Bioactive Compound Analysis by Gas Chromatography–Mass Spectrometry (GC-MS) and Elemental as Well Proximate Composition of Leaf *Terminaliacatappa*

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Abstract:- **Medicinal plants have been identified and used throughout human history to treat aliment and diseases. Plants have ability to synthesize a wide variety of chemical compound. Many of which are efficacious and contain substances that are potential drugs that require further examinations. Chemical compounds in plants mediate their effects on the human body by binding to receptor molecules present in the body; Also, many of the herbs and spices used by humans to season food yield useful medicinal compounds. *Terminaliacatappa* Linn (Indian almond) is a Combretaceae plant (tropical almond family), Usually a small to medium-sized tree 30–50 ft (9–15 m) high and 1 ft (0.3 m) in trunk diameter, but sometimes much larger in diameter and with slight buttresses, evergreen except in areas with a marked dry season. This study was designed to evaluate proximate content, elemental contentsas well as Gas-chromatography analysis of *Terminaliacatappa* leaf. One thousand grammes (1000g) of the powdered leaf of *Terminaliacatappa* was extracted with methanol using cold infusion (maceration) method. Fresh leaf of *Terminaliacatappa* was collected from Bolori ward Maiduguri Borno state and it was identified by Professor S. S. Sunusi of Department of Biological Science, Faculty of Science, University of Maiduguri. Eighty three point eight two grammes (83.82g) of the dark green in colour gummy in texture of methanol crude extract was obtained, which was further partitioned with n-hexane, ethyl acetate, n-butanol and water to give n-hexane portion (1.638% $^{W}/_{W}$), dark green in colour, oily in texture, ethyl acetate portion (0.075% $^{W}/_{W}$), black in colour, gummy in texture, n-butanol portion (0.777% $^{W}/_{W}$), brown in colour, oily in texture and finally aqueous portion (2.997% $^{W}/_{W}$), dark brown in colour, powdered in texture. The concentration levels of macro-elements (Ca, Mg, Na, K) and micro-elements (Cd, Cu, Ni, Zn, Fe, Mn) were analyzed using Atomic Absorption Spectrophotometer and the anions (Cl^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}) were estimated using smart spectrophotometer. The leaf of *Terminaliacatappa* indicated the presence ofcalcium (19.68µg/ml), cadmium (0.12µg/ml), copper (6.84µg/ml), iron (10.67µg/ml), potassium (18.90µg/ml), magnesium (10.27µg/ml), manganese (1.27µg/ml), sodium (15.30µg/ml) nikkel (1.00µg/ml), zinc (4.17µg/ml), chloride (0.72µg/ml), nitrate (46.00µg/ml), phosphate (70.00µg/ml) and sulphate (227.33µg/ml). However, only phosphate and sulphate exceeded the permissible limit of world health organization (WHO) standard. The results of proximate content evaluation showed that moisture, was 7%, crude protein, 10.37%, fat, 1%, crude fibre, 21%, ash, 10% and carbohydrate, 51%. Purification of compound was done by using column and thin layer chromatography method. After pooling and recombination with different solvent system of the n-butanol extract, three compounds T_{CA}, T_{CB} and T_{CC} were obtained with melting points T_{CA}(286.00-287.00), T_{CB} (278.00-279.00) and T_{CC} (260.00-262.33). All the melting points were shape and uncorrected. The Gas Chromatography-Mass Spectrometry of the compound T_{CA} revealed the presence of fatty acid derivaties such as octadecanoic acid 4-hydroxybutyl ester, tetradecanoic acid 2-hydroxyl, pentanoic acid, 2,2 4-trimethyl-3-carboxy isopropyl, isobutyl ester, octadecanoic acid (2-phenyl 1-3-dioxolan -4-yl) methyl ester cis.**

Keywords: **Terminalia, extract purification, isolation, proximate elemental**

I. INTRODUCTION

Medicinal plants have been in used throughout human history to treat aliment and deseases. Plants have the ability to synthesize a wide variety of chemical compounds[1]. Many of which are efficacious and contain substances that are potential drugs that require further examinations[2]. Plants have evolved with the ability to synthesize chemical compounds that can help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals[3]. By chance, some of these compounds, whilst being toxic to plant predators, turn out to have beneficial effects when used to treat human diseases. Such compounds called secondary metabolites are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen – substituted derivatives[4]. Chemical compounds in plants mediate their effects on the human body by binding to receptor molecules present in the body; such processes are identical to those already well understood for conventional drugs and as such herbal medicines do not differ greatly from conventional drugs in terms of how they work. Also, many of the herbs and spices used by humans to season food yield useful medicinal compounds[5].*Terminalia Catappa* is a large, deciduous tree with smoothgrey bark and whorled branches that form a canopy and is found in tropical and subtropical

regions, It is widely planted throughout the tropics as an ornamental tree for shade for the edible nuts. *Terminaliacatappa* contains hydrolyzable tannins punicalagin (major tannin), punicalin, terflavins A and B, tergallagin, tercatain, chebulagic acid, geraniin, granatin B, corilagin), flavanoids (isovitexin, vitexin, isoorientin, rutin) and triterpinoids (ursolic acid, 2α, 3β, 23-trihydroxyurs-12-en-28oic acid and asiatic acid)[67]. The leaves, bark and fruit of the tree *Terminaliacatappa* L.(Combretaceae) have been commonly used as a folk medicine for antidiarrhea, antipyretic and haemostatic purposes[8]. The leaves of *T. catappa* have been used for the preventionand treatment of hepatitis and liver-related diseases[9].

II. EXPERIMENTATION

Sample collection and Identification

Fresh leaf of *Terminaliacatappa* was collected from Bolori ward of Maiduguri Borno State, Nigeria, and identified by a Plant Taxonomist, in the Department of Biological Science, Faculty of Science, University of Maiduguri. The plant leaf material was air-dried in the laboratory at room temperature. The leaf of the plant was ground to fine powder using wooden mortar and pestle and the sample was given a voucher number (562C), stored in the research laboratory of the Chemistry Department, University of Maiduguri for further analysis.

Determination of Proximate Content

The ground air-dried leaf of *Terminaliacatappa* was processed for dry matter, crude protein, crude fibre, ether extract or fat, ash, carbohydrate and nitrogen free extract (N.F.E) according to method described by Association of Analytical Chemist[10].

Determination of Elemental contents

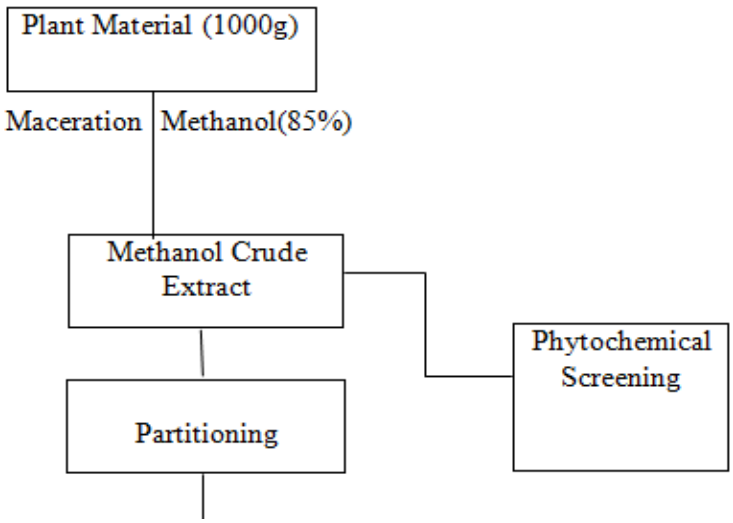
The air-dried plant leaf was ground and placed in a porcelain crucible and put into muffle furnace at a temperature 500^{o}C for about 3 hours after the sample has been ashed, it was removed and cooled in a desicator for further analysis. Zero point five grams (0.5g) of the ashed sample was weighed into 250ml beaker. 10ml of 6 mole of hydrochloric acid was added. The beaker was covered with watch glass and heated for 15 minutes, 1ml of concentrated nitric acid was added and the sample was heated to evaporate to dryness. 1m of 6 mole of hydrochloric acid and 10ml of distilled water was added and heat again on a hot plate to complete dissociation. It was left to cool and then filtered with Whatman filter paper into 100ml volumetric flask and made up to the mark with distilled water and transfer into polythene bottle for analysis. Micro and macro elements were analysed using Atomic Absorption Spectroscopy (AAS) using standard procedure and anions was analysed using spectrophotometer following guided protocols[11].

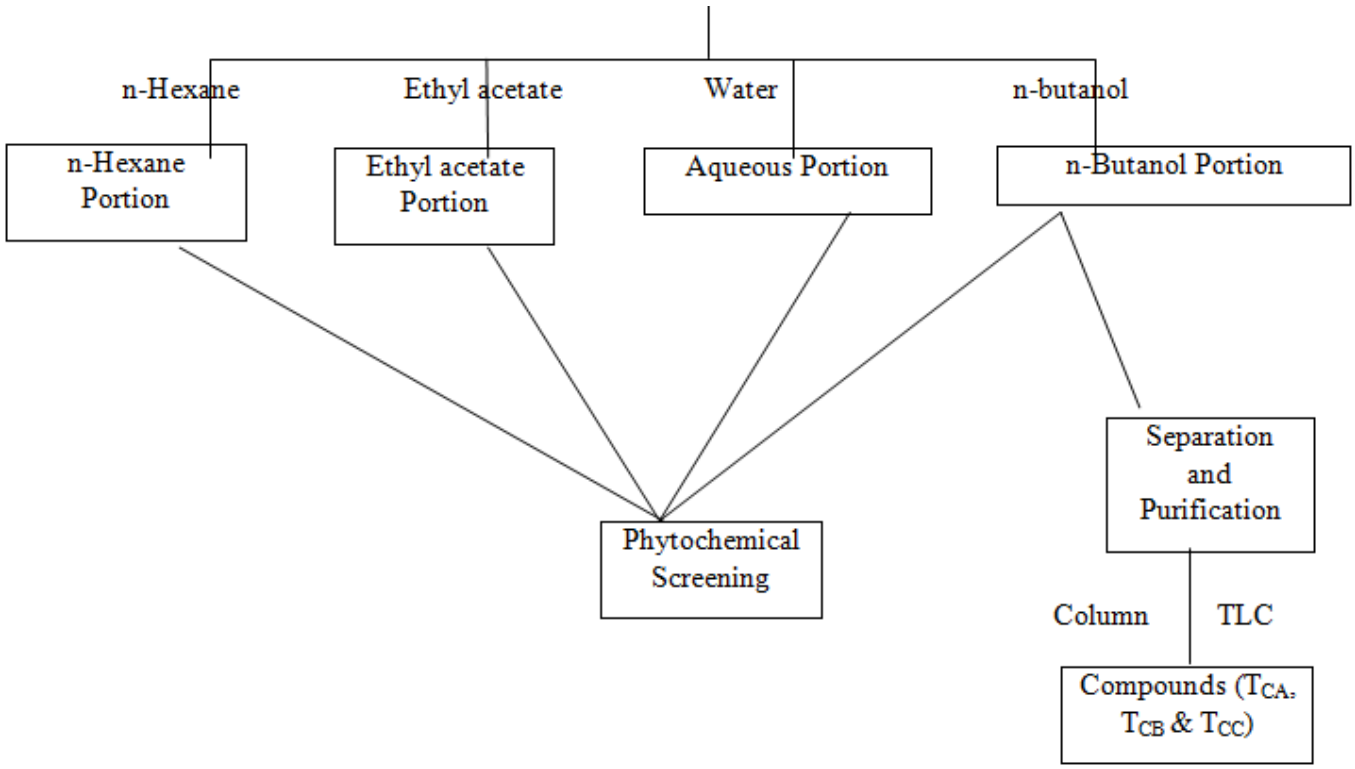
Sample Extraction

The ground leaf material (1,000g) was extracted with 85% methanol using maceration (cold infusion) method for 72 hours. The crude extract was concentrated under reduced temperature. The crude extract was then stored in a desiccator. The chaff was socked in distilled water for three hours and the mixture was filtered, concentrated and stored under pressure andreduced temperature.

Partitioning of the Extract

The methanol extract of *Terminaliacatappa* was partitioned with n-hexane until exhaustion. The aqueous fraction was partitioned with ethyl acetate and also partitioned exhaustively with n-butanol. The n-butanol portion and the aqueous portion were then evaporated using oven under pressure and reduced-temperature. The resulting masses were then weighed and kept in a desiccator for further analysis.





Scheme 3.0: Schematic Diagram (Summary) of *Terminaliacatappa* Chemical analysis

Isolation and Gas-Chromatographic Analysis (GC-MS) analysis

The n-butanol partitioned portion was subjected to column chromatography using the Gradient elution protocol through suitable mixed solvents system, ethyl acetate and n-butanol in the following ratios 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 100 and from each 100ml aliquotes was collected (Libikas*et al* 2005). It was further subjected and purified using preparative thin layer chromatography (T.L.C) with a suitable mixed solvent of dichloromethane (92.5%), methanol (5%) and acetic acid (2.5%). The isolated compound(s) was subjected to Gas Chromatography-Mass Spectrometry to identify possible compound(s) using standard protocols.

Preliminary phytochemical screening

The methanol crude extract together with the partition portions were subjected to phytochemical screening using standard procedures to identify the constituents [121314]

III. RESULT AND DICUSSION

Table 1: The Weight, Percentage Yield, Colour and Texture of the Methanol Crude extract and Partitioned portions of *Terminaliacatappa*.

S/NO.	Extract	Weight (g)	Percentage yield (%)$^{W}/_{W}$	Colour	Texture
1.	MCE	83.82	8.382	Dark green	Gummy
2.	NHP	16.38	1.638	Dark green	Oily
3.	EAP	0.75	0.075	Black	Gummy
4.	NBP	7.77	0.777	Brown	Oily
5.	AQP	29.97	2.997	Dark brown	Powdered

Key: MCE = Methanol Crude Extract, AQP = Aqueous Portion, NHP = n-Hexane Portion, NBP= n-Butanol Portion and EAP = Ethyl Acetate Portion.

Table 2: Proximate Content Analysis of the Leaf of *Terminaliacatappa*

S/NO.	Proximate Content	Percentage Content (%)
1.	Dry Matter	93.00
2.	Moisture Content	7.00
3.	Crude Protein	10.00
4.	Ether Extract	1.00
5.	Crude Fibre	21.00
6.	Ash	10.00
7.	Carbohydrate	51.00

Table 3: Analysis of Macro Element, Micro Elements and Anions *Terminaliacatappa* Leaf

S/NO.	Elements	Concentration level (µg/ml)	WHO Standard (1996) (µg/ml)
1.	Ca	19.68	3600-80000
2.	Cd	0.12	10-35
3.	Cu	6.84	100-300

4.	Fe	10.67	50-5000
5.	K	18.90	10-100
6.	Mg	10.27	100-200
7.	Mn	1.27	100-20000
8.	Na	15.30	400-500
9.	Ni	1.00	10-50
10.	Zn	4.17	150-20000
11.	Cl^-	0.72	250
12.	NO_3^-	46.00	50
13.	PO_4^{3-}	70.00	0.40
14.	SO_4^{2-}	227.33	200

Key: Ca = Calcium, Cd = Cadmium, Cu = Copper, Fe = Iron, K = Potassium, Mg = Magnesium, Mn = Manganese, Na = Sodium, Ni = Nickel, Zn = Zinc, Cl^- = Chloride, NO_3^- = Nitrate, PO_4^{3-} = Phosphate, SO_4^{2-} = Sulphate

Table 4: Phytochemical Screening of *Terminaliacatappa* Methanol Crude extract, n-Hexane Portion, Ethyl acetate Portion, n-Butanol Portion and Aqueous Portion.

S/NO.	Test	MCE	NHP	EAP	NBP	AQP
1.	Carbohydrate	+	-	-	+	+
2.	Soluble starch	-	-	-	-	-
3.	Phlabotannins	-	-	-	-	-
4.	Glycosides	-	-	-	-	-
5.	Cardiac glycoside	+	-	-	+	+
6.	Flavonoid	+	-	-	+	+
7.	Terpenoid	+	+	+	-	+
8.	Saponins	+	-	-	-	+
9.	Alkaloid	+	-	-	+	-
10.	Tannins	+	-	-	+	+

Key: MCE = Methanol Crude Extract, NHP = n-Hexane Portion, EAP = Ethyl Acetate Portion, NBP= n-Butanol Portion, AQP = Aqueous Portion, (+) = Present and (-) = Absent.

Table 5: Compounds, Retardation factor and Melting point of Compound T_{CA}, T_{CB} and T_{CC}

S/NO.	Compound	Retardation factor (R_f)	Melting point (^oC)
1.	T_{CA}	0.4	286.00-287.00
2.	T_{CB}	0.7	278.00-279.00
3.	T_{CC}	0.9	260.00-262.33

Compound T_{CA} was having melting point of **(**286.00-287.00)^oC with R_f value 0.4, compound T_{CB} was having (278.00-279.00)^oC with R_f value 0.7 and compound T_{CC} showed the melting point (260.00-262.33)^oC with R_f value 0.9.

CHEMISTRY ANALYTICAL LABORATORY

(GC7890B-MSD-5977A-AGILENT TECH USA)

(Componud T_{CA})

Chromatogram

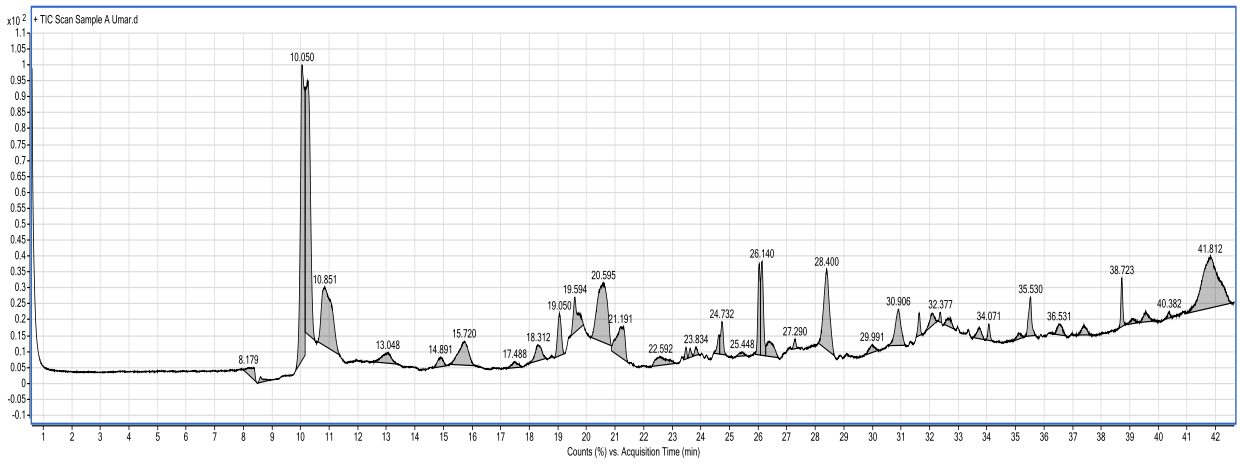


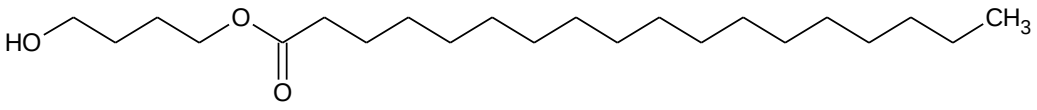
Fig. .1: Chromatogram of compound TCA

Table 6: GC-MS Analysis of Compound TCA

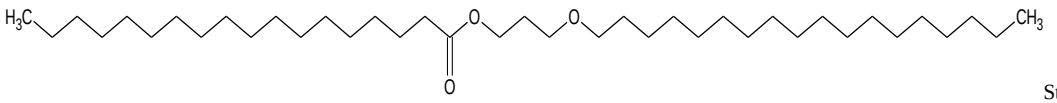
Peak no.	Retention time	Area	Height	Width	Name
Peak 1	8.179	487043.04	20163.72	0.452	Octadecanoic acid, 4- hydroxybutyl ester
Peak 2	8.608	150764.06	15371.33	0.416	Pseudoephedrine
Peak 6	13.048	673816.16	29188.22	0.782	d- mannitol, 1- decylsulfonyl
Peak 7	14.891	364283.59	26720.18	0.454	Octadecanoic acid, 4- hydroxybutyl ester

Peak	8	15.72	1604936.72	67047.33	0.848	α-β- glucopyranoside, O-α-D-glucopyranosyl (1.fwdarw. 3)-β-D fructol
Peak	10	18.312	717134.5	43927.4	0.569	4- methyldocasone
Peak	11	19.05	1071590.44	119608.91	0.333	Stearic acid, 3- (octadecyloxy) propyl ester
Peak	14	21.19	1818588.84	85902.41	0.575	Tetradecanoic acid, 2- hydroxyl
Peak	15	22.592	683906.97	24506.43	0.827	2- Cyclohexylpiperidine
Peak	18	23.834	143747.13	23092.29	0.195	Estra- 1,3,5 (10)- Trien-17β-Ol
Peak	20	24.732	605445.34	92123.18	0.234	Phen-1-4 diol, 2,3-dimethyl-5-trifuoromethyl
Peak	23	26.14	1705234.27	264790.25	0.183	Pentanoic acid , 2,2,4- trimethyl-3-carboxylisopropyl, isobutyl ester
Peak	25	27.29	155432.31	26650.47	0.275	Vitamin A palmitate
Peak	26	28.40	3219126.74	226251.26	0.418	Cinamic acid, 4 hydroxy-3-methoxy-(5-hydroxy-2-hydroxymethyl-6-(2-(4-hydroxy-3-methoxyphenyl) ethoxy)-4-(6-methyl-3,4,5 trihydroxy tetrahydropyran-2-yloxy) tetrahydropyran-3yl) ester
Peak	27	29.991	282948.66	21855.16	0.418	1,3,-Dioxane, 5-(hexadecyloxy)-2-pentadecyl-trans
Peak	29	31.639	332313.54	62893.62	0.197	Beta-methasonevalerate
Peak	34	34.071	241437	46442.04	0.216	9.10 secocholesta-5,7,10(19)-triene-1,3-diol 25-[(trimethylsilyl)oxy]- (3β, 5Z, 7E)
Peak	36	35.53	845780.6	109859.97	0.338	Dasycarpidan-1-methanol, acetate (ester)
Peak	42	40.382	100263.08	18347.05	0.183	1-monolinoleoylglycerol trimethylsilyl ether

This table 6 contains the compounds in T_{CA}. The chromatogram revealed the presence of forty two compounds, but nineteen compounds were having the comparison from the NIST library such as Octadecanoic acid, 4- hydroxybutyl ester with peak number one retention time 8.179 and Phen-1-4 diol, 2,3-dimethyl-5- trifuoromethyl with peak number 23 retention time 26.14, other peaks could not be identified from the library.

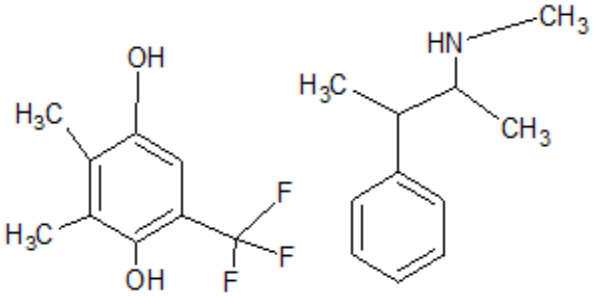


Octadecanoic acid, 4-hydroxybutyl ester



Stearic acid,

3-(octadecyloxy) propyl ester



Phen-1-4 diol, 2,3-dimethyl-5- trifuoromethyl Pseudoephedrine

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GCMS ANALYSIS
(GC7890B-MSD-5977A-AGILENT TECH USA)
(CompoundT_{CB})

Chromatogram

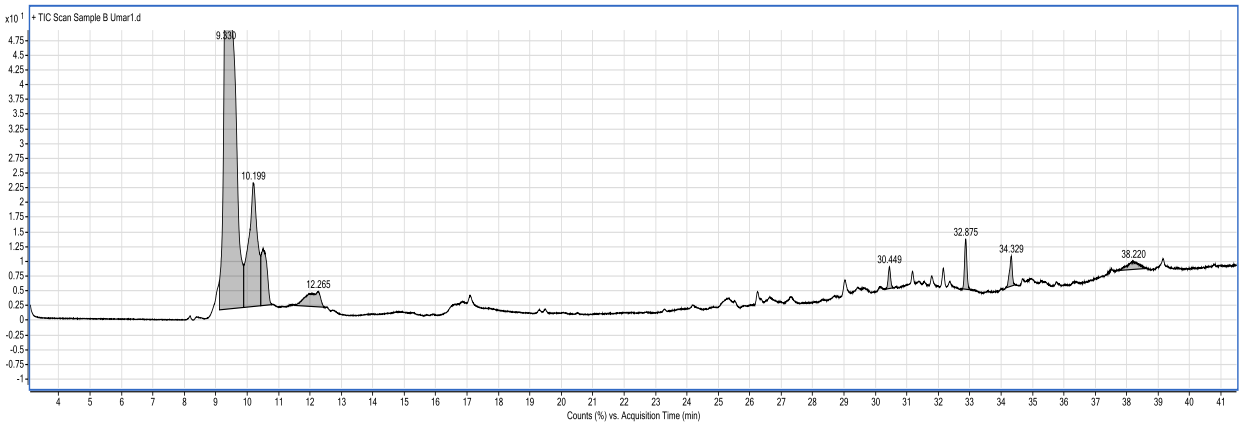
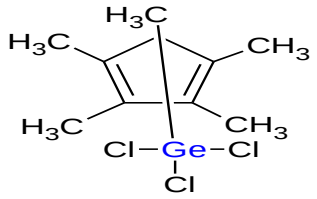


Fig. 2: Chromatogram of compound T_{CB}

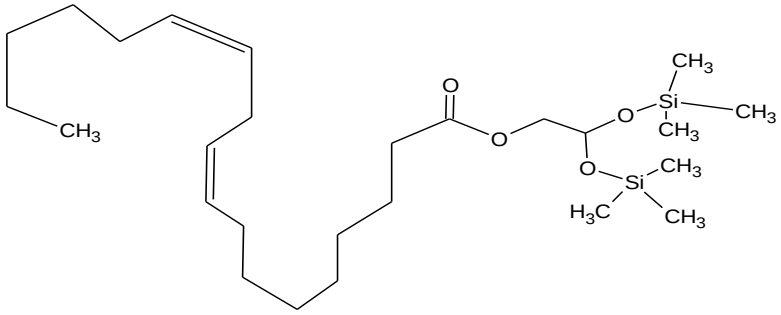
Table 7: GC-MS Analysis of Compound T_{CB}

Peak no.	Retention time	Area	Height	Width	Name
Peak 6	32.875	788420.70	151457.96	0.246	Trichloro- (1,2,3,4,5-pentamethylcyclopenta-2,4 dimethyl germen
Peak 7	34.329	570517.61	90888.84	0.307	Dasycarpidan-1-methanol, acetate (ester)
Peak 8	38.22	619582.29	24479.47	0.801	1-Monolinoleoyl glycerol trimethylsilyl ether

This table 7 contains the compounds in TCB. The chromatogram revealed the presence of eight compounds, but three compounds were having the comparison from the NIST library such as Trichloro- 1,2,3,4,5- pentamethylcyclopenta-2,4 dimethyl germen with peak number 6 retention time 32.875, Dasycarpidan-1-methanol, acetate (ester) with peak number 7 retention time 34.329 and 1-Monolinoleoyl glycerol trimethylsilyl ether with peak number 8 retention time 38.22. Other peaks could not be identified from the library.



Trichloro- (1,2,3,4,5- pentamethylcyclopenta-2,4 dimethyl)- germen



1-Monolinoleoyl glycerol trimethylsilyl ether

CHEMISTRY ANALYTICAL LABORATORY
GCMS ANALYSIS
(GC7890B-MSD-5977A-AGILENT TECH USA)
(CompoundT_{CC})

Chromatogram

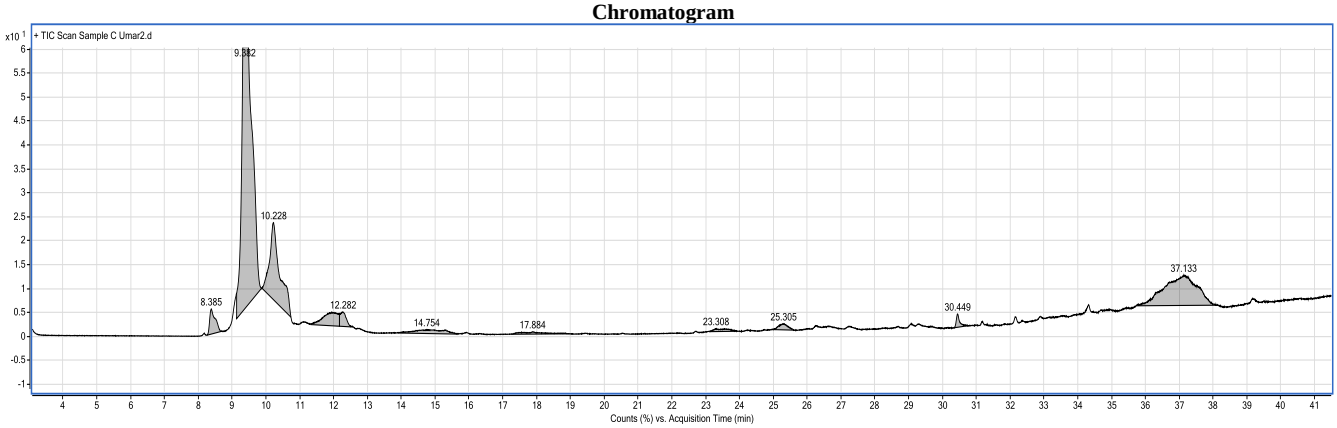
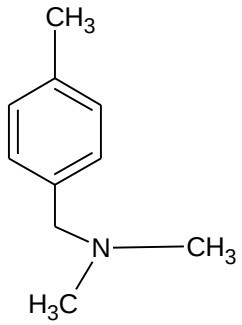


Fig. 4.: Chromatogram of compound T_{CC}

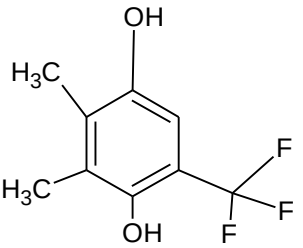
Table 5: GC-MS Analysis of Compound T_{CC}

Peak no.	Retention time	Area	Height	Width	Name
Peak 1	8.385	1239283.31	101256.17	0.411	Hordenine
Peak 7	17.884	450668.55	10124.21	1.688	Androstane-11, 17-dione, 3-(trimethylsilyl) oxyl-17-(O-(phenylmethyl)oxime), (3α, 5α)
Peak 8	23.308	351305.76	12735.59	0.79	1,2- Benzenedicarboxylic acid mono (2-ethyhexyl) ester
Peak 9	25.305	498526.74	26093.62	0.594	3H- Cycloocta(c) pyran-3-one,5,6,7,8,9,10 hexahydro-4-isopropyl-1-phenyl
Peak 10	30.449	406874.89	57795.14	0.386	Phen-1, 4diol, 2,3 dimethyl-5-trifluoromethyl

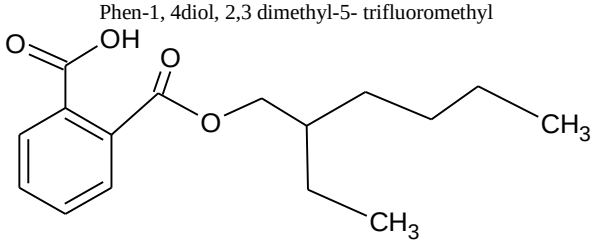
This table 8 contains the compounds in T_{CC}. The chromatogram revealed the presence of eleven compounds, but five compounds were having the comparison from the NIST library such as Hordenine with peak number 1 retention time 8.385, Androstane-11, 17-dione, 3-(trimethylsilyl) oxyl-17-(O-(phenylmethyl)oxime), (3α, 5α) with peak number 7 retention time 17.884 and 1,2- Benzenedicarboxylic acid mono (2-ethyhexyl) ester with peak number 8 retention time 23.308. Other peaks could not be identified from the library.



Hordenine



Phen-1, 4diol, 2,3 dimethyl-5- trifluoromethyl



1,2- Benzenedicarboxylic acid mono (2-ethyhexyl) ester

Discussions

The proximate analysis indicates that the dry matter content in *Terminaliacatappa* leaf is 93.0%, moisture content is 7.0%, ether extract is 1.0%, crude fibre is 21.0%, ash is 10.0%, crude proteinis 10.00%, carbohydrate is 51.00% and therefore

this plant can be used as a good source of energy and nutrients.

The elemental analysis indicates the presence of calcium (19.68µg/ml), cadmium (0.12µg/ml), copper (6.84µg/ml), iron (10.67µg/ml), potassium (18.90µg/ml), magnesium (10.27µg/ml), manganese (1.27µg/ml), sodium (15.30µg/ml) nikkel (1.00µg/ml), zinc (4.17µg/ml), chloride (0.72µg/ml), nitrate (46.00µg/ml), phosphate (70.00µg/ml) and sulphate (227.33µg/ml). However, only phosphate and sulphate exceeded the permissible limit of world health organization (WHO) standard, although they are important nutrient in less concentration.

The results of phytochemical screening of *Terminaliacatappa* leaf extract indicate that the plant contains many secondary metabolites. The methanol crude extract showed the presence of tannins, cardiac glycoside, flavonoid, terpenoidsaponins and alkaloid while glycoside, phlabotannins and were not found in the extract. The *n*-hexane and ethyl acetate partitioned portion showed the presence of terpenoid only, while tannins, cardiac glycoside, flavonoid, saponins, alkaloid, glycoside, phlabotannins and soluble starch were not found. The *n*-butanol partitioned portion was found to contain carbohydrate, tannins, cardiac glycoside flavonoid and alkaloid. However, metabolites such as phlabotannins, terpenoid, saponins, soluble starch and glycosides were not found. Aqueous partitioned portion was also found to contain carbohydrate, tannins, cardiac glycoside, terpenoid, saponins and flavonoid. However, metabolites such as soluble starch, alkaloid, phlabotannins and glycosides were not found. The presence of secondary metabolites in the *Terminaliacatappa* such as tannins, cardiac glycoside, flavonoid, saponnins and Phenolic compound, indicates or implicate the medicinal value of it. These compounds have been reported to have antioxidants property and exhibit a wide range spectrum of medicinal properties such as anti-cancer, anti-inflammatory and anti-diabetes [1516]. The plants that contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, and tannins), nitrogeneous compounds (such as alkaloids and amines), vitamins, terpenoids (including carotenoids) and some other metabolites are found to be reached in antioxidant activity[17].

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