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GC-MS ANALYSIS OF THE CHLOROFORM EXTRACT OF BARK OF *TERMINALIA TRAVANCORENSIS* WIGHT & ARN. (COMBRETACEAE)

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ABSTRACT: Gas Chromatography-Mass Spectrometry (GC-MS) studies play an important role in isolation and identification of phytocompounds. Since, the chemical composition of the plants usually determines its biological activity, phytochemicals of possible pharmaceutical importance can be subjected to a detailed analysis. The present study is concerned with the GC-MS analysis of chloroform extract of the bark of *Terminalia travancorensis*, an evergreen tree, endemic to the Western Ghats. Reports on the benefits and or medicinal properties of *T. travancorensis* are rare or even absent. In this context, the present study was carried out to determine the chemical constituents of the bark by GC-MS analysis. Twenty two components were identified by GC-MS analysis in the chloroform extract. The major components detected were Friedelan-3-one (15.93%), gamma-sitosterol (10.48%), alpha -octadecene (8.54%), n-Tetracosanol-1 (8.21%), Phenol, 2,4-bis(1,1-dimethylethyl) (7.77%), 1-Nonadecene (7.49%), Heptacosanol (6.16%), 1-Heptadecene (5.25%) and stigmast-4-en-3-one (5.46 %). Friedelan-3-one, the major compound is a triterpenoid possessing antifeedant, anti-inflammatory, anticancer, hepatoprotective, antimicrobial and anticandidal activities. The second major compound, gamma-sitosterol plays a vital role in the structure and dynamics of membranes, also involved in the embryonic growth of plants. The remaining compounds are also reported to have various biological activities.

INTRODUCTION: Plants are capable of synthesizing a variety of low-molecular weight compounds called secondary metabolites. The most important among these secondary metabolites are the alkaloids, terpenoids, flavonoids and phenolic compounds. They have interesting biological activities owing to their complementary and overlapping mechanism of action and therefore serve as sources of many potent and powerful drugs.

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Knowledge of the chemical constituents of plants is helpful in the discovery of new sources of drugs/therapeutic agents and other economically useful materials [1, 2, 3].

Chromatographic techniques are used for the separation of plant compounds. The GC instrument is effective in separating compounds but cannot be used for reliable identification of specific substances. The MS instrument provides specific results but produces uncertain qualitative results. Gas chromatography-Mass spectroscopy is a hyphenated technique which couples the powerful separation potential of gas chromatography with the specific characterization ability of mass spectrometry. GC-MS provides a detailed chromatographic profile of the sample and

consequently measurements of the relative or absolute amounts of the components Thus the GC-MS is a tool for conclusive proof of compound identity [4].

The present study focused on the identification of the phytoconstituents present in *Terminalia travancorensis* Wight & Arn., with the aid of GC-MS. *Terminalia travancorensis* commonly known as 'Kattu Kadukka/Pei Kadukka or Choolamaruthu, is one among the ten species of *Terminalia* in the state of Kerala [5]. All the species of *Terminalia* are very popular in the traditional medicine practice [6]. *Terminalia travancorensis*, is an evergreen tree species, buttressed, upto 35m tall and endemic to the Western Ghats (occasional in South Sahyadri and rare in Southern part of central Sahyadri) [7]. A perusal of literature revealed that this species of *Terminalia* is the least studied. Information on *T. travancorensis* is restricted to its occurrence at various locations in Kerala followed by short morphological descriptions [8, 9, 10, 11]. Reports on phytochemical analysis is also almost absent [12, 13]. Lack of information on the species is probably due to the difficulty in identifying the material or due to the lack of sufficient material for study.

MATERIALS AND METHODS:

Collection of plant parts: Plant parts of *T. travancorensis*, required for the present study were collected from Arippa forest division, Thiruvananthapuram. Specimens of *T. travancorensis* (**Fig. 1**) were identified and authenticated by Sri. B. N. Nagaraj, Divisional Forest Officer, Malayatoor Forest Division, Cochin, Kerala. Voucher specimens have been submitted in the herbarium of the Department of Botany for future reference (KUBH 6030). The collected fresh bark of *Terminalia travancorensis* was dried initially in sunlight, later in shade and ground into a fine powder.

FIG. 1: THE BUTTRESSED ROOT OF *T.TRAVANCORENSIS*

Extraction of plant sample: About 20gm of powdered sample was extracted with 300ml chloroform in the soxhlet extractor. The extract was filtered, concentrated and stored in refrigerator until use [14]. Two microlitres of the extract was taken for GC-MS analysis.

Gas Chromatography Mass Spectrometry analysis of chloroform extract: Instrument Details: GC-MS analysis was carried out on a GC-MS-QP 2010, Shimadzu, Tokyo, Japan Model

equipped with Column type-VF 5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25µm film thickness, operating in electron impact mode at 70eV. Helium (99.99%) was used as carrier gas@ constant flow rate of 1.51 ml/min. Injector and mass transfer line temperature were set as 200°C and 240°C respectively. The oven temperature was programmed from 70 to 220°C @

10°C/min., held isothermal for three minutes and finally raised to 300°C at 10°C/min.

Sample injected was two microlitre in a split mode with a scan range of 40 – 1000 m/z and run for 30 minutes.

Identification of components: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standards and Technology (NIST08 LIB) [15] and Registry of mass spectral data, Wiely, New York (WILEY8 LIB) [16]. The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns. The spectrum of unknown components was compared with the spectrum of the known components stored in the computer library. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage of the extract was expressed as percentage with peak area normalization.

Results: The compounds identified from the chloroform extract of bark of *T.travancorensis*, their retention time and peak area percentage are given in **Table 1.** The study revealed the presence of 22 compounds (**Fig.2**).

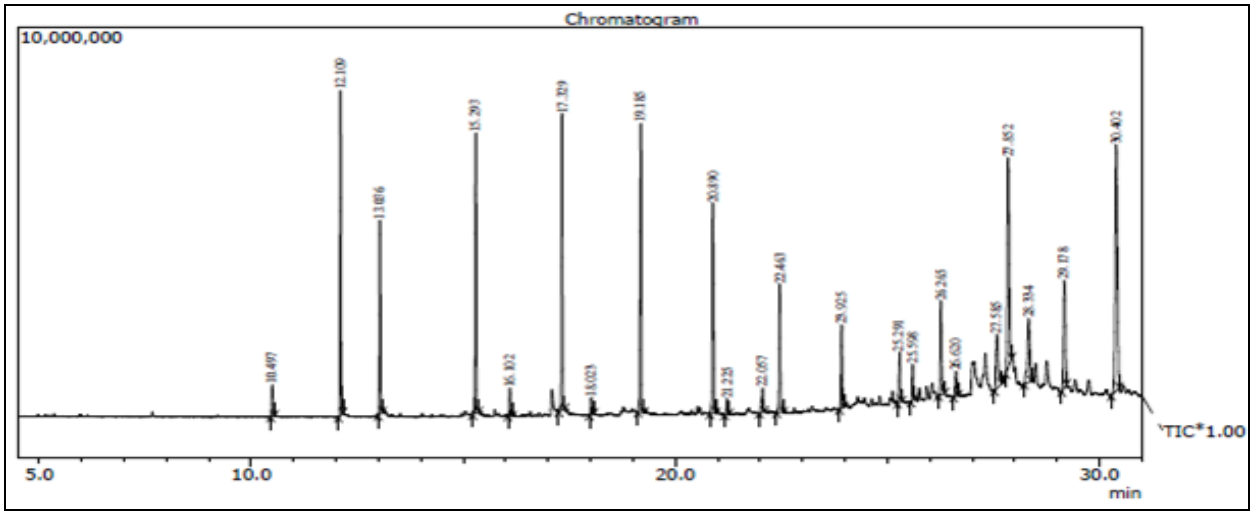

FIG. 2: GC-MS CHROMATOGRAM OF CHLOROFORM EXTRACT OF BARK SAMPLE OF *T. TRAVANCORENSIS*

TABLE 1: PHYTOCOMPONENTS IDENTIFIED BY GC-MS ANALYSIS OF THE CHLOROFORM EXTRACT OF BARK OF *T. TRAVANCORENSIS*

Peak No.	Retention Time	Peak Area	Peak Area%	Name of the Compound
1	10.497	1331221	0.89	1-Pentadecene
2	12.109	11568213	7.77	Phenol, 2,4-bis(1,1-dimethylethyl)-
3	13.036	7818488	5.25	1-Heptadecene
4	15.293	11152249	7.49	1-Nonadecene
5	16.102	1113138	0.75	Phthalic acid, diisobutyl ester
6	17.329	12711984	8.54	Alpha.-octadecene
7	18.023	779549	0.52	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1 methylethyl ester
8	19.185	12224930	8.21	n-Tetracosanol-1
9	20.89	9171620	6.16	Heptacosanol
10	21.225	742230	0.5	Hexadecanal
11	22.057	1453401	0.98	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
12	22.463	6065700	4.07	1-Heptacosanol
13	23.925	3789553	2.55	Heptacosan-1-ol
14	25.291	2352407	1.58	Eicosyl heptafluorobutyrate
15	25.598	1696478	1.14	6-methoxy-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chromane
16	26.265	4921728	3.31	alpha.-Tocopherol-.beta.-D-mannoside
17	26.62	1444030	0.97	Heptacosanol
18	27.585	5040892	3.39	n-Tetracosanol-1
19	27.852	15610966	10.48	gamma.-Sitosterol
20	28.334	6060313	4.07	Lanosterol
21	29.178	8126971	5.46	Stigmast-4-en-3-one

22	30.402	23713364	15.93	Friedelan-3-one
		148889425	100.00	

DISCUSSION: The GC-MS analysis of the chloroform extract of bark of *T. travancorensis* showed the presence of 22 compounds. Of these, Friedelan-3-one, gamma. -Sitosterol, alpha.-octadecene, n-Tetracosanol-1, Phenol, 2,4-bis(1,1-dimethylethyl)-, 1-Nonadecene, Stigmast-4-en-3-one, 1-Heptadecene were predominant in terms of the percentage. These compounds have been reported to possess certain biological actions such as anti-inflammatory, analgesic antipyretic, antimicrobial, antioxidant, anti tumour, anti diabetic activities [17, 18]. The major group of compounds and their biological activities are given in **Table 2.**

TABLE 2: BIOACTIVITY OF THE IMPORTANT COMPOUNDS DETECTED IN *T. TRAVANCORENSIS*

Major compounds	Major group	*Biological activity
Friedelan-3-one	Terpenoid (Triterpenoid)	Analgesic, antipyretic, antifeedant, anti-inflammatory, anticancer, hepatoprotective, antimicrobial and anticandidal activities
Gamma.-Sitosterol	Sterol (C-24 isomer of betasitosterol)	Growth hormone of animals and plants, anti-ulcer, antimicrobial, antipyretic and hypolipidemic agent
Alpha.-octadecene	Hydrocarbon (A long-chain hydrocarbon and an alkene)	Compatible with oleic acid.
n-Tetracosanol-1	Fatty acid derivative (Fatty alcohol derived from the fatty acid Lignoceric acid).	Anti-oxidant, lowering cholesterol, inhibiting lipid peroxidation, enhancing immune functions, inhibits platelet aggregation, cardiovascular
Phenol, 2,4-bis(1,1-dimethylethyl)-	Phenol	Antibacterial and antioxidant
1-Nonadecene	Alkene	Artificial ripening of fruits, metabolism of aromatic compounds in animals and antimicrobial
Stigmast-4-en-3-one	Sterol	Antitumour, hypoglycaemic and antidiabetic
1-Heptadecene	Alkene	Artificial ripening of fruits, metabolism of aromatic compounds in animals antimicrobial

*Activity source: Dr Duke's Phytochemical and Ethnobotanical data bases.

GC-MS analysis has been conducted earlier in almost all the species of *Terminalia* using the extracts obtained from a range of solvents (non-polar to polar) and in all the plant parts (stem, leaves, fruits and bark). In general, almost all the previous results revealed the presence of hydrocarbons, alcoholic compounds, flavanoids, alkaloids, ketones, carbohydrates, fatty acid ester, alkenes compounds and fatty acids. The results of the present study are in agreement with previous reports. Most of the previous studies on the genus *Terminalia* also included the antimicrobial and anti-oxidant activities of the crude extracts, implying the potent biological activities of these extracts [19, 20, 21, 22]. The results of the present study highlight the utility of *T.travancorensis* just as any other species of *Terminalia* in possessing bioactive secondary metabolites

CONCLUSION: This is the first report of GC-MS analysis in *T. travancorensis.* Presence of triterpenoids, sterols, phenolics and alkenes was noted. Literature compilations and database records reveal the association of the specific compounds detected in the present study with numerous biological activities**.** The study thus provides new information on the phytochemical constitution of *T. travancorensis,* a rare endemic species, before being lost from science.

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