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Assessment of Mineral and Essential Oil Composition of Anonna Muricata Leaves

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Abstract: *The qualitative phytochemical, mineral and components of essential oil of the leaves of A. muricata were analysed. The qualitative phytochemical analysis showed the presence of Alkaloids, Flavonoids, Carbohydrate, Tannins, Steroids, Terpenoids, Cardiac glycoside, Saponin, Carboxylic acid, Aldehyde/ketone and Phenols. The mineral analysis using Atomic absorption spectroscopy (AAS) showed the presence of Magnesium, potassium, calcium, sodium, Phosphorus, zinc, copper, manganese, chromium and iron. The GC-MS analysis of the essential oil revealed thirteen different compound; Heptadecane, Methyl tetradecanoate, Octadecane, Nonadecane, Hexadecanoic acid, methyl ester, Eicosane, Methyl 10-trans,12-cis-octadecadienoate, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z), Phytol, Methyl stearate, Octadecane,2,6,10,14-tetra methyl, Tricosane and Phthalic acid, di(oct-3-yl) ester.*

Keywords*: Phytochemicals, Minerals, GC-MS analysis and Essential Oil.*

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I. Introduction

Annona muricata generally called graviola, guanabana (Adefegha et al., 2015) or sour sop (shawashop in Eastern Nigeria) is a green leafy plant belonging to annonaceae family (Florence et al., 2014). It is a fruit tree found mostly in the tropics and literature has it that it is native to Central America (Kossouoh et al., 2007). It is generally cultivated in South America, some parts of Africa, Asia and Australia (Kossouoh et al., 2007). A.muricata is also grown in different parts of Nigeria as a garden plant.Due to the medicinal importance of A.muricata, a lot of research has been focused on its chemical composition. Research has shown that almost every part of A.muricata has rich deposits of plant chemicals which have therapeutic effects. These phytochemicals include; alkaloids, tannin, megastigmanes, flavonol, triglycosides, flavonoids, alkaloids, steroids, triterpenoid (Hardoko et al., 2015), phenolic compounds and cyclopeptides (Moghadamtousi et al., 2015). Florence et al., 2014 noted the presence of tannins, coumarins, stearic acid, myristique acid, and ellagic acid in A. muricata. Adefegha et al., 2015 demonstrated that the total phenol compounds present in the Pericarp, Pulp and Seed of A.muricata are 560.21mg/g, 430.29mg/g and 50.51mg/g respectively while the total flavonoid compounds present in the Pericarp, Pulp and Seed of A.muricata are 275.45mg/g, 100.01mg/g and 85.65mg/g respectively.hytochemicals are plant chemicals which are richly deposited in plants (Onuah et al., 2016). Onuah et al; 2016, noted that plants rich in phytochemical can be used in the treatment of cancer, paracetamol induced toxicity, stroke and other diseases.inerals are chemical substances which exist naturally in the soil, plants and ingested by man as nutrients. Inorganic minerals are found in the soil while organic minerals are gotten from the plants. Minerals form a part of food nutrient and are required by animals for normal functioning of the body. Iron is needed for the transportation of blood; calcium is needed for strong bones and teeth. Potassium play a big role in skeletal and muscle contraction (Farid and Neda, 2014). Chromium is essential for insulin function, hence improves the ability of insulin to metabolise glucose (Staniek and Krejpcio, 2017). Mineral nutrients are divided into macro and trace minerals. Macro minerals are required in large quantities; they include calcium, phosphorus, magnesium, sodium, potassium, chloride and sulphur. Trace minerals include iron, manganese, copper, iodine, zinc, cobalt, fluoride and selenium. Minerals are important in the protection of the body against different diseases. Lack of minerals has been linked to different diseases including goitre, osteoporosis, anaemia and many others.Essential oils or volatile oils are oils present in different parts of a plant. They comprise of complex volatile compounds, synthesized naturally during secondary metabolism (Swamy et al., 2016). It can be extracted from the leaves, stem, roots, seeds, flowers and other parts of a plant. Essential oil comprises of different kinds of volatile aromatic compounds from plants which are useful in pharmaceutical, medical and cosmetic industries.

Most essential oils are composed of phytochemicals known as terpenes and terpenoids which have been reported severally as antioxidants. They also contain low molecular weight aromatic and aliphatic

compounds (Swamy et al., 2016). They form the major components used in the branch of medicine known as aromatherapy. They relief pain, serve as anti-depressant and help to reduce stress (Kuriyama et al., 2005).
Essential oils possess antibacterial, antifungal, anti-viral activity and cytotoxic effect (Swamy et al., 2016), (Mith et al., 2014), (Tilaoui et al., 2015). Antibacterial activity of essential oil has been widely studied and reported. According to Thompson et al., 2013 essential oil from peppermint, lemon balm and coriander seed exhibited antibacterial activity against Escherichia coli gram positive bacteria responsible for irritable bowel syndrome. Essential oil of Callistemon citrinus (Curtis) leaves has wide range of antibacterial action against Aeromonas hydrophila, Escherichia coli, Salmonella typhi, Listeriamonocytogenes, Vibro alginolyticus, Staphylococcal enteritis and Staphylococcus aureus (Larayetan et al., 2017). Eucalyptus globulus (eucalyptus) oil and Anthemis nobilis has proven to be effective antimicrobial and anti-inflammatory oils. Mith et al., 2014 demonstrated antibacterial activity of cinnamaldehyde, carvacrol, thymol, and eugenol against major bacterial that causes food borne diseases including L. monocytogenes, S. typhimurium, and Escherichia coli which are essential oil of cinnamon, oregano, thyme, and clove respectively.

Antifungal activity of essential oil from different plants has been reported; Swamy et al., 2016 noted that essential oil from Coriandrum sativum, Ocimum sp, M. communis thyme and clovecan inhibit the growth of different kinds of fungi including Candida species, Penicillium notatum, Rhizopus stolonifer, Trichophyto rubrum and Cryptococcus neoformans.wamy et al., 2016 also noted that 2,5,5-Trimethyl-3,6-heptadien-2-ol, eucalyptol, eugenol, thymol, estragole, E-caryophyllene and β-caryophyllene possess anti-viral activity against herpes simplex virus, Avian influenza and Japanese encephalitis virus.

The potential use of essential oil as anticancer regimen has been studied and documented; anti-tumour toxicity test of different parts of Artemisia herba alba oils has shown cytotoxic effect against P815 and BSR cell lines with essential oil from the leaves showing more cytotoxic effect. This the Author attributed to the greater concentration of sesquiterpenes in the plant (Tilaoui et al., 2015). Essential Oil of Pallenis spinosa has cytotoxic activity against leukemic (HL-60, K562 and Jurkat) and solid tumor cells (MCF-7, HepG2, HT-1080 and Caco-2). The Author also noted that the major component of the essential oil of this plant is sequiterpene (Al-Qudah and Saleh, 2017).Essential oil from different parts of plants has been incorporated in many beauty products due to their antiseptic properties; thus they are useful in the treatment of skin infection (Orchard and Vuuren, 2017). Essential oil of Abies balsamea, Acacia dealbata, Achillea millefolium, Allium sativum, Apium graveolens, Citrus limon and many others are used in the treatment of skin infection, including ;Abscesses, acne, athlete's foot, blisters, boils, cuts, insect bites, sores, pimples, and ulcers (Orchard and Vuuren, 2017).
Antioxidant activity of essential oil has been studied; Citrus aurantium (neroli) and Callistemon citrinus has strong antioxidant activity. The free radical scavenging and antioxidant potential of Callistemon citrinus activity has been attributed to its high Eucalyptol content (Larayetan et al., 2017). Essential oil of Pallenis spinosa exhibits antioxidant activity, essential oil from the arial part of dried Pallenis spinosa has stronger DPPH and ABTS scavenging activity than the oil from fresh ariel parts (Al-Qudah and Saleh, 2017). The free radical scavenging activity of the plant is due to its high content of oxygenated terpene (Al-Qudah and Saleh, 2017).
Terpine-4-ol which is the major constituent of M. alternifolia can hinder some cytokines including tumour necrosis factor (TNF), interleukin-1, interleukin-8, and interleukin-10, and prostaglandin E2 (Orchard and Vuuren, 2017). Kuriyama et al., 2005 reported that massaging with essential oils reduces anxiety and also increases $CD8^+$ and $CD16^+$ lymphocyte.

II. Materials And Methods

2.1 Collection and identification of plant material:

The leaves of A. muricata Linn, were collected from Abuja park of University of Port Harcourt, Choba Rivers State Nigeria. The plant was identified in the Herbarium of Department of Plant Science and Technology, University of Port Harcourt. The Plant sample was washed and air dried under shade. The dried sample was homogenized to fine powder and stored in sterile air tight bottles for the experimental work.

2.2 Qualitative determination of the phytochemical contents

Qualitative phytochemical content of the leaves of A. muricata was determined using a standard method according to AOAC (AOAC, 1980)

2.3Determination of mineral contents of the plant

The mineral content of the plant sample was determined using atomic absorption spectrophotometer (AAS) (AVANTA GBC Ver. 2.02) GBC Scientific equipment USA. (AOAC, 1980)

Procedure:

Wet Digestion Method

A total volume of 100ml of H_2SO_4, HNO_3, and HCL in the ratio of 40%:40%:20% were mixed together. About 1g of the sample was weighed into a conical flask and 2ml of the mixed solution was added.

The sample was digested in a fume cupboard with hot plate until white fumes appeared. It was cooled and filtered into a 100ml volumetric flask and made up to mark with distilled water.
The dilute filtrate solution was used for analysis of elements of interest (magnesium, potassium, calcium, sodium, phosphorus, zinc, copper, manganese, chromium and iron)using suitable hollow cathode lamps. Air acetylene flame was used for determination of mineral content of the plant. The inert argon gas flow and the temperature parameters were set as recommended by manufacturer. The flame was ignited and allowed to stabilize for few minutes. The blank was aspirated to zero the instrument. The standard solution was aspirated and the aspiration rate of the nebulizer adjusted to obtain maximum sensitivity. The burner was adjusted both vertically and horizontally to obtain maximum response. The blank was aspirated again to re-zero the machine. A standard with a concentration near the middle of the linear range was aspirated and the absorbance recorded.

Identification of essential oil components of the plants using Gas Chromatography Mass Spectroscopy (GC-MS)

The essential oil component of the plant was analysed using a combined gas chromatograph Model 7890A (GC) and Mass Selective Detector model: 5975C (MSD) by Agilent Technologies. The electron ionization was at a 70v with an ion source temperature at 250 ^{o}C. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5 (30mm X0.25mm X 0.320µm) was the stationary phase. The oven temperature was at 60^{o}C held for 0.5 minute and ramped to 140 ^{o} C at the rate of 4 ^{o} C/minute holding for a minute, then ramped to 280 degrees while holding for 5 minutes at the rate of 8^{o}C /minutes. The milled sample was extracted in dichloromethane after soaking for 5days. 10g of the sample was weighed into a well stopper bottle and 20mls of the organic solvent was added. The mixtures were vigorously agitated and were left to stand for 5days. The crude extract was collected by filtering into a quartz beaker; the process was repeatedly carried out for two more consecutive times. The combined aliquot collected was concentrated on a steam berth to about 5ml. This was purified by passing through a pasture pipette packed with silica gel and anhydrous sodium sulphate on a membrane and air dried to about 2ml for gas chromatographic analysis.

III. Result And Discussions

The preliminary qualitative screening of A. muricata leaves revealed the presence of alkaloid, flavonoid, carbohydrate, tannins, steroids, Cardiac glycoside, Saponin, Terpenoids, Carboxylic acid, Aldehyde/ketone,Phenol as shown in Table

Table 1. Phytochemical content of the leaves of A. muricata

Phytochemicals	Status
Alkaloids	2+
Flavonoids	1+
Carbohydrate	3+
Tannins	1+
Steroids	1+
Terpenoids	2+
Cardiac glycoside	1+
Saponin	1+
Carboxylic acid	2+
Aldehyde/ketone	1+
Phenols	2+

No of + indicates the level of occurrence

Table 2: Result of Macro mineral content of the leaves of A. muricata

Minerals	Quantity (ppm)
Magnesium (Mg)	18.43018
Poatassuim (K)	51.60357
Calcium (Ca)	73.49241
Sodium (Na)	30.52650
Phosphorous (P)	12.12716

Table 3: Result of the trace mineral content of the leaves of A. muricata

Minerals	Quantity (ppm)
Zinc (Zn)	2.69700
Copper (Cu)	7.59013
Manganese (Mn)	0.46115
Chromium (Cr)	0.29738
Iron (Fe)	26.43842

Table 3: Result of GC-MS Analysis of the constituent oil from Annona muricata

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula (g/mol)	Molecular weight
1	Heptadecane	13.134	1.053	$C_{17}H_{36}$	240.4677
2	Methyl tetradecanoate	13.509	1.583	$C_{15}H_{30}O_2$	242.3975
3	Octadecane	14.523	2.214	$C_{18}H_{38}$	254.4943
4	Nonadecane	15.849	3.447	$C_{19}H_{40}$	268.5209
5	Hexadecanoic acid, methyl ester	16.197	26.309	$C_{17}H_{34}O_2$	270.4507
6	Eicosane	17.116	3.060	$C_{20}H_{42}$	282.5475
7	Methyl 10-trans,12-cis-octadecadienoate	18.271	7.583	$C_{19}H_{34}O_2$	294.4720
8	9,12,15-Octadecat rienoic acid, methyl ester, (Z,Z,Z)-	18.346	27.134	$C_{19}H_{32}O_2$	292.4562
9	Phytol	18.490	14.304	$C_{20}H_{40}O$	296.5310
10	Methyl stearate	18.664	6.722	$C_{19}H_{38}O_2$	298.504
11	Octadecane,2,6,10,14-tetramethyl-	19.489	2.445	$C_{22}H_{46}$	310.6006
12	Tricosane	20.603	1.809	$C_{23}H_{48}$	324.627
13	Phthalic acid, di(oct-3-yl) ester	23.378	2.338	$C_{24}H_{38}O_4$	390.5561

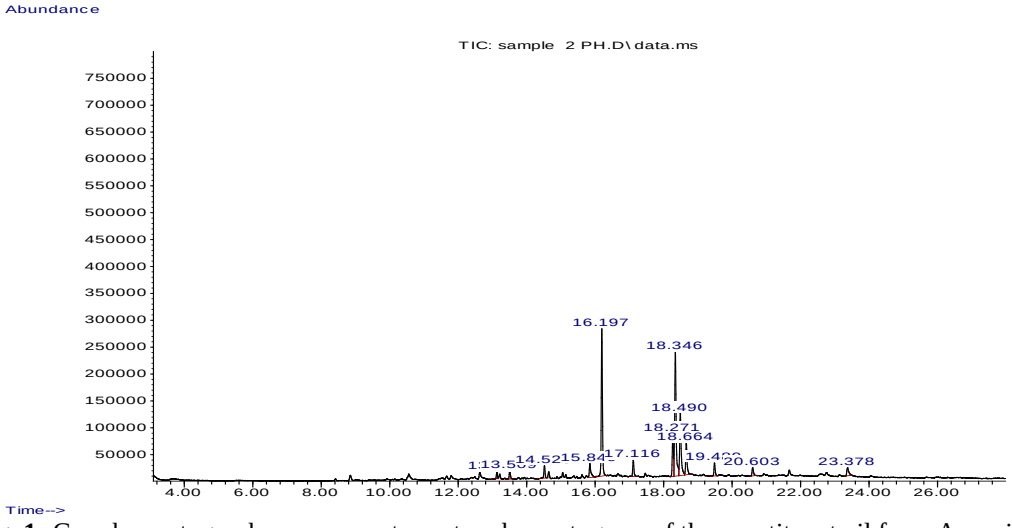
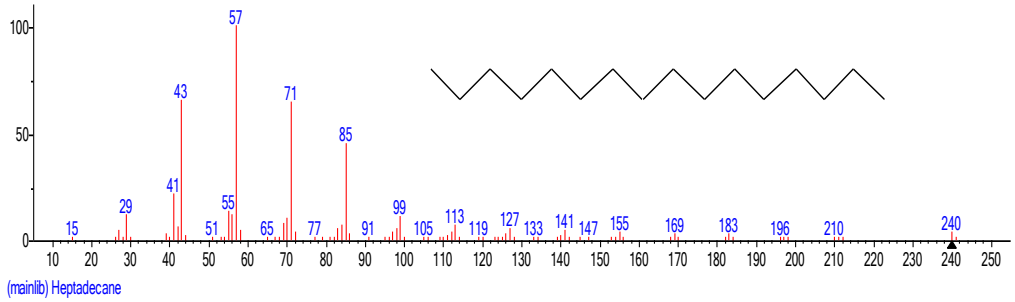
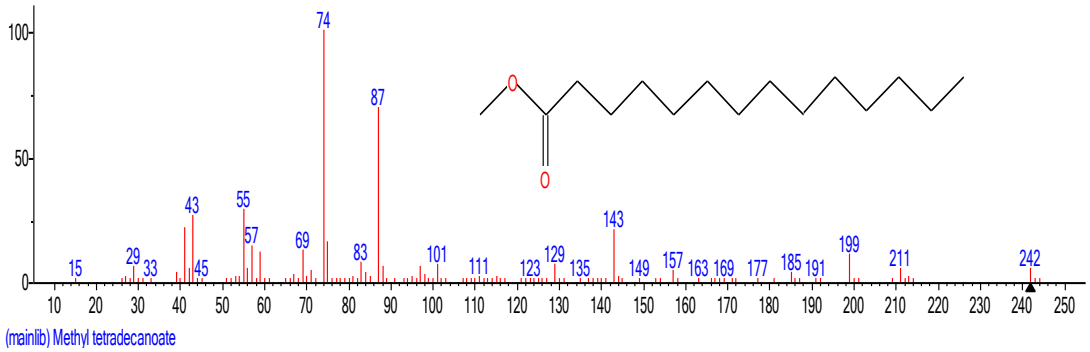


Fig. 1: Gas chromatography-mass spectrometry chromatogram of the constituent oil from A.muricata

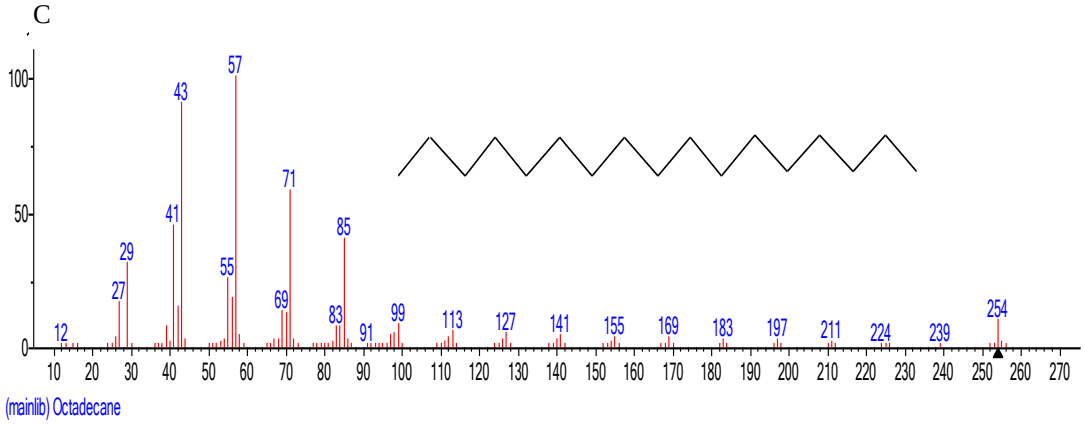
A


(mainlib) Heptadecane

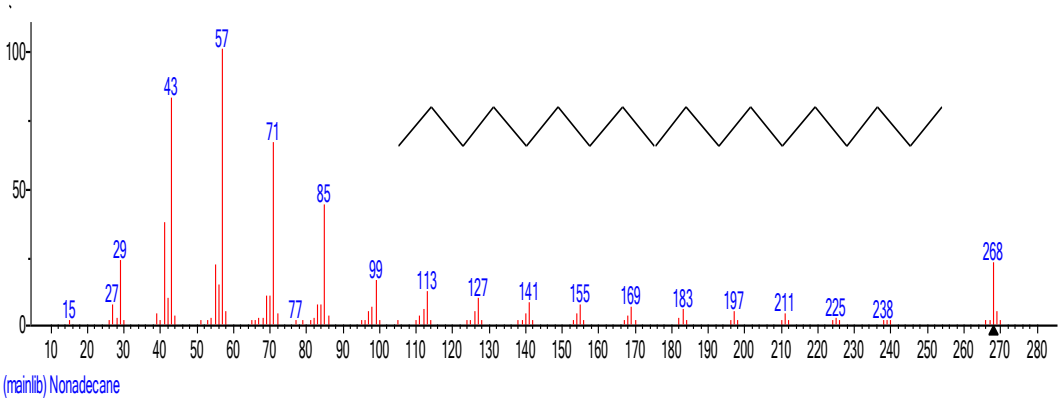
B


(mainlib) Methyl tetradecanoate

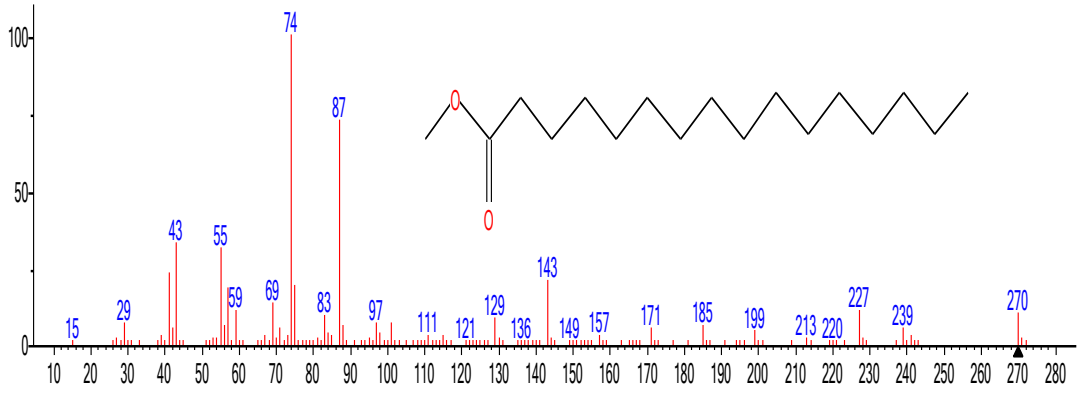
C


(mainlib) Octadecane

D

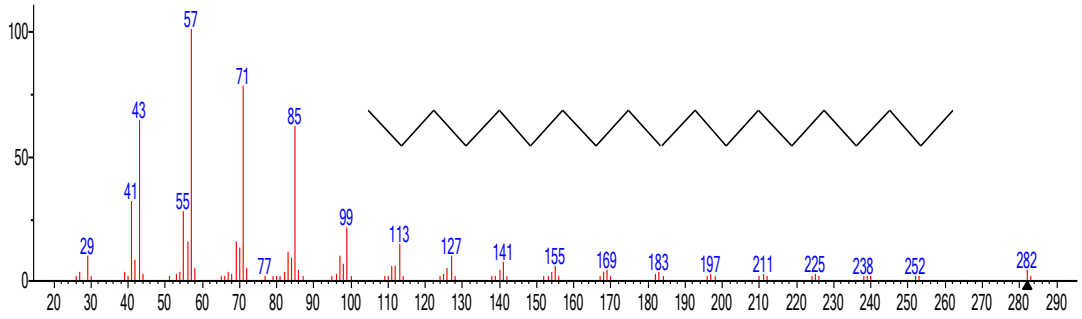

(mainlib) Nonadecane

E



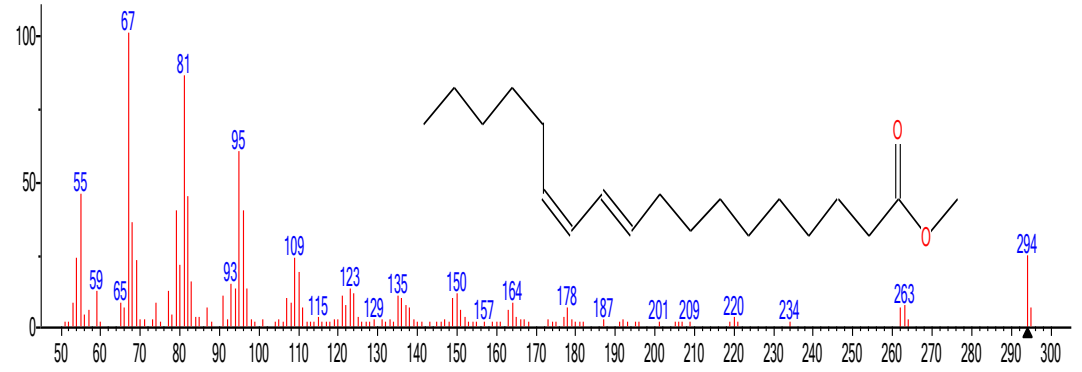
(mainlib) Hexadecanoic acid, methyl ester

F



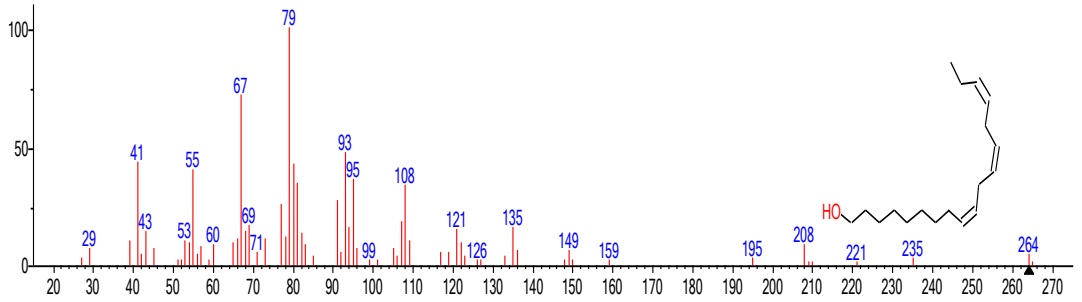
(mainlib) Eicosane

G



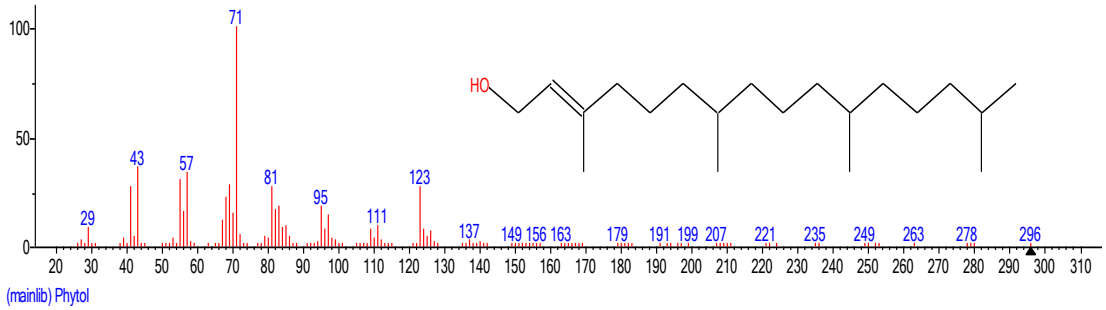
(mainlib) Methyl 10-trans,12-cis-octadecadienoate

H



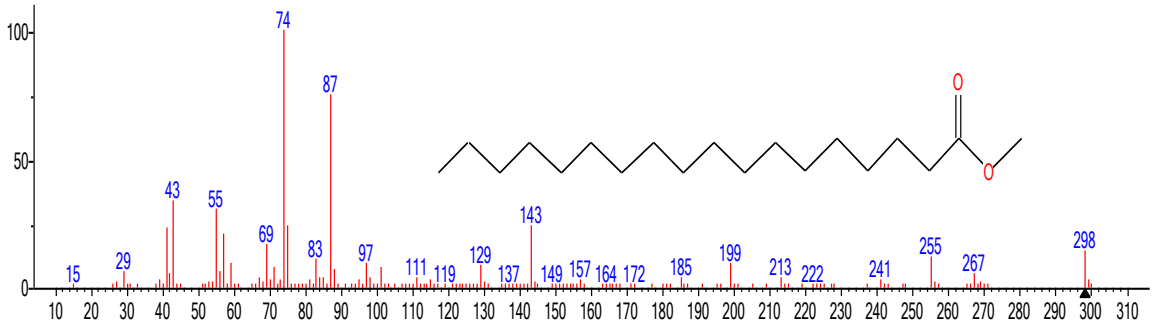
(mainlib) 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-

I



(mainlib) Phytol

J

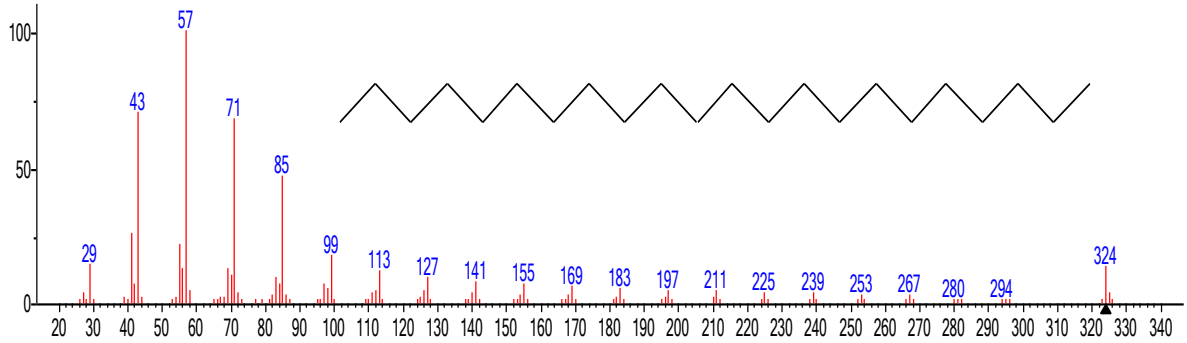


(mainlib) Methyl stearate

K

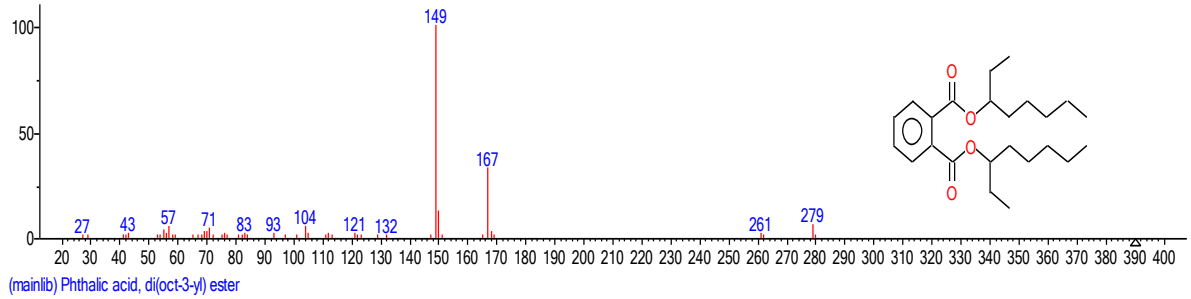
(mainlib) Octadecane, 2,6,10,14-tetramethyl-

L



(mainlib) Tricosane

M



IV. Discussion

In this study the mineral analysis of the plant showed the presence of both macro and trace minerals, Magnesium, potassium, calcium, sodium, Phosphorus, zinc, copper, manganese, chromium and iron respectively. The result showed that the leaves of A. muricata has a high concentration of Calcium, potassium, iron and magnesium with values of 7.39, 51.60, 30.53, 26.44 and 18.43 ppm respectively. Minerals form part of food nutrients and are required by animals for normal functioning of the body. Iron is needed for the transportation of blood; calcium is needed for strong bones and teeth. Potassuim play a big role in skeleton and muscle contraction (Farid and Neda, 2014).The qualitative phytochemical screening of the plant revealed the presence of alkaloids, flavonoids, carbohydrate, tannins, steroids, terpenoids, cardiac glycosides, saponin, carboxylic acid, aldehyde/ketone and phenols. Phytochemicals act as antioxidants, meaning that they have the potentials to reduce oxidative stress (Onuah et al., 2016). Many of the plant products are rich in phytochemicals which are effective in the treatment of different kinds of diseases including diabetes mellitus, cardiovascular diseases, liver toxicity, malaria and many others (Onuah et al., 2016). Flavonoids which are mostly present in the leaf extract of the plant have proven effective in the prevention and treatment of different kinds of diseases (Onuah et al., 2016).In the present study, the spectrum profile of GC-MS confirmed the presence of thirteen different compounds in the leaves of the plant. The compounds identified include Heptadecane, Methyl tetradecanoate, Octadecane, Nonadecane, Hexadecanoic acid, methyl ester, Eicosane, Methyl 10-trans,12-cis-octadecadienoate, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z), Phytol, Methyl stearate, Octadecane, 2,6,10,14-tetra methyl, Tricosane and Phthalic acid, di(oct-3-yl) ester as shown in table 3. The compounds that has the highest concentration include, Hexadecanoic acid, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) and Phytol with a concentration of 26.309%, 27.134% and 14.304% respectively. The individual fragmentation of the components is illustrated in Figures 2A-2M. 9,12,15-Octadecatrienoic acid, a methyl esther of linoleic has various biological activity including anti-inflammatory, Hypocholesterolemic, Cancer preventive, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antieczemic and anticoronary. It also serve as a nematocide and insecticide (Devi et al., 2014) .Phytol a diterpene has antimicrobial, anti-inflammatory and anticancer activity (Devi et al., 2014). The cytotoxicity of phytol against HT-29 human colon cancer cells, MG-63 osteosarcoma cells and AZ-521 gastric cancer cells has been reported (Keawsa-ard et al., 2012). Hexadecanoic acid has been reported to possess anti-inflammatory, antioxidant, hypocholesterolemic, anti-androgenic, flavor, hemolytic, 5-alpha reductase inhibitor as well as potent mosquito larvicide, nematicide, pesticide and anticancer activity (Abubakar and Majinda, 2015), (Keawsa-ard et al., 2012). Shibula and Velavan, 2015 reported the presence of Phytol, Hexadecanoic acid and 9,12,15-Octadecatrienoic acid in the methanolic extract of the leaves of A.muricata.

V. Conclusion

The present study has shown that the leaves of A.muricata are rich in phytochemicals which are known for their different biological activity. They also contain substantial amount of both macro and trace minerals which are required for the normal functioning of the body.Thirteen essential oils were extracted and have been implicated in the treatment of different kinds of diseases and infections, a branch of medicine known as aromatherapy. Studies have shown that essential oils have anti-cancer, antimalarial, anti-inflammatory and antioxidant activity. The present study justifies the traditional use of this plant in treatment of different kinds of diseases.

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