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A STUDY OF THE PHYTOCHEMICAL CONSTITUENTS IN THE LEAF FRACTIONS OF *COMBRETUM MOLLE* (R.Br. Ex.G.Don) BY GC- MS ANALYSIS

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ABSTRACT

The aqueous, n-butanol and the ethyl-acetate fractions of the leaf of *Combretum molle* were analyzed for their phytochemical content using Gas chromatography –mass spectrometry (GC-MS). Ethanol was used as solvent for extraction, after which differential fractionization was carried out using distilled water, ethyl acetate and *n*-butanol. Aqueous, ethyl-acetate and *n*-butanol fractions of the leaf of *C. molle* were screened for secondary chemicals. GC-MS revealed the presence of 17 compounds in the n-butanol leaf fraction, 15 compounds in the ethyl-acetate leaf fraction and 11 compounds in the aqueous leaf fraction.

Key words: *Combretum molle*, Gas chromatography mass spectrometry, Plant fractions

INTRODUCTION

Traditional medicine has remained the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities and local therapy is the only means of medical treatment for such communities (Yinger and Yewhalaw, 2007). According to Alaribe (2008) about 80% of Nigerian homes, maintain some sort of private family traditional medicine practitioner. Existing data and contemporary researchers seem to authenticate the assumption for general health improvement of the masses by traditional healers. Plants have broader uses than just food and genetic reservoirs. Medicinal plants have been used for centuries to treat a wide variety of ailments (Vaidya and Devasagayam, 2007). The presence of secondary metabolites in plants has been associated in most of their therapeutic activities (Ogunleye and Ibitoye, 2003). Herbal medicines are now considered a part of Complementary and Alternative medicine (CAM) and are gaining popularity due to their potent antioxidant activity, minimal side effects and economic viability (Auddy *et al*., 2003). Active principles from natural sources have contributed significantly to the

development of new drugs from medicinal plants (Cox and Balick, 1994).

MATERIALS AND METHODS

Source and preparation of plant materials

The plant materials were collected from neighboring communities near ABU dam, in Samaru, Zaria (latitude 11.07^o N, longitude 7.73^o E and altitude 613meters), Nigeria. These were brought and identified by a Taxonomist with voucher number 900191 at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria. The plant parts were air-dried for two weeks at room temperature (25^{o}C) in the laboratory and then ground to powder.

Extraction procedures

The ground plant parts were extracted at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, following the methods of Sofowora (2006).

Preparation of Ethanol Extraction of *C. molle*

Approximately 800 g of the dried leaves and roots of *C. molle* were extracted with 10 litres of 80% (v/v) ethanol by maceration at (25^{o}C) for 3days. The total The mixture then is strained and filtered. The filtrate was concentrated to dryness on a water bath at 100^o C so as to obtain the dry extract after which was stored at -20^{o}C for further studies.

Differential Fractionation of the Ethanol Extract of *C. molle* in Different Solvents

The dried ethanol extract obtained from both the roots and leaf of *C. molle* (50 g) were each suspended in 1 litre of distilled water and partitioned in sequence with ethyl acetate (1 litre), and *n*-butanol (1 litre). The different solvent fractions were concentrated on a water bath at 100^o C so as to obtain the dry extract after which was stored at -20^{o}C.

Gas chromatography–mass spectrometry (GC-MS)

N-butanol fraction, ethyl acetate, and aqueous fractions from the stem and roots of *C. molle* was subjected to gas chromatography – mass spectrometry analysis, (GCMS-QP2010 PLUS SHIMADZI, JAPAN) Column of 0.25diametre and 30mm length was used at column oven temperature of 60^{o}C and an injection temperature of 250^{o}C and a pressure of 100.2 kPa, a column flow of 1.61mL/min and a total flow of 6.2 ml/ min. Content identified was compared with the database of the NIST library. Compounds with close similarity index where identified and recorded.

RESULTS

The chromatograms of the fractions of *C. molle* were obtained from the gas chromatography – mass spectrometry analysis with the following peaks respectively as can be seen in Figure 1, 2 and 3. Tributyl-acetyl citrate had the highest peak of 99 while 1-hydroxy-2-methylbenzene had

the least mass peak of 32 for the n-butanol leaf fraction. 5-Cholestene had the highest peak of 136 while 1H-Pyrrole had the least mass peak of 21 for the ethyl-acetate leaf fraction. Octadecanoic acid had the highest peak of 146 while cyclopropane had the least mass peak of 49 for the aqueous leaf fraction. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K. It is used along with simple sugar or corn syrup as a hardener in candies.

Table 1 shown the GC MS result of n-butanol fraction of the leaf of *C. molle*. Total of seventeen compounds comprising of various fatty acids (such as Octadecanoic acid, Hexadecenoic acid and Phthalic acid) and hydrocarbons (such as 1-hydroxy-2,3-dimethylbenzene and 1-dodecene). GC MS result of ethyl-acetate fraction of the leaf of *C. molle* was shown in Table 2 shows a total of fifteen compounds comprising of various fatty acids (such as pentadecanoic acid and 11-octadecanoic acid) and hydrocarbons (such as 1H-Pyrrole and Tridecane). GC MS result of aqueous fraction of the leaf of *C. molle* of Table 3 shows a total of eleven compounds comprising of various fatty acids (such as 9,12, octadecanoic acid and 9,12,15, octadecanoic acid) and hydrocarbons (such as cyclopropane and 2,4, Decadienol).

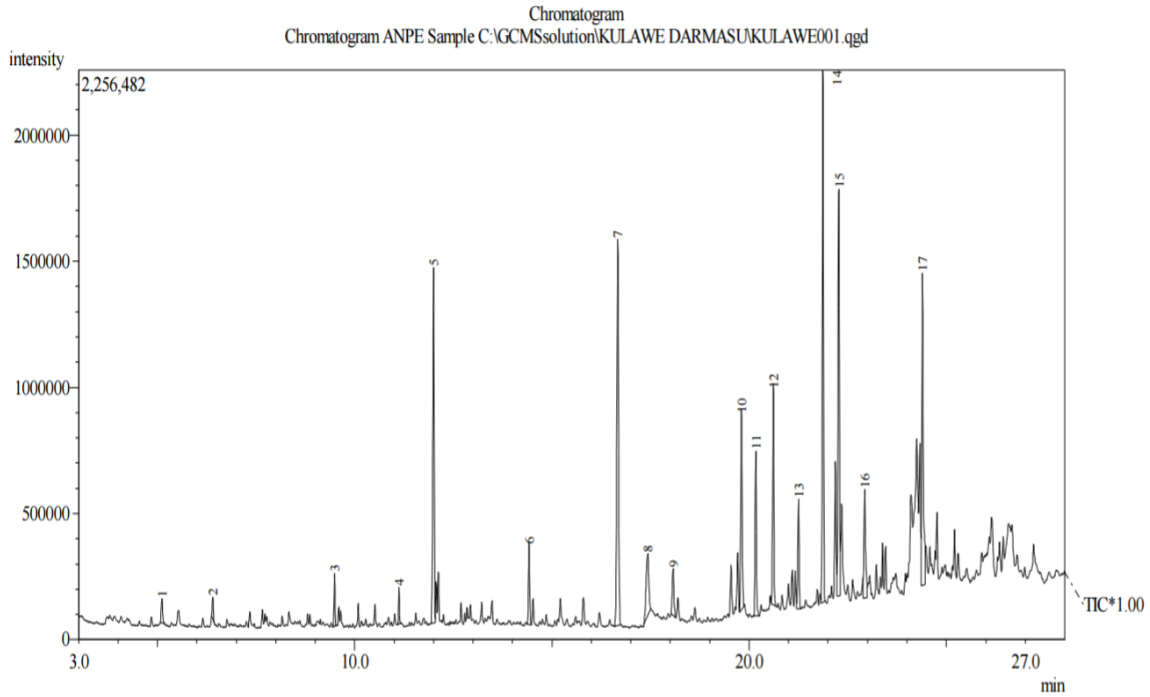

Figure 1: GC-MS Chromatogram of N-butanol leaf fraction of *Combretum molle*

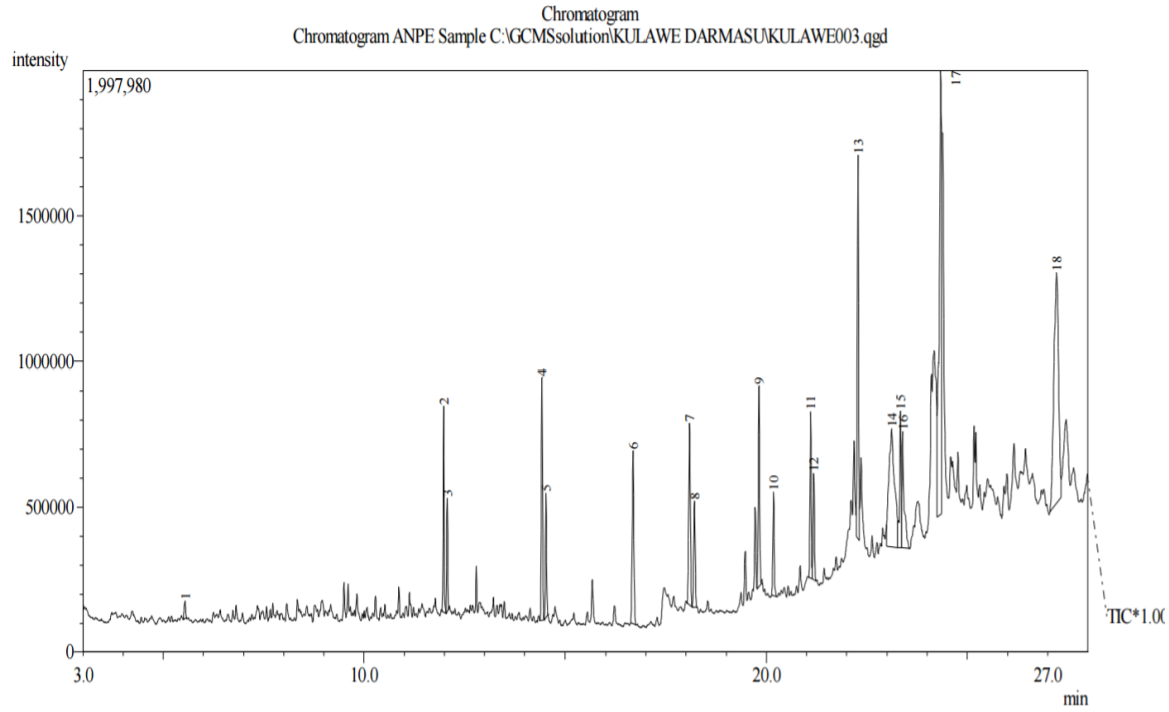

Figure 2: GC-MS Chromatogram of Ethyl-acetate leaf fraction of *Combretum molle*

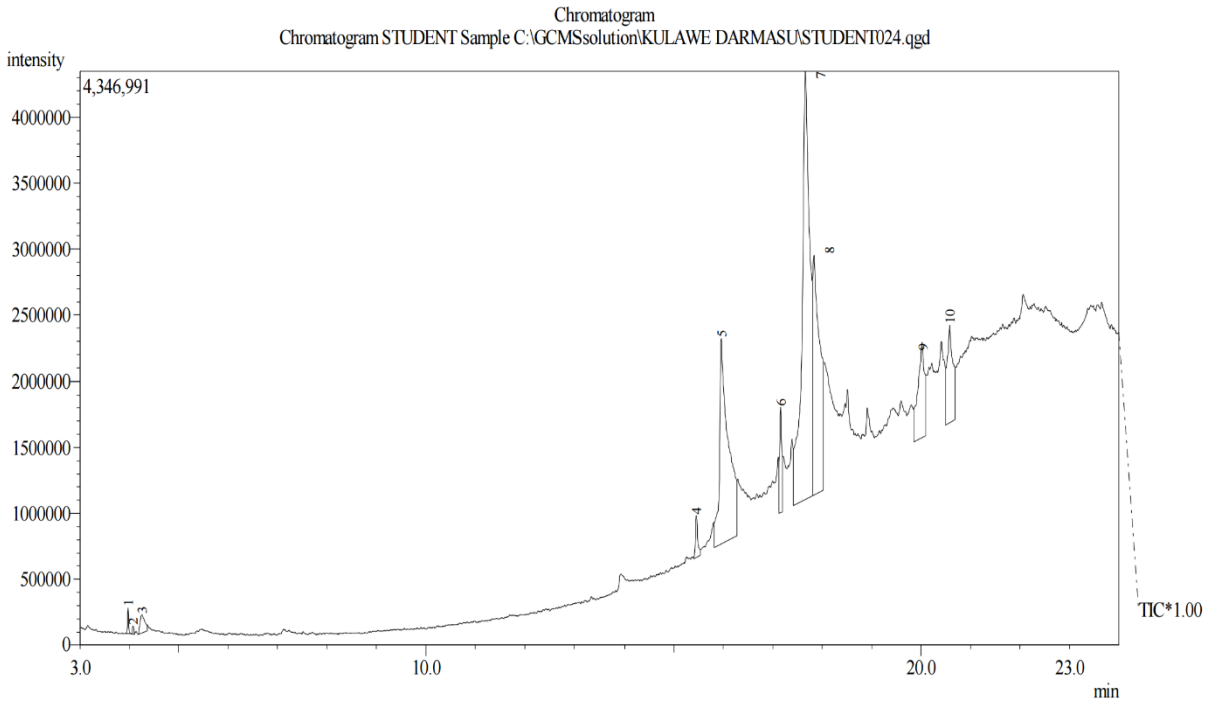

Figure 3: GC-MS Chromatogram of the Aqueous leaf fraction of *Combretum molle*

Table 1: GC MS result of n-butanol fraction of the leaf of *C. molle*.

Line	Compound Name	Formula	Mol. weight	Retention time (Min.)	Similarity index(%)
1.	1-hydroxy-2-methylbenzene	C_7H_8O	108	5.108	90
2.	1-hydroxy-2,3-dimethylbenzene	$C_8H_{10}O$	122	6.400	86
3.	1-dodecene	$C_{12}H_{24}$	168	9.492	95
4.	Tridecanoic acid	$C_{14}H_{28}O_2$	228	11.125	91
5.	Phthalic acid	$C_{14}H_{14}O_4$	246	12.000	76
6.	1-hexadecene	$C_{16}H_{32}$	224	14.425	95
7.	Pentadecanoic acid	$C_{17}H_{34}O_2$	270	16.667	93
8.	1,2benzenedicarboxylic acid	$C_{16}H_{22}O_4$	278	17.433	77
9.	1-pentadecene	$C_{15}H_{30}$	210	18.075	95
10.	11-Octadecanoic acid	$C_{19}H_{36}O_2$	296	19.808	92
11.	Octadecanoic acid	$C_{19}H_{38}O_2$	298	20.167	91
12.	1-propane-1,2,3-tricarboxylic acid	$C_{18}H_{30}O_6$	342	20.617	78
13.	Acetic acid	$C_{20}H_{40}O_2$	312	21.250	93
14.	Tributyl acetylcitrate	$C_{20}H_{34}O_8$	402	21.867	88
15.	Hexadecanoic acid	$C_{37}H_{74}NO_8P$	691	22.275	86
16	4,8,12,16-Tetramethyl heptadecan-4-olide	$C_{21}H_{40}O_2$	324	22.925	87
17.	Hexadecanoic acid	$C_{19}H_{34}O_4$	330	24.392	89

Table 2: GC MS result of ethyl-acetate fraction of the leaf of *C. molle*.

Line	Compound Name	Formula	Mol. weight	Retention time (Min.)	Similarity index(%)
1.	1H-Pyrrole	$C_4H_6N_2O$	98	5.542	74
2.	2-Tridecene	$C_{13}H_{26}$	182	11.983	94
3.	Hexadecene	$C_{16}H_{34}$	226	12.075	96
4.	1-Pentadecene	$C_{15}H_{30}$	210	14.425	95
5.	Pentadecanoic acid	$C_{17}H_{34}O_2$	270	16.675	93
6.	1-Hexadecene	$C_{16}H_{32}$	224	18.083	95
7.	11-Octadecanoic acid	$C_{19}H_{36}O_2$	296	19.817	92
8.	Octadecanoic acid	$C_{19}H_{38}O_2$	298	20.175	91
9.	1-Tetracosanol	$C_{24}H_{50}O$	354	21.100	95
10.	Tridecane	$C_{13}H_{28}$	184	21.175	88
11.	Hexadecanoic acid	$C_{39}H_{34}O_4$	284	22.275	84
12.	1-Iodo-2-methylnonane	$C_{10}H_{21}I$	268	23.117	78
13.	Eicosane	$C_{20}H_{42}$	282	23.392	94
14.	9,12,15-Octadecatrienoic acid	$C_{25}H_{40}O_6$	436	24.342	85
15.	5-cholestene-3-ol	$C_{28}H_{48}O$	400	27.225	80

Table 3: GC MS result of aqueous fraction of the leaf of *C. molle*.

Line	Compound Name	Formula	Mol. weight	Retention time (Min.)	Similarity index(%)
1.	Cyclopropane	C_7H_{12}	198	7.725	88
2.	2,4,Decadienol	$C_{10}H_6O$	158	8.133	87
3.	Benzene methanol	$C_{10}H_{14}O$	150	8.383	81
4.	Hexadecanoic acid	$C_{19}H_{30}O_2$	330	17.922	91
5.	9,12,octadecanoic acid	$C_{19}H_{34}O_4$	294	19.925	95
6.	9,12,15,octadecanoic acid	$C_{19}H_{34}O_2$	292	20.033	91
7.	9-Hexadecanoic acid	$C_{16}H_{32}O_2$	254	20.975	92
8.	Octadecanoic acid	$C_{19}H_{38}O_2$	298	21.150	92
9.	Decane	$C_{11}H_{24}$	156	22.483	81
10.	9,12,octadecadienoylchloride	$C_{18}H_{31}ClO$	284	24.292	90
11.	11-Octadecanoic acid	$C_{19}H_{36}O$	280	26.358	88

DISCUSSION

Muthezilan *et al.* (2012) reported that the main components of 9, 12 octadecadienoic acid has potential antioxidant and anticancer activities. Linolenic acid is known for its potential antibacterial, antifungal, anti-arthritic and anti-inflammatory activities. Homo-γ-linolenic acid has gained importance due to its anti-inflammatory and anti-cancer action, and also it has been used in the treatment of rheumatoid arthritis (Inoue *et al*., 2005). Oyewo, *et al*. (2012) reported that Palmitic acid possess antibacterial and cholesterolaemic effects. significant cytotoxicity against the MCF-7, WRL- 68, CaCo2, Colo-320 DM cancer cell lines and hepatoprotection against galactosamine.

CONCLUSION

It was concluded that fractions of the leaf of *C. molle* possesses various potent bioactive compounds and is recommended as a plant of phyto-pharmaceutical importance. Further subjects are required to explore the potential compounds responsible for the biological activity from *C. molle* for application in drug delivery, nutritional or pharmaceutical studies.

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