preventive, Diuretic ,Antiinflammatory
6	17.49	2-Aminononadecane	Not Found	Not Found
7	18.85	Octadecane,6-methyl-	1-(ethenyloxy)-	Ether Antisepsis
8	19.52	Didodecyl phthalate	Plasticizer	Antimicrobial Antifouling
9	20.23	1-Nonodecanol	Not Found	Not Found
10	21.61	Valeric acid, 2-Pentadecyl ester	Fatty acid ester	No activity reported
11	23.16	Squalance	Triterpene	Pesticide,Sunscreen, Antibacterial,Anticancer, Antioxidant,Chemo preventive,Antitumour
12	29.75	Z-8-Methyl-9-tetradeconic acid	Not Found	Not Found
13	30.77	Heptadecanoic acid, heptadecyl ester	Palmatic acid	Antioxidant,Nematicide Pesticides,Hypocholesterolmic

of plant, it was found that most of the biologically active phytochemicals were present in the methanolic extract of *canthium coromandelicum* leaves. In other words, the results confirmed the presence of therapeutically potent compounds in leaf extract of *canthium coromandelicum*. It revealed that alkaloids Resins, saponins tannins, and quinines were predominantly found in all the seven extracts, followed by flavonoids which were found in five extracts and cardiac glycosides, steroids, phenol and resins were found in four extracts used for phytochemical screening.

GC-MS: Phytocomponents in Methanolic Extract of *Canthium Parvifloram* by GC-MS Report

The GC-MS analysis revealed the presence of thirteen compounds from the methanolic leaf extract of *canthium coromandelicum.* the major components were Squalence (34.94),Z-8-Methyl-9-tetradecenoic acid, Heptadecanoic acid (30.37),3,7,11,15-Tetramethyl -2-hexadecen-1-ol (7.94), heptadecyl ester(7.90). Along with other miner constituents were also present. The GC-MS chromatogram [fig1] shows the peak area separation.

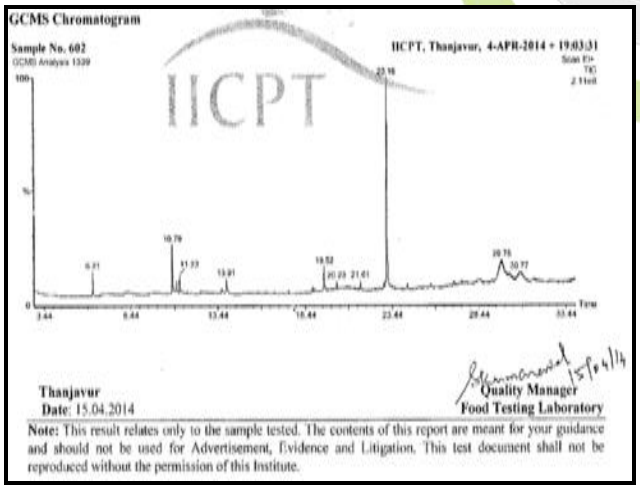

Figure 1: Shows GC-MS chromatogram of methanolic leaf extract of *canthium coromandelicum*

The chromatogram figure1 shows the fourth prominent peaks in the retention time range. The peak at the 23.16 (RT) is having the peak area 34.94. This largest peak is due to the presence of Squalene. The second prominent peaks at 29.75(RT) is having the peak area 30.37, is due to the presence of Z-8-Methyl-9-tetradecenoic acid. The third prominent peaks at prominent peaks at 10.79(RT) is having the peak area 7.94, is due to the presence of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, the fourth prominent peaks at prominent peaks at 30.77 (RT) is having the peak area 7.90, is due to the presence of Heptadecanoic acid, heptadecyl ester. The other less prominent peak at other retention times are given in Table 2. The nature of the chemical compound and its therapeutic activity is depicted in table 3.

DISCUSSION

There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease[6].

In recent years, the interest for the study of the organic compounds from plants and their activity has increased. The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative and quantitative analysis for volatile and semi-volatile compounds. From the above results, the results of our present study is further supported to the similar reports prevent by[7].

The results from the table clearly emphazises the wide store of phytochemicals included by *Canthium coromandelicum* in different solvents. There by it can serve as effective therapeutic agent against various infectious diseases. Significant antioxidant and diuretic activity was exhibited by extracts of leaves[8].

The aim of the present study was to develop a rapid method for the quantitative determination of organic compounds in plant and to confirm the phytochemicals present in the plant extracts. Diversity of medicinal plants and herbs containing various phytochemicals with biological activity can be of valuable therapeutic key. Different phytochemicals have been found to have a broad range of activities, which may help in protection against chronic diseases[9].

In the present investigation, the GC-MS analysis revealed the presence of thirteen compounds from the methanolic leaf extract of *canthium coromandelicum*. The presence of phyto-components reveals the importance of the plant as medicinally used. So, it is recommended as a plant of phyto - pharmaceutical importance, however, further studies will need to be undertaken to ascertain fully its pharmacological activity. From our investigation, the results confirm the presence of therapeutically potent compounds in leaf extract of *canthium coromandelicum*. Similar to our study,[10] also characterized two major compounds through GC-MS analysis of *Rhinacanthus nasutus* leaves;[11] characterized thirteen compounds from methanolic leaf extracts of *Naringi crenulata*[12]; identified fourteen compounds by GC-MS analysis in *Caralluma fimbriata*[13]; identified twenty chemical compounds from ethanolic extract of *Mussaenda frondosa*;[14] found abundance of phyto-components, such as alkaloids and majorly flavonoids;[15] identified twelve chemical constituents from ethanolic extract of aerial parts of *Albizia rocera* (Roxb,) Benth. by GC-MS analysis;[16] identified the presence of atleast thirteen compounds from the ethanolic extract of *H. Enneaspermus.*

CONCLUSION

The result of the present investigation reveals that the methanolic extracts of *canthium coromandelicum* possessed significant anticancer activity which was analyzed by GC-MS analysis. Phytol and Squalene which was present in *canthium coromandelicum* may be responsible for anticancer activity. The GC-MS analysis of the ethanolic extract of *canthium coromandelicum* reveals the presence of phytoconstituents belonging to the type acids, esters, alcohols, ethers, *etc.* Thus, the medicinal plant *canthium coromandelicum* is found to possess significant phytoconstituents. The presence of such a variety of phytochemicals may be attributed to the medicinal characteristics of this plant *canthium coromandelicum.*

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