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Vol. 13(22), pp. 2260-2264, 28 May, 2014
DOI: 10.5897/AJB2013.13192
Article Number: 7E30DDA44906
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Analysis and identification of oils from seed extract of *Anthonotha macrophylla* using gas chromatography-mass spectrometry (GC-MS)

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Received 23 August, 2013; Accepted 2 May, 2014

The volatile components obtained from the seed extract of *Anthonotha Macrophylla* were analyzed using gas chromatography-mass spectrometry (GC-MS). Prior to GC-MS analysis, Soxhelt extraction was carried out from the seeds of *A. Macrophylla.* Agilent GC-MS system comprising 6890GC model coupled with 5973 n mass selective detector was used for analysis. The GC is equipped with Agilent 7673 autosampler and a 30 m 0.25 id DB-1 MS dimethylpolysiolaxane capillary column. The MS source temperature was set at 230C and electron energy at 70V. The ionisation mode was electron ionization and the mass range was 50 to 550 while the scan time was 1 scan/min. The different compounds were identified by matching their mass spectra with the MS spectra in the NIST library. Various compounds were separated and identified but eight of these were at an elevated level. These include: n-hexadecanoic acid, n-octadecadienoic acid; cis-vaccenic acid; octadecanoic acid; hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester; campesterol; stigmasterol and gamma-sitosterol.

Key words: Gas chromatography-mass spectrometry, determination, evaluation, composition, *Anthonotha macrophylla*, seed oil.

INTRODUCTION

More than 80% of the world's population rely on Traditional medicine for their primary healthcare needs (Pierangeli, 2009). This is particularly so for the African continent (Addae-Mensah, 1992; Cunningham, 1993). Unfortunately, most of these herbal remedies have not been scientifically explored and exploited. In Africa, natural products have proven to be great sources of essential oils which combine the function of healthcare and nutrition. Essential oils have other uses such as in foods, drinks, cosmetics and medicine especially with aromatherapy becoming increasingly popular (Reische et al., 1998; Lis-Balchin et al., 1999; Ghelardini et al., 1999).

Nigeria and Africa at large are endowed with seed-bearing plants, which over the years have served various purposes and yet quite a number of them remain untapped. One of such underutilised plant is *Anthonotha*

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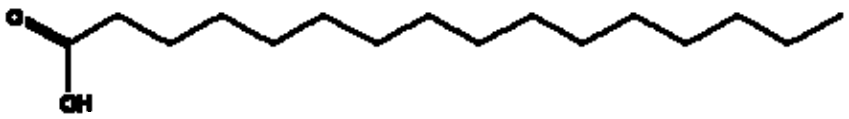

Figure 1. Chemical structure of n-hexadecanoic acid

macrophylla (AM) P. Beauv; a member of the family Leguminosae - Caesalpinioideae. AM is a small tree which is common throughout the rain forests, a medium-sized tree up to 20 m high, tree trunk seldom reaching 50 cm diameter, often multi-stemmed and low-forked, bearing a wide-spreading crown, common in the under storey of the rain-forest from Guinea to Western Cameroons and Fernando Po, and extending to Zaïre. It abounds in Nigeria with common names: (Yoruba: abata; Igbo: ububa - ikpa). Several ethnomedicinal claims have been attributed to various parts of this plant. The bark has been claimed to be useful in the treatment of venereal diseases and as vermifuges. The roots are used for intestinal related discomfort. Gums extractable from the bark have analgesic properties while the leaves could be useful as anti-diarrhoea, dysentery, skin infections, as antidotes in venomous stings and bites (Keay, 1989; Burkill, 1985). The seeds are also useful as general food. Previous studies reveal that the oil extracted from the milled dry seed gave iodine value of 1.013 ± 0.01%, free fatty acid 2.334 ± 0.04%, saponification value 5.394 ± 0.23%, un-saponifiable matter 8.33 ± 0.01% and refractive index of 1.472 ± 0.07% (Durunna, 2006).

So far, to the best of our knowledge, no data has been recorded on the profile of the oils contained in the seeds of *A. macrophylla*. The present work is aimed at identifying the fatty acids in the oil of *A. macrophylla* using gas chromatography-mass-spectrometry (GC-MS) method thereby laying a good foundation for future studies on the medicinal uses of this plant.

EXPERIMENTALS

Plant material

The matured seeds of *A. macrophylla* used for this study were collected from the forest in Akatta, Oru-East Local Government Area, Imo State of Nigeria.

Extraction

The seeds of *A. macrophylla* were shade-dried until constant weight at room temperature and milled in a hammer Mill (Thomas Willey, model 4, USA). Extraction was carried out using a standard Soxhlet apparatus (Haake FK, Germany). The Soxhlet thimble was charged with 500 g milled seeds of mean particle size (0.3 to 0.5 mm), and extracted with 1000 ml petroleum spirit (40 to 60°C) during each complete extraction step. The oil was extracted from the distillate using hexane and then dried over anhydrous sodium sulphate. After filtration, the solvent was removed by distillation under reduced pressure in a rotary evaporator at 35°C and the pure oil kept in sealed glass at 4°C in the dark, until the moment of analysis.

Gas chromatography-mass spectrometry analysis (GC/MS) conditions

The extracted oil was diluted with n-heptane in the ratio (1:50) and subjected to GC-MS. The analysis was carried out on Agilent GC-MS system comprising 6890 GC model coupled with 5973 n MSD. The GC is equipped with agilent 7673 autosampler and a DB-1 MS dimethylpolysiolaxane capillary column (30 m × 0.32 mm; film thickness 1.00 µm). The instrument operating conditions were: Carrier gas was helium at flow rate of 1.0 ml/min and at a constant pressure mode of 2.56 psi. Injector temperature was 250°C; injector volume was 1 ul in the split mode. The initial temperature was 120°C for 5 min and ramped up at 3°C/min with Nitrogen as make-up gas. The final temperature, 350°C was held for 5 min. The run time was 62 min. The MS source temperature was 230°C; electron energy was 70 V. The ionisation mode was electron ionization and the mass range of *m/z* 50 to 550 while the scan time was 1 scan/min.

Identification of phytocompounds

The interpretation of GC-MS spectra was done using the database of the National Institute of Standards and Technology (NIST). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The nomenclatures, molecular weights and structures of the component compounds in the oil of *Anthonotha macrophylla* were ascertained. The different compounds were identified by matching their mass spectra with the MS spectra in the NIST02 library.

RESULTS AND DISCUSSION

With the help of MS spectra in the NIST library, some of the fatty acids separated in the seed extract of *A. macrophylla* were identified. Various compounds were separated and identified but eight of these were present at elevated levels and future work will focus on these compounds. Figures 1 to 8 give the chemical structures of these compounds. They include: n-hexadecanoic acid, n-octadecadienoic acid, cis-vaccenic acid, octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, campesterol, stigmasterol and γ-sitosterol. No attempt was made at quantifying the fatty acids in the oil. The relative abundance of these compounds were: 9,12-Octadecadienoic acid (33.38%); n-hexadecanoic acid (17.33%); γ-sitosterol (14.84%); 9Z- Octadec-9-enoic acid (12.85%); Campesterol (4.32%); Stigmasterol (3.63%) and Octadecanoic acid (2.97%) (Table 1). Very

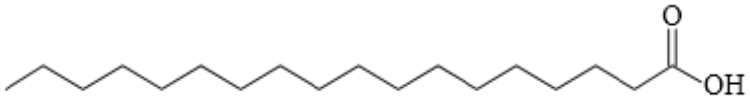

Figure 2. Chemical structure of octadecanoic acid (stearic acid)

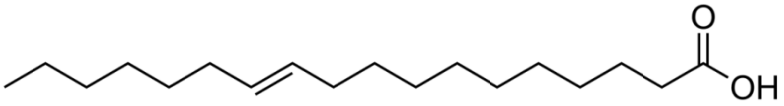

Figure 3. Chemical structure of cis-veccenic acid

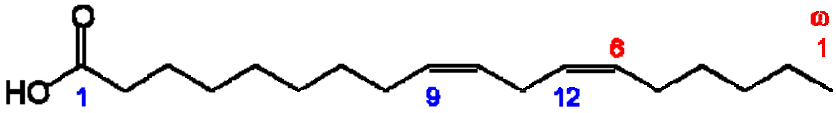

Figure 4. Chemical structure of n-octadecadienoic acid (linoleic acid)

Figure 5. Chemical structure of octadecanoic acid

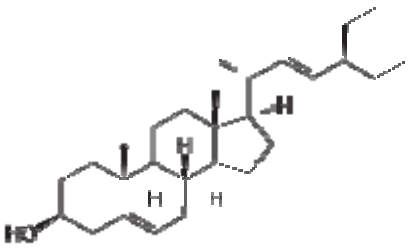

Figure 6. Chemical structure of stigmasterol

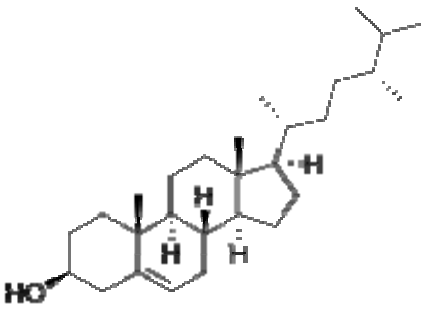

Figure 8. Chemical structure of Compesterol

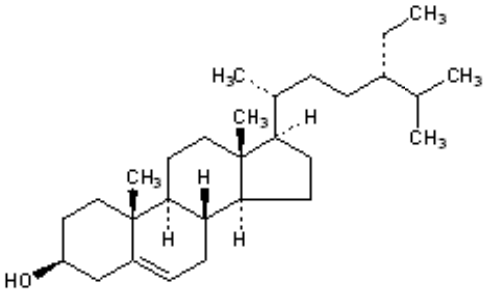

Figure 7. Chemical structure of sitosterol

many other compounds were present in the sample.

Anthonotha macrophylla oil contains n-Hexadecanoic acid (palmitic acid), a fatty acid reported to show anti-inflammatory properties by Aparna et al., (2012). From structural and kinetic studies they concluded that n-hexadecanoic acid is a potent inhibitor of phospholipase A2, an inflammatory compound. However, a downside of this phytocompound is that reported by the WHO (2003) technical report, of the convincing evidence that consumption of palmitic acid increases the risk of developing

Table 1. Phytoconstituents identified from the methanolic extract of *Anthonotha macrophylla*.

Retention time	Phytocompound	Relative Percentage (%) composition
10.04	n-Hexadecanoic acid	17.33
16.03	Octadecanoic acid (stearic acid)	2.97
	Cis-Vaccenic acid	12.85
	hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	0.61
47.59	Campesterol	4.32
	n-Octadecadienoic acid	33.38
48.61	Stigmasterol	3.63
50.42	γ - Sitosterol	14.84

cardiovascular diseases, placing it in the same evidence category as trans fatty acids, myristic acid, high sodium intake, overweight and high alcohol intake. Also, present is the common monoenoic fatty acid of bacterial lipids, cis-Vaccenic acid (cis-11-octadecenoic acid). It occurs as a minor component of most plant and animal tissues. Previous research have shown that cis-Vaccenic acid, within the range of the concentrations used, produces a proportionate inhibition of growth and respiration of *Bacillus subtilis*, thus suggesting its antibiotic effect with the cis- isomer having about twice the activity of the trans-isomer. A recent study (Djoussé et al., 2013) suggested that higher plasma levels of phospholipid cis-vaccenic acid are associated with reduced odds of heart failure with antecedent coronary heart disease.

The cholesterol-lowering potential of dietary plant sterols has been known for over 50 years (Pollack, 1953). Despite the daily consumption of plant sterols in food, the amounts are usually not high enough to have significant blood cholesterol-lowering effect. The major plant sterols are sitosterol (approx. 80%), campesterol and stigmasterol (Rao and Janezic, 1992; Ikeda et al., 2006; Phuruengrat and Phaisansuthichol, 2006). Our study showed *A. macrophylla* to contain all 3 major phytosterols in the oil.

Plant sterols are similar in structure to cholesterol (cholest-5-en-3beta-ol). The structural similarity of plant sterols to cholesterol enables them to compete with cholesterol for incorporation into the micelles, the particles that transport lipids and cholesterol into the intestinal mucosa. This competition reduces the absorption of dietary and biliary cholesterol in the gastrointestinal tract (Clifton, 2002; Lichtenstein, 2002). Phytosterols and phytostanols both inhibit the uptake of dietary and biliary cholesterol, and thus reduce the levels of low density lipoproteins and total serum cholesterol. Because the structure of β-sitosterol is similar to that of cholesterol, β-sitosterol takes the place of dietary and biliary cholesterol in micelles produced in the intestinal lumen (Moreau et al., 2002). The structural difference is the presence and positioning of methyl or ethyl moieties in their side chains.

Phytosterols possess biological functions, such as anticarcinogenic (Li et al., 2001), anti-inflammatory, antibacterial and antifungal activities (Padmaja et al., 1993) and anti-angiogenic activities (Jung-Min et al., 2007). In the mid 20th century, studies have shown plant sterols to be beneficial in lowering low density lipo-proteins and cholesterol (Farquhar and Sokolow, 1958). Since then, numerous studies have also reported the beneficial effects of the dietary intake of phytosterols, including campesterol. It is thought that the campesterol molecules compete with cholesterol and thus reduces the absorption of cholesterol in the human intestine (Heggen et al., 2010). There is rising evidence that campesterol exhibits chemopreventive effects against many cancers, including prostate (McCann et al., 2005), lung (Schabath et al., 2005) and breast (Awad et al., 2000) cancers. Our study also showed *A. macrophylla* oil to contain gamma-sitosterol and stigmasterol. Stigmasterol is reported to inhibit cholesterol biosynthesis via inhibition of sterol Delta (24)-reductase in human Caco-2 and HL-60 cell lines. A study (Batta et al., 2006) carried out on the effect of feeding 0.5% stigmasterol on plasma and liver sterols and intestinal cholesterol and sitosterol absorption in 12 wild-type Kyoto (WKY) and 12 Wistar rats showed that stigmasterol lowered plasma cholesterol levels, inhibited intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Wistar as well as WKY rats. Thus, stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the animal study.

The most extensively studied members of the phytosterols, beta and gamma sitosterols, are stereoisomers and differ only in the spatial configuration of the C-17 side chain. The structural similarity of Stigmasterol and β-sitosterol strengthens the hypocholes-terolemic potential of sitosterol. Matsuoka et al. (2008) in their work reported that β-Sitosterol inhibits cholesterol absorption in the intestine. The absorbed sterol in the intestine is transported by lipoproteins and incorporated into the cellular membrane (Awad and Fink, 2000). The overall consequence being reduced cholesterol, lowered

low density lipoproteins (LDL) and fewer incidences of artherosclerosis. Sitosterol (though the β-isoform) has been reported to be beneficial to urinary and overall prostate health (Wilt et al., 1999). The GC-MS analysis of *A. macrophylla* oil of study reveals the presence of these phytosterols, suggesting that the plant will definitely find place in the scheme of drug development programmes as a useful lead.

Conclusion

With the aid of GC/MS, eight major chemical constituents have been identified from the extract of the seed oil of *A. macrophylla.* The presence of these bioactive compounds justifies the ethnomedicinal use of the plant for various ailments by traditional practitioners. This study has opened up various research opportunities on this plant and its plant parts with the aim of maximizing its potential as a possible lead in drug discovery.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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