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Spectrophotometric Analyses of Fatty Acids Extracts of Alstonia boonei De Wild Leaves

Ibukun E.O. Durojaiye O. Oladipo G.O.* Nlekerem C.M. Fasomoyin S. M.
Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria

Abstract
The study aimed at profiling the fatty acids in methylated n-hexane extract of *Alstonia boonei* leaf using Gas Chromatography coupled with Mass Spectrophotometer (GC-MS). The qualitative and quantitative evaluations of the oil extracted from the leaves revealed the fatty acids contents and presented a ω-6: ω-3 PUFA ratio of 2.77. Thus, these study revealed the various bioactive compounds in *Alstonia boonei* leaf n-hexane and inferred its utilization to effect pharmaceutically relevance.
Keywords: *Alstonia boonei* leaf; fatty acids; gas chromatography-mass spectrophotometry (GC-MS),

Introduction

Dietary fats and oils provide calories and essential fatty acids and are sources of fat- soluble vitamins. Certain types of fat, however can increase risk of chronic cardiovascular diseases that affect the heart, blood vessels, and brain. The type of fat that is consumed can have either positive or negative effects on risk of cardiovascular disease (CVD). Saturated fatty acids (SFA) and trans-fatty acids are generally considered as unhealthy; whereas, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are considered beneficial (USDA). The effect of dietary SFA on CVD is partially mediated by effects on blood lipids, in particular, increased total cholesterol, and low density lipoproteins (LDL) cholesterol. Elevated blood LDL cholesterol increases artherosclerotic lipid accumulation in blood vessels and is an intermediate marker of CVD progression. Therefore reduction in SFA intake has been a key component of dietary recommendations to reduce the risk of CVD (USDA).

Upon consumption, the body converts alpha-linolenic acid (ALA), the primary Omega-3 fatty acid, to eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), these two types of omega-3 (ω-3) fatty acids are more readily used by the body. Researches indicate that omega-3 fatty acids reduce inflammation and help prevent certain chronic diseases like heart disease, stroke, arthritis, skin disorders, diabetes, high cholesterol, high blood pressure, breast, colon and prostate cancer. Linoleic Acid is the primary Omega-6 (ω-6) fatty acid. A person of good health and fit nutrition will convert linoleic acid into gamma linolenic acid (GLA), which will later be manufactured with EPA from the Omega-3 group into eicosanoids (the body's cellular check and balance system.). The ratio of omega-6 to omega-3 essential fatty acids have been shown to provide a clue about the health status of an individual.

Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines, among those plants whose parts have found wide application in phytotherapy is *Alstonia boonei* De Wild, a large evergreen tree belonging to the family Apocynaceae. It is one of the widely used medicinal plants in Africa and beyond. It grows up to an altitude of about 45m and 1.2m diameter. It is distributed throughout the tropics and the rain forest of west and Central Africa Akinloye *et al.* (2013). Almost all the plant parts viz. leaves, stem bark; root and inflorescences have been used and are further under investigative study. Specifically, *the* stem bark, has been reported to possess anti-inflammatory, analgesic and antipyretic activities, antifungal, antibacterial, antiviral, antithrombosis, anti-tumor and antioxidant activities (Akinmoladun *et al.,* 2007). Raji and Akinsomisoye (2005) reported that the methanolic extract of *A. boonei* has reversible antifertility effects in male rats. The stem bark is commonly used in treating malaria, toothache and rheumatism (Akinmoladun *et al.,* 2007). The chloroform and methanol extracts from the roots showed activity against *Staphylococcuc aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* (Deepthi *et al.*, 2008). The hypoglyceamic activity of stem bark aqueous extract of *A. boonei* was also reported by Akinloye *et al.* (2013). Thus as the plant possesses immense medicinal properties, the aim of the present work was to identify the compounds present in the chloroform-methanol-hexane extract of *Alstonia boonei* leaf by Gas Chromatography coupled Mass spectrum (GC-MS) analysis.

Materials and method

Plant collection

The leaves of *Alstonia boonei, were* collected in bulk from Egun farm settlement, Ifaki-Ekiti, Ekiti State, and authenticated at the Department of Crop, Soil and Pest Management, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure. After authentication, the leaves were washed under running tap water to remove adhering dirt followed by rinsing with distilled water, air dried in shadow and grinded by mixer grinder.

Lipid Extraction

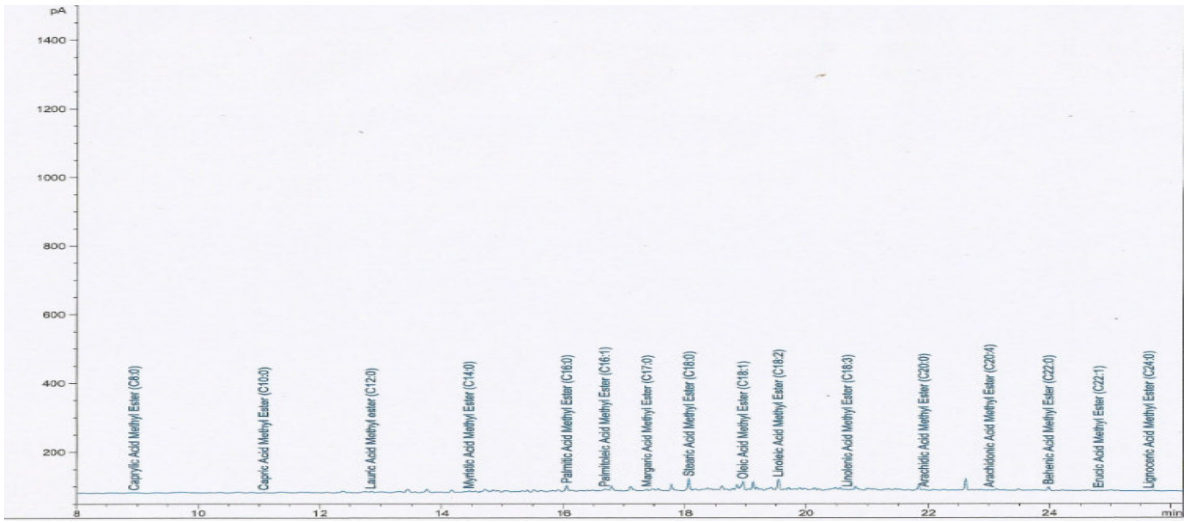
Lipid from the dried Alstonia boonei leaves sample was extracted using AOAC (1990) with little modification. 300g of dried and grounded leaves was dissolved in chloroform (300ml) in a test tube fitted with a condenser, and 1% sulfuric acid in methanol (150ml) was added, mixture was vortexed for 2 hours and 50ml of sodium chloride solution (5% w/v) was later added and the required esters was extracted with 50ml of hexane. Pasteur pipettes was then used to separate the layers. The hexane layer was washed with 50ml of 2% w/v potassium bicarbonate solution and dried over anhydrous sodium sulfate. The solution was then harvested to remove the drying agent, and the solvent removed under reduced pressure in a rotary film evaporator. The lipid extract was stored in an air -tight container in a desiccator at 4^{o}C until when needed. The lipid extract was further methylated using standard laboratory protocol. 50mg of the extracted fat content of the extract was saponified (esterified) for five (5) minutes at 95^{0}C with 3.4ml of the 0.5M KOH in dry methanol. The mixture was then neutralized using 0.7M HCl. 3ml of the 14% boron triflouride in methanol was later added. The mixture was heated for 5 minutes at the temperature of 90^{0}C to achieve complete methylation process. The fatty acid methyl esters was thrice extracted from the mixture with redistilled n-hexane. The lipid extract was used to profile the fatty acids present in the sample using Gas Chromatography and Mass Spectrophotometer and administered to evaluate the dietary relevance.

Gas Chromatography and Mass Spectrophotometry

The content was concentrated to 1ml for gas chromatography analysis and 1µl injected into the injection port of GC. The GC equipment used was HP 6890 powered with HP chemstation Rev. A09.01 (1206) software. The split ratio will be 20:1, the carrier gas was nitrogen at inlet temperature of 250^{0}C with a column type of HP INNOWax and column dimensions of 30m x 0.25mm x 0.25µm. the oven program parameters included initial temperature at 60^{0}C, first ramping at 12^{0}C/minutes for 20minutes, maintained for 2minutes and second ramping at 15^{0}C/min for 3minutes, maintained for 8minutes. The detector used was FID at 320^{0}C at hydrogen pressure 22psi and compressed air of 35psi

Results and Discussion

The gas chromatography analysis of the lipid (hexane-chloroform) extract of *A. boonei* leaves resulted in sixteen peaks as obtained on the chromatogram of the oil. These peaks indicated the presence of sixteen compounds in the chloroform-hexane-methanol extract of the leaf. The qualitative and quantitative evaluations of fatty acids in the sample revealed the following fatty acids in g/100g of samples caprylic acid (c8:0), 0.005; capric acid (c10:0), 0.004; lauric acid (c12:0), 0.013; myristic acid (c14:0), 1.216; palmitic acid (c16:0), 30.097; palmitoleic acid (c16:1), 2.291; margaric acid (c17:0), 0.072; stearic acid (c18:0), 4.513; oleic acid (c18:1), 6.165; linoleic acid (c18:2), 39.735; linolenic acid (c18:3), 14.362; arachidic acid (c20:0), 0.349; arachidonic acid (c20:4), 0.083; behenic acid (c22:0), 0.665; erucic acid (c22:1), 0.331; and lignoceric acid (c24:0), 0.100. The extract contained the ω-3 fatty acids precursor, *α*-linolenic acid; Linoleic acid, a precursor of ω-6 fatty acids; and arachidonic acid, a ω-6 fatty acid, in amounts of 14.362, 39.735 and 0.083 g/100g respectively. By calculation, *Alstonia boonei* leaf had an ω-6 to ω-3 PUFA ratio of 2.77.



GC-MS chromatogram for *Alstonia boonei* leaf fatty acids profile

The roles of PUFAs become more important as they are not synthesized in human organism and have

to be delivered with food (Ibukun and Oladipo, 2016). The ω-6 and ω-3 eighteen-carbon polyunsaturated fatty acids compete for the same metabolic enzymes; thus the ω-6: ω-3 ratio of ingested fatty acids has significant influence on the ratio and rate of production of eicosanoids, a group of hormones intimately involved in the body's inflammatory and homeostatic processes, which include the prostaglandins, leukotrienes, and thromboxanes, among others. Altering this ratio can change the body's metabolic and inflammatory state. Metabolites of omega-6 are more inflammatory (esp. arachidonic acid) than those of ω-3. This necessitates that ω-6 and ω-3 be consumed in a balanced proportion; healthy ratios of ω-6: ω-3, according to some authors, range from 1:1 to 1:4 (an individual needs more omega-3 than omega-6). (Lands, 2005). Other researchers believe that ratio 4:1 (when the amount of ω-6 is only 4 times greater than that of ω-3) is already healthy (*Daley, 2004; Simopoulos, 2002)*. Studies suggested the evolutionary human diet, rich in game animals, seafood, and other sources of omega-3, may have provided such a ratio. (Simopoulos, 2003). The importance of the ratio of ω-6/ω-3 essential fatty acids as established by comparative studies showed an ω-6: ω-3 ratio under 4:1 may contribute to improved health (Simopoulos, 2002). Also, preliminary research indicated that ω-3 fatty acids in diets lowered the risk of heart attacks and that ω-6 fatty acids may also reduce the risk of cardiovascular disease (Okamoto et al., 2007).

The result also revealed the presence of Stearic acid (4.513g per 100 g of oil extracts), a saturated fatty acid (SFA), which had been shown to have recommendable effects on blood total and low density lipoprotein (LDL) cholesterol levels (Mensink, 2005), based on this, it had been shown to alleviate cardiomyopathy (Kris-Etherton *et al.,* 2005). However, this effects of stearic acid on high density lipoprotein cholesterol (HDL-c) and triglyceride levels were inconsistent (Mensink, 2005; Kris-Etherton *et al.,* 2005). Palmitoleic acid is a monounsaturated fatty acid resembling saturated fatty acids in its ability to lower LDL-C (Nestel *et al.,* 1994). The result also revealed palmitoleic acid (2.291g per 100g), a beneficial fatty acid which has been shown to increase insulin sensitivity by suppressing inflammation, as well as inhibit the destruction of insulin-secreting pancreatic beta cells (Yang *et al.,* 2011). Oleic acid also has an amount of 6.17 g per 100 g of lipid extract and it had been reported as LDL-C lowering fatty acid (Nestel *et al.,* 1994). Palmitic acid, the most common fatty acid (saturated) found in animals, plants and microorganisms (Gunstone *et al.*, 2007), occurred in high amount of 30.10 g per 100 g of oil extract has been reported to reduce LDL as linoleic acid increases (French *et al.,* 2002). Lauric, myristic, palmitic and stearic acids had been shown to exert a neutral or hypocholesterolemic effect on blood cholesterol levels in experimental animals (Kris-Etherton *et al*., 2005). However, myristic acid has a sufficiently high hydrophobicity to become incorporated into the fatty acyl core of the phospholipid bilayer of the plasma membrane of the eukaryotic cell. In this way, myristic acid acts as a lipid anchor in biomembranes.

In conclusion, the fatty acid profile obtained in this GC–MS analysis revealed promising evidence for the effects of the extract of *A. boonei* to lower the risk of cardiovascular diseases (CVD); and to bring about other beneficial pharmacological effect like anti-inflammation and malignant growth suppressing activity. These components include essential fatty acids, PUFA, ω-6 and ω-3 which contribute to or ensure improved health.

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