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Phytochemical, Antimicrobial and Gc-Ms of African Nutmeg *(Monodora Myristica*).

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ABSTRACT: *The quest to continue searching for new antibiotic, anti-parasites necitate this project as African nutmeg is a very useful plants medically and all its parts are useful. The major aim of this research work was to examine the monodora myristica for its secondary metabolites, antimicrobial analysis and to characterize the oil using gas chromatography mass spectrophotometer. The antimicrobial screening was done using agar method and the isolates used were obtained from the microbiology laboratory, Federal University of Technology, Akure, saponin, alkaloid, tannin and flavonoids were determined using standard methods. The results of the characterization of oil showed that organic compounds and fatty acids were present, the fatty acids present included, Palmitic acid, Eicosanoic acid, stearic acid, oleic acid, the results showed that the plants contained essential fatty acids useful for both adult and infant. The result of antimicrobial screening showed that the oil was very sensitive against the tested isolates. After 24hours incubation, the zone of inhibitions against Escherichia coli was (11millimetre), Bacillus substilis (8millimetre) and Staphylococcus aureus (11millimeter). The result compares favorably with standard streptomycin, the result of zone of inhibition against Escherichia coli of the sample (11millimetre) was better than that recorded for standard streptomycin (7millimetre).These may be as a result of presence various secondary metabolites which were discovered in the sample during analyses. These are flavonoids, tannin, saponin and alkaloids.*

Keywords: *Monodora myristica, antimicrobial, phytochemical, oleic acid, stearic acid, palmitic acid, eicosanoic acid*

I. INTRODUCTION

African nutmeg *(Monodora myristica)*, belongs to the Ananacea family, it is one of the most important trees of the evergreen forest of West Africa and its mostly prevalent in the Southern part of Nigeria (Adegoke and Akinsanya, 1970). Almost every part of the tree has economic importance (Okafor, 1987; Okigbo, 1977). However, the most economically essential parts are the seeds which are embedded in the white sweet-smelling pulp of the sub-spherical fruit. After harvesting, between April to September every year (Ejiofor *et al*., 1998), a series of unit operations (fermentation, washing, drying and cracking) are carried out. The kernel is obtained by cracking the nuts, which is easier done by heating. The kernel, when ground to powder, is a popular condiment used to prepare pepper soup as a stimulant to relieve constipation and to control passive uterine hemorrhage in women immediately after child birth (Okafor, 1987; Iwu *et al*., 1987; Udeala *et al*., 1980). It also has diuretic properties and is used for mild fever (Iwu *et al*., 1987; Udeala *et al*., 1980). Disease continues to be a great barrier to progress in the advancement of humankind. Problems of ecology, culture and habit peculiarities contribute to making people more susceptible to diseases. Medicinal plants have been used in many forms over the years to cure, manage or control man's ailments. Any effort to further maximize the output of medicinal or natural products from the botanical floral and so to improve health-care delivery certainly deserves great attention. Plants have been used since antiquity for medicinal purposes by diverse peoples and cultures throughout the world. Indeed, the recorded use of natural products as a source of relief from illness dates back at least four thousand years, and it can be assumed that unrecorded practices are as old as mankind (Christophersen *et al*., 1991). The use of plants for medicinal purposes continues to this day, usually in the form of traditional medicine, which is now recognized by the World Health Organization (WHO) as a building block for primary health care (Akerele, 1988 ; WHO, 2005). The vibrant healing power of herbs had been recognized since creation and hence botanical medicine is one of the oldest practiced professions by mankind (Van Wyk, and Gericke, 2000; Iwu, 1993). It has been estimated that 25% of prescribed medicines today are substances derived from plants (Hamburger and Hostettmann, 1991). These include about 119 plants derived chemical compounds of known structures which are currently used as drugs or as biodynamic agents that affects human health. Less than a dozen of these compounds are produced by chemical synthesis or semi-synthesis, the rest being extracted and purified directly from plants (Farnsworth, 1990). Well-known examples of drugs with plant origins includes

aspirin, atropine, digoxin, ephedrine, morphine, quinine, reserpine, vincristine and vinblastine, as well as several plant steroidal2sapogenins which serve as semi-synthetic precursors to the steroidal drugs. The study of plants of medicinal importance in the first years of the nineteenth century led to the isolation in crystalline form of such complex substances as Strychnine ($C_{21}H_{22}O_2N_2$),Quinine ($C_20H_{24}O_2N_2$) and Morphine ($C_{17}H_{19}O_3N$) which have physiological actions in man and animals (Farnsworth and Buigel, 1977).Herbal medicine has for too long been neglected in favor of synthetic drugs of which its misuse or abuse, and cases of side effects have become a social evil (Farnsworth, 1990). It has also been reported that 'nearly' half the prescriptions written annually in the United States of America contain a drug of natural origin, either as the sole ingredient or as one of the two main ingredients (WHO, 2002). The structure of many synthetic drugs resulted directly from an observation of some biologically active plants materials (Farnsworth, 1990). The plant Kingdom has long served as a prolific source of useful drugs, foods, additives, flavoring agents, lubricants, colouring agents and gums from time immemorial (Keay et al, 1964). The World Health Organization (WHO) has reported that about 80% of the world's population depends mainly on traditional medicine and the traditional treatment involves mainly the use of plant extracts (WHO, 2005).

However, the objective of this study was to assess the powdered sample and the oil of the African nutmeg for its antimicrobial strength, the biologically active compounds and fatty acid compositions present.

II. MATERIALS AND METHODS

Fresh African nutmeg fruits were collected from Aponmi in Akure South Local government area, Ondo state, Nigeria, on the 30th August, 2011. The seeds were peeled, sun dried for eight days and then grinded into powder form using blender.

Oil Extraction And GC Analysis

The oil of the sample was extracted using soxhlet extractor and petroleum ether as solvent. The composition of the fatty acids was determined using gas chromatography of the purified methylated sample(methylation was done using BF3 methanol), 1µl was injected into a HP 5890 series II gas chromatograph fitted with a FID and an HP-FFAP capillary column. The helium carrier gas flow was 8ml/min. The detector temperature was 280^{o}C. The injector split 1:50 at 220^o**C**. A temperature programmer was used with an initial temperature of 160^o**C** held for 5min, raised from 200to 220^{o}C at a rate of 2^{o}C/ min and 220^{o}C held for 30min. The fatty acid methyl esters were identified by comparison with the retention times of the standards.

Antimicrobial Analysis

Agar plug method was adopted, the micro-organisms used for the antibacterial screening were, *Staphylococcus aureus*, *Substilis aureus* and *Escherichia coli*. Two (2ml) of the test organisms was aseptically injected into the sterilized plate, 20ml of the sterilized nutrient agar were poured on top of the test organisms aseptically after it has been cooled to 45^{o}C. Sterilized cork borer of 5mm diameter was used to make 4 wells on the solidified agar into which 0.5ml of the isolates were aseptically introduced into the well with the use of sterilized clinical syringe. The plates were incubated at 37^{o}C for 24 hours. Zone of inhibition were observed around each well after 24hours and were recorded. The results were quoted as the radii (millimetre) of the zone of inhibition around the well.

PHYTOCHEMICAL ANALYSIS

(a)Tannin determination

Finely grounded sample was weighed (0.2g) into a 50ml sample bottle. Ten of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2hours at 30^{0}C .The solution was then centrifuge and the supernatant stored in ice, 0.2ml of the solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannin acid solution was prepared from a 0.5mg/ml of the stock and the solution made up to 1ml with distilled water, 0.5ml of Folinciocateau reagent was added to the sample and standard followed by 2.5ml of 20% Na_2CO_3 the solution was then vortexed and allow to incubate for 40minutes at room temperature, its absorbance was read at 725nm against a reagent blank concentration of the same solution from a standard tannic acid curve prepared (Markkar and Goodchild,. 1996).

(b) Saponin determination

The spectrophotometric method of Brunner (1984) was used. Two gram of the finely grinded sample was weighed into a 250ml beaker and 100ml of Isobutyl alcohol was added. Shaker was used to shake the mixture for 5hours to ensure uniform mixing. The mixture was filtered using No 1 Whatman filter paper into 100ml beaker containing 20ml of 40% saturated solution of magnesium carbonate. The mixture obtained again was filtered using No 1 Whatman filter paper to obtain a clean colourless solution. One (1ml) was added into 50ml volumetric flask using pipette, 2ml of 5% iron (iii) chloride ($FeCl_3$) solution was added and made up to the

mark with distill water. It was allowed to stand for 30min for the color to develop. The absorbance was read against the blank at 380nm.

$$saponin = \frac{Absorbance\ of\ sample \times conc.of\ s\tan dard}{Absorbance\ of\ s\tan dard} \quad \text{……..equation 1}$$

(c) Alkaloid determination

Five gram of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4minutes, this was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid which was dried and weighed (Harbone 1973).

$$\%\,Alkaloid = \frac{W_3 - W_2}{W_1} \times 100\,\% \quad \text{…… equation 2}$$

Where:
W_1 =initial weight of sample
W_2 =weight of the extract
W_3 = final weight of the residue

Total Flavonoid Determination

The flavonoid content of the extract was determined using a colorimetric assay developed by (Bao. J.Y et al 2005), 0.2ml of the extract was added to 0.30ml of 5% NaNo3 at zero time. After five minutes, 0.6ml of 10% AlCl3 was added and after six minutes, two ml of 1M NaOH was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm against the reagent blank**.**

III. RESULTS AND DISCUSSIONS

Antimicrobial Results

From the results of figure one, the antimicrobial screening of the sample, it was found that the oil of *Monodora myristica was* very active against the tested organisms**.** After 24hrs incubation, the zone of inhibition against *Escherichia coli* was 11millimetre, *Bacillus substlus*, it was 8millimetre, and with *Staphylococcus aureus*, the zone of inhibition was 11millimetre. The result compared favorably with standard *streptomycin*, the result of zone of inhibition against *Escherichia coli* of the sample (11millimetre) was better than that recorded for standard *streptomycin* (7millimetre).The zone of inhibition of oil sample of wild yam (Discorea villosa) when tested against isolates, *Eschericia coli, staphylococcus aureus* and *Bacillus substilis* were 6millimetre, 8millimetre and 8millimetre (Adewole, 2010),also when compared with that of Breadfruit, the zone of inhibition were: Eschericia coli (12.5millimetre), staphylococcus aureus (21millimetre) and Bacillus substilis (10millimetre).These strong antimicrobial properties of African nutmeg when compared with other plants, showed that it possesses more beneficial medicinal effects and these is as a results of secondary metabolites present.

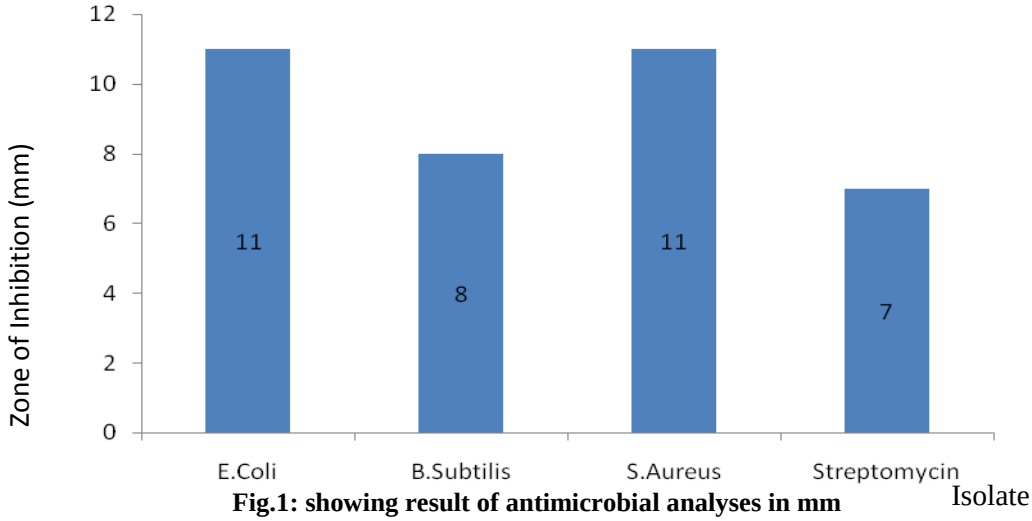


Fig.1: showing result of antimicrobial analyses in mm

Phytochemical Results

The results of figure two, phytochemical analysis showed alkaloid having (12.62%), saponin (2.273mg/g ±0.01), tannin (0.002mg/g ±0.02) and flavonoid (0.541mg/g±0.01). The phytochemical test of *Geranium incanum showed that* alkaloid was absent, but tannin and flavonoids were present in different concentrations (Buckingham, 1996; Bruneton 1999; Amabeoku, 2009).The presence of these photochemical compounds in African nutmeg is an indication that novel compounds may be discovered if various chromatographic techniques were employed to separate the bioactive compounds and then characterized using various spectroscopic techniques like 13C and 1H- NMR, FT/IR and mass spectroscopy, phytochemical compounds like flavonoids, alkaloids, tannin and saponin have been found to contain many different compounds when the sample have been isolated and purified and they are found useful in the formulation of drugs and anti-parasitic agents etc.

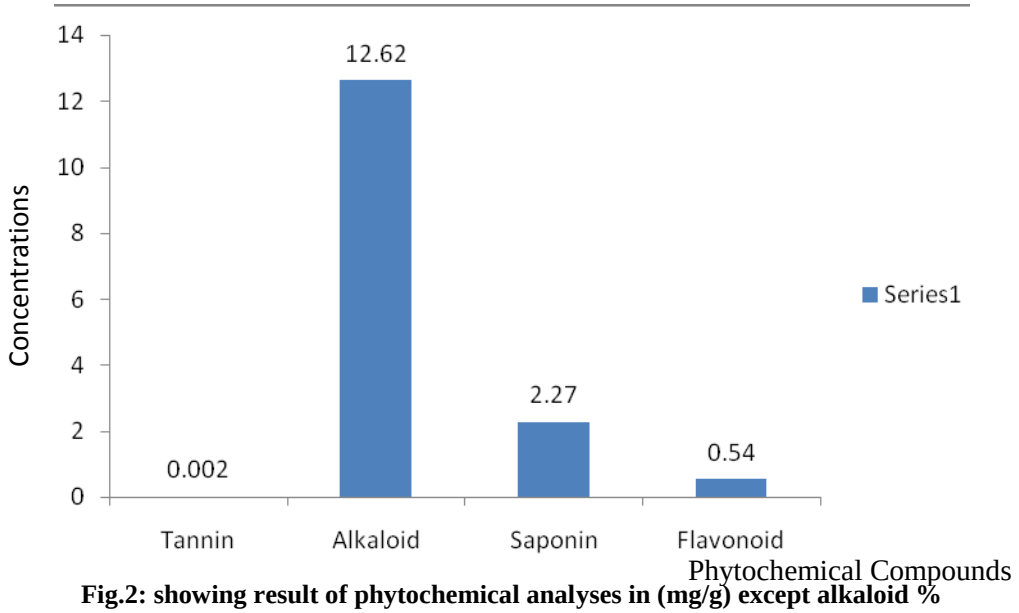


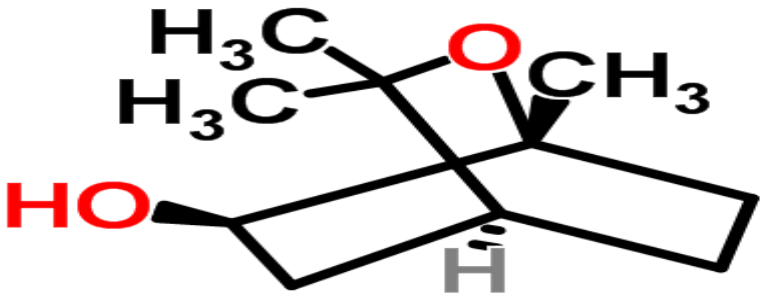
Fig.2: showing result of phytochemical analyses in (mg/g) except alkaloid %

Characterization of oil Results

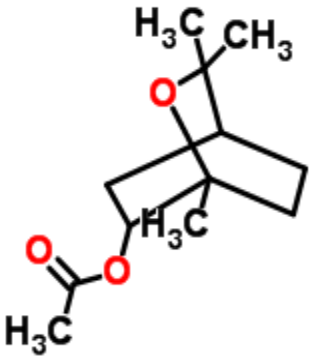
The various compounds shown through the Gas chromatography-Mass Spectroscopy include: 1, 3, 3-Trimethyl-2-Oxabicyclo [2,2,2] octan-6- ol, or eucalyptol a natural organic compound which is a colorless liquid. It is used as an ingredient in many brands of mouthwash and cough suppressant. Eucalyptol was found to control airway mucus hyper secretion and asthma, because it can suppress arachidonic acid metabolism and cytokine production in human monocytes (Juergens et al.,2003; Juergens et al 1998). Also, because of its pleasant spicy aroma and taste, eucalyptol is used in flavorings, fragrances and cosmetics (Harborne 1973). This compound is also used as an insecticide and insect repellent (Sfara et al., 2009; Klocke et al., 1987). 1,3,3-Trimethyl-2-Oxabicyclo[2,2,2] oct-6-yl acetate, and 4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methyl-2-butanon are used as flavoring, fragrances and cosmetics (Harbone 1973), n-Hexadecanoic acid (palmitic acid), this is the most common fatty acid found in animals, plants and microorganisms (Gunstone et al., 2007). It is also the major component of the oil from palm trees (palm oil, palm kernel oil and coconut oil). However, palmitic acid can also be found in meats, cheeses, butter, and dairy products. It has been shown (in rats fed on a 20% fat (palmitic acid), 80% carbohydrate diet) to alter aspects of the central nervous system responsible for the secretion of insulin and to suppress the body's natural appetite-suppressing signals from leptin and insulin -- the key hormones involved in weight regulation (Benoit *et al*., 2009). Furthermore, excess carbohydrates in the body are converted to palmitic acid. Palmitic acid is the first fatty acid produced during fatty acid synthesis and the precursor to longer fatty acids. Palmitate negatively feeds back on acetyl-CoA carboxylase (ACC), which is responsible for converting acetyl-CoA to malonyl-CoA, which in turn is used to add to the growing acyl chain, thus preventing further palmitate generation.[8] In biology, some proteins are modified by the addition of a palmitoyl group in a process known as palmitoylation. Palmitoylation is important for membrane localization of many proteins (Gunstone et al., 2007). N-Pentadecanoic acid is a saturated fatty acid, rare in nature, being found at the level of 1.2% in the milk fat from cows (Jost, 2002). The butterfat in cow's milk is its major dietary source (Smedman, et al., 1999) and it is used as a marker for butterfat consumption. Moreover, n-octadecanoic acid (stearic acid) is one of the most common saturated fatty acids found in nature following palmitic acid (Gunstone et al., 2007), it can be used as a binding agent for products like lotions, soaps, deodorants and candles. It also serves the same function in food products, such as butter flavoring, vanilla flavoring, chewing

gum, fruit waxes and butter. Eicosanoic acid, heptadecanoic acid or margaric acid, is a saturated fatty acid. It occurs as a trace component of the fat and milk fat of ruminants,(Hansen, et al., 1957) but it does not occur in any natural animal or vegetable fat at concentrations over half a percent (Beare-Rogers et al., 2001). 9-Octadecenoic acid ethyl ester (oleic acid) is a monounsaturated fatty acid and one of the major components of membrane phosphorlipids, it inhibits collagen-stimulated platelet aggregation by approximately 90% when used at a concentration of 10Aµg/ml (siafaka-Kapadai *et al*., 1997) and Cis-9-Hexadecenal. The presences of these compounds are good indication that if the oil is isolated using various types of chromatographic techniques, well purified and modified, it will be of high value in pharmaceutical, food component and agrochemical industries

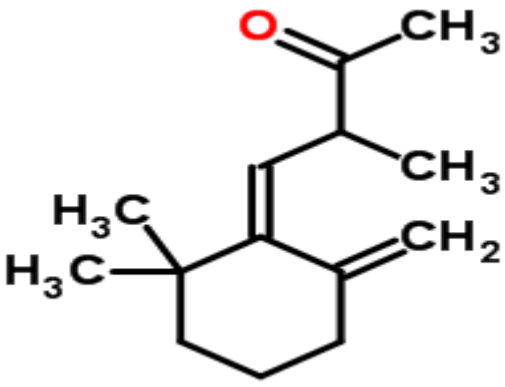
Structures of the compounds



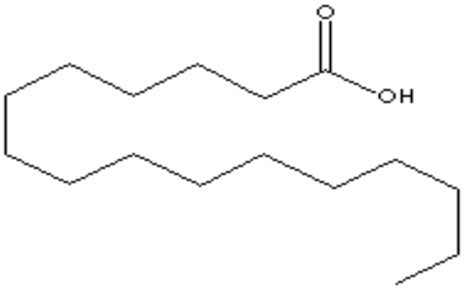
1,3,3- Trimethyl-2-Oxabicyclo[2,2,2] octan-6- ol



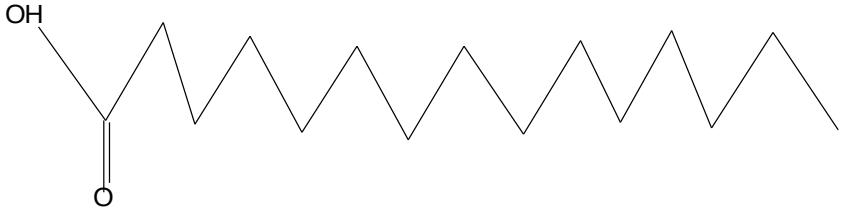
1,3, 3- Trimethyl-2-Oxabicyclo[2,2,2] oct-6-yl acetate



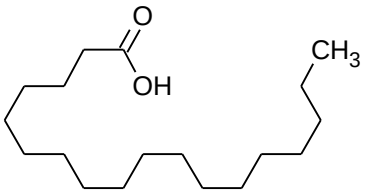
4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methyl-2-butanon



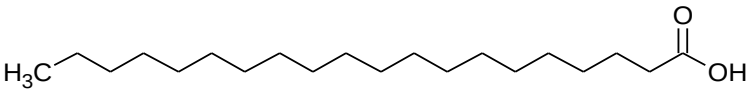
N-Hexadecanoic acid (palmitic acid)



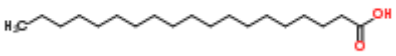
N-Pentadecanoic acid



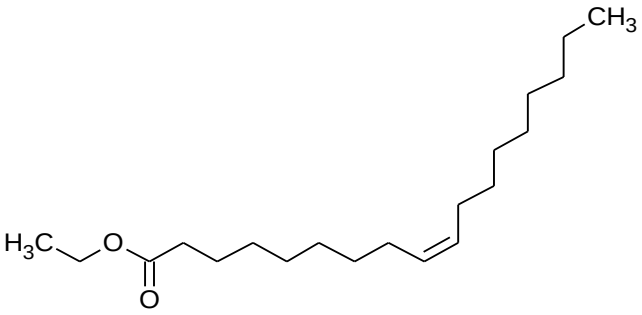
N-octadecanoic acid (stearic acid)



Eicosanoic acid



Nonadecylic acid



9-Octadecenoic acid ethyl ester (oleic acid)

Cis-9-Hexadecenal

Table 1: properties of the identified compounds in the oil of *Monodora myristica*

Compound S/NO	Compound Name	Molecular formula	Molecular weight	CAS
A	**1,3,3- Trimethyl-2-Oxabicyclo[2,2,2] octan-6- ol**	**$C_{10}H_{18}O_2$**	**170**	**18679-48-6**
B	**1,3,3- Trimethyl-2-Oxabicyclo[2,2,2] oct-6-yl acetate**	**$C_{12}H_{20}O_3$**	**212**	**57709-95-2**
C	**4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methyl-2-butanon**	**$C1_4H_{22}O$**	**206**	**93175-74-7**
D	**n-Hexadecanoic acid(palmitic acid)**	**$C_{16}H_{32}O_2$**	**256**	**57-10-3**
E	**n-Pentadecanoic acid**	**$C_{15}H_{30}O_2$**	**242**	**1002-84-2**
F	**n-octadecanoic acid(stearic acid)**	**$C_{18}H_{36}0_2$**	**284**	**57-11-4**
G	**Eicosanoic acid**	**$C_{20}H_{40}O_2$**	**312**	**506-30-9**
H	**Nonadecylic acid**	**$C_{19}H_{38}O_2$**	**298**	**18281-05-5**
I	**9-Octadecenoic acid ethyl ester (oleic acid)**	**$C_{20}H_{38}O_2$**	**310**	**111-62-6**
J	**Cis-9-Hexadecenal**	**$C_{16}H_{30}O$**	**238**	**56219-04-6**

IV. CONCLUSION

The antimicrobial strength, phytochemical compounds and the various organic compounds found in African nutmeg is an eye opener for the discovery of novel bioactive compounds if the plant can be subjected into intensive research and making use of various spectroscopic techniques like 13C and 1H NMR, FT/IR and mass spectroscopy to characterize the compounds to be isolated in order to discover known and new antibiotics and anti-parasitic agents.

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