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RESEARCH ARTICLE

Fatty Acid Composition of *Aspilia africana* (Nigerian)

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ABSTRACT

Fatty acids composition of Aspilia africana leaf oil was analyzed by gas chromatography (GC) and sixteen acids were identified with seven fatty acids found in trace amounts while nine were in various appreciable amounts. Polyunsaturated essential fatty acids identified are linoleic (C18:2) an "omega-6" is 50.28% and linolenic acid (C18:3) an "omega-3" is 0.28%. The monounsaturated fatty acids oleic acid (C18:1) an "omega-9" and erucic acid C22:1 was found in concentrations of 18.85 and 0.22% respectively. Five saturated fatty acids stearic (C18:0), palmitic (C16:0), behenic (C22:0), arachidic (C20:0) and myristic (C14:0) were obtained in concentrations of 15.10, 11.77, 2.95, 0.54 and 0.001% respectively. The physicochemical analysis gave the free fatty acid (oleic) 8.84%, acid value as 0.86mg KOH/g of oil, iodine value 79 (g of 1/100g of oil), peroxide value 16.2meq of active oxygen/Kg oil and saponification value of 201.21mg KOH/g of oil. The results of the mineral elements concentration showed potassium as the predominant element (13,800.37ppm) while copper had the least concentration (15.46ppm).

INTRODUCTION

Various plants (*Gongronema latifolia, Asmina triloba, Aspilia africana, Azadirachta indica, citrus aurantifolia,* etc) have been used in traditional medicine for the treatment of several ailments in different parts of the world Nwachukwu *et. al.* [1] and Agonihotri *et. al.* [2]. Ailments such as gonorrhea, tuberculosis, cough, rheumatic pains, stomach trouble, corneal opacity, wounds and insect bites have been treated [3,2]. *Aspillia africana* C.D. Adams (compositae) is a tropical shrub which grows wildly at roadsides in Nigeria and popularly used as fodder in most villages has been established as an effective medicinal plant. It is commonly known as yurinyun by the Yorubas, Orangila by Igbos, Tozakin by Hausas and Edemedong by Efiks [3] while in Ekpeye it is called Uwhoridhoatu and Eutung of Cross Rivers State (all of Nigeria) call it Ottabi. The leaf extract and fractions of *A. africana* effectively arrested bleeding from fresh wounds, inhibited microbial growth of known wound contaminants and accelerated wound healing process; Okoli *et. al.* [4]. Evaluation of the potentials of *A. africana* in wound care showed that the leaf extract and fractions exhibited haemostatic, antimicrobial and wound healing activities suggesting that the constituents of the leaves may play a useful role in wound care. The extract and fractions arrested bleeding from fresh wounds by reducing bleeding/clotting and whole blood coagulation time which are important indices of haemostatic activity[5]. It is also used to clean the surfaces of sores, in the treatment of rheumatic pains, bee and scorpion stings and for removal of opacities and foreign bodies from the eyes [4]. These studies indicate that leaf extracts of *A. africana* have good potentials for use in wound care and further provide a rationale for the use of the leaves of this plant in wound management in traditional medicine practice. Anti-inflammatory activity of hexane leaf extract of *Aspilia Africana* has also been reported; Okoli *et. al.* [6] and Agonihotri *et. al.* [2].

Phytochemical analysis of the extract and fractions indicated the presence of typical plant constituents such as alkaloids, saponins, sterols, terpenoids, carbohydrates, glycosides and tannins[6,7]. These metabolites are usually responsible for the pharmacological activities of medicinal plants.

The ethnomedicinal uses of this plant suggest its usefulness in wound treatment and stimulated our interest to study the fatty acids and some physico-chemical properties of *A. africana* for potential application in other areas of health care.

MATERIALS AND METHODS

Collection of plant material

Aspilia africana leaf was obtained from the hedges of a field at University of Port Harcourt. It was duly identified by the Curator at the Herbarium, Department of Plant Science and Biotechnology, University of Port Harcourt.

Extraction of oil

1.0kg *Aspilia africana* leaf was crushed with a mortar and pestle. The oil was extracted with petroleum ether using Soxhlet after 3hrs reflux. Solvent was removed using a rota evaporator to give 4.7g oil. It was dried ($MgSO_4$) and stored in a desiccator until needed.

Derivatization of fatty acid of the oil for GC analysis

To 0.35g of the oil extract in a 50ml round bottom flask was added 6ml 0.5m methanolic NaOH solution. The mixture was refluxed until the droplet of oil disappeared. 7ml 14% BF_3 in methanol was added and refluxed for 2mins before adding 5ml heptane. The mixture was refluxed for additional three hours, allowed to cool and saturated with a solution of NaCl with swirling. The organic layer was collected, dried ($MgSO_4$) and stored in a desiccator until needed for GC analysis.

GC condition for analysis of oil

Gas Chromatography analysis was accomplished with HP 6890 Powered by HP Chem Station Rev. A 09.01 [1206] Software and HP 5MS capillary columns (30m x 0.25mm x 0.25µm film thickness). The program temperature is 60 ^{O}C per 3min, 8 ^{O}C/min to 140 ^{O}C. Injector and detector temperature were maintained at 230 ^{O}C and 275 ^{O}C respectively; the carrier gas is hydrogen (1.0ml/min), detector dual, FID. Volume injected was 0.5µl. Identification of components was obtained by comparison of their retention time with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of n-hydrocarbons.

N. B: *Extraction was done at Department of Pure and Industrial Chemistry, University of Port Harcourt, Port Harcourt, Rivers State while gas chromatography (GC) analysis was done at Multi Environmental Management Consultant, 20 Daniel Makinde Street, Ketu, Lagos, Nigeria.*

CHEMICAL ANALYSIS

1. Peroxide value

To 1.0g oil sample in 200 ml conical flask was added 20ml of acetic acid:chloroform (2:1) solvent. 1.0ml of potassium iodide was added and the mixture was kept in the dark for about 1 minute before 30.0ml of de-ionized water was added. The mixture was titrated against sodium thiosulphate ($Na_2S_2O_3$) solution in the conical flask using starch indicator.

The same procedure was repeated for the blank.

$$\text{Peroxide Value} = \frac{100\ N(V_1 - V_2)}{W} \quad meq/Kg$$

where

N = Normality of thiosulphate
V_1 = Volume of thiosulphate used in the test
V_2 = Volume of thiosulphate used in the blank
W = Weight of oil sample

2. Acid Value

To 0.50g oil sample in a conical flask was added 5.0ml ethanol:diethyl ether (1:1). The mixture was homogenized and titrated to phenolphthalein end-point with NaOH.

$$\text{Acid Value} = \frac{56.1\ x\ N\ x\ V}{W}$$

where

N = Normality of sodium hydroxide
V = Volume of sodium hydroxide used in the test
W = Weight of sample

3. Iodine Value

To 1g oil sample was added 10ml carbon tetrachloride and 20ml Wiji solution in 100ml volumetric flask. It was stoppered and allowed to stand for two hours in darkness at the room temperature; this is

to allow the chlorination of any double bond. 20ml 10% KI solution and 100ml water were added. The liberated I_2 was titrated with $0.1MNa_2S_2O_3$ solution using the starch indicator. The same procedure was repeated for the blank.
where

$$\text{Iodine Value} = \frac{12.69 \quad N \quad (V_2 - V_1)}{W}$$

N = Normality of thiosulphate
V_1 = Volume of thiosulphate used in the test
V_2 = Volume of thiosulphate used in the blank
W = Weight of sample

Saponification Value
To 2.0g oil sample was added 25ml ethanolic potassium hydroxide in 500ml round bottom flask and refluxed for 30 minutes to cause saponification reaction to occur. The unreacted KOH was back-titrated with 0.5M HCl using phenolphthalein as indicator.
The same procedure was repeated for the blank.
where

$$\text{Saponification Value} = \frac{56.\ 1N\ (V_1 - V_2)}{W}$$

N = Normality of hydrochloric acid
V_1 = Volume of hydrochloric acid used in the test
V_2 = Volume of hydrochloric acid used in the blank
W = Weight of sample

Metal ion analysis
1.0g of sample was digested using mixed acid method and analyzed for the desired metal ions by Atomic Absorption Spectrometer

RESULTS AND DISCUSSION
The relative fatty acid composition and concentration of *Aspilia Africana* is presented in Table 1 and sixteen fatty acids were found in *Aspilia Africana* with nine in quantifiable amounts (99.99%) while seven were in trace quantity (0.01%). **Fatty acid** composition of *Aspilia Africana* is in the order of polyunsaturated (50.56%) > saturated (30.36%) > monounsaturated (19.07%).
Palmitic acid C16:0 **(**$CH_3(CH_2)_{14}COOH$**)**, also known as hexadecanoic acid is a saturated fatty acid whose concentration in *Aspilia Africana* is 11.77%. This value is similar to *Cultivars sinnensis* seed oil (11.50%) but lower than *Citrullus lanatus* seed oil (17.71%), *Trichosanthes cucumerina* seed oil (19.15%) and G*ardinia jasminoide* 20.87% [8,9]
Stearic Acid (C18:0) ($C_{18}H_{36}O_2$) or octadecanoic acid is a saturated fatty acid with a concentration of 15.10% in our sample. In epidemiologic and clinical studies stearic acid was associated with lowered low-density lipoprotein cholesterol in comparison with other saturated fatty acids. These findings may indicate that stearic acid is less unhealthy than other saturated fatty acids [10]. Stearic acid is used for many different household products. It is used as a lubricant, a hardener, and an emulsifier, a chemical that allows oils and water to mix.
Oleic Acid (C18:1) ($C_{18}H_{34}O_2$) is monounsaturated omega-9 fatty acid. It is the only monounsaturated fatty acid found in *Aspilia Africana* at a concentration of 18.84%. Dietary intake of oleic acid which can be found in avocado pear, peanuts, cashew, olive oil (extra virgin or virgin), olives etc. is encouraged because it aids in cancer prevention, lowers heart attack risk and arteriosclerosis [11]. Oleic acid concentration in *Aspilia Africana* is lower than its value in *Trichosanthes cucumerina* seed oil (19.29%), *Gardenia jasminoide* (23%), *Citrullus lanatus* seed oil (24.77%) and *Cultivars sinnensis* seed oil (34.77%) [12,8,9].
Linoleic Acid (C18:2) ($C_{18}H_{32}O_2$), a poly-unsaturated fatty acid (PUFA) is also called *cis*,*cis*,-9,12-octadecadienoic acid was found in 50.28% in our sample. Gamma-linolenic acid (GLA) is an omega-6 fatty acid that is found mostly in plant-based oil and are considered essential fatty acids necessary for human health but the body can't make them. PUFAs help stimulate skin and hair growth, maintain

bone health, regulate metabolism, and maintain the reproductive system. Along with omega-3 fatty acids, omega-6 fatty acids play a crucial role in brain function as well as normal growth and development. Clinical studies suggest that children with ADHD have lower levels of essential fatty acids (EFAs), both omega-6s and omega-3s [13]. For overall healthy living, a balance of omega-6 and omega-3 fatty acids is proposed in a ratio in the range of 2:1 - 4:1, omega-6 to omega-3.

Linolenic Acid concentration in *Aspilia africana* is 0.28%. Linolenic acid (C18:3) is a polyunsaturated fatty acid (PUFA) and technically, it refers to either an Omega-3 (*cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid) fatty acid (Alpha-linolenic acid "ALA") found in vegetable oils or an Omega-6 fatty acid (*cis*-6, *cis*-9, *cis*-12-octadecatrienoic acid, Gamma-linolenic acid "GLA") found in sources such as primrose oil. GLA is normally used by the body to make prostaglandins (hormone-like substances) and they are believed to be involved in many processes in the body, including regulation of the immune system. GLA's anti-inflammatory properties have led it to be used to treat eczema and psoriasis, rheumatoid arthritis, nerve pain caused by diabetes and chronic fatigue syndrome. GLA helps lower cholesterol and maintain proper levels of various blood fats. ALA is considered an essential fatty acid because the body cannot produce it naturally but requires it for various body functions and overall health. ALA also can help prevent and even reverse atherosclerosis, or hardening of the arteries. ALA helps contribute to the production of the protective outer layers of the skin, helping it look and feel healthier and absorb moisture more efficiently. Linolenic acid must be added to the diet through easily absorbed nutritional sources such as seed oils and nuts. Dietary sources of omega-3 fatty acids include fish oil (docosahexaenoic acid "DHA" and eicosapentaenoic acid EPA") and certain plants oils (α-linolenic acid) Harris *et. al.* [14]. Among the common sources of linolenic acid are flaxseed or flaxseed oil, canola oil and walnut oil. A linolenic acid deficiency will result in hair loss, mood disorders, poor wound healing and scaly dermatitis. Linolenic acid is used in the manufacture of paints, coatings, and vitamins.

Arachidic Acid (C20:0) also known as eicosanoic acid has a concentration of 0.54% in our sample. Arachidic acid is a saturated fatty acid found in vegetable, peanut, and fish oils. Arachidic acid is present in the membranes of the body's cells and is highly enriched in the brain. It is as a minor constituent of peanut oil (1.1%–1.7%) and corn oil (3%) [15]. It can be formed by the hydrogenation of arachidonic acid and its reduction yields arachidyl alcohol.

Behenic Acid (C22:0) $C_{22}H_{44}O_2$ also known as docosanoic acid is a saturated fatty acid obtained from plant sources and its concentration in *Aspilia Africana* is 2.95%. At 9%, it is a major component of ben oil (or behen oil), which is extracted from the seeds of the ben oil tree (*Moringa oleifera*). Behenic acid is present in some oils and oil-bearing plants, including rapeseed (canola) and peanut oil and skins. It is estimated that one ton of peanut skins contains 13 pounds of behenic acid[15]. Commercially, behenic acid is used to manufacture waxes, plasticizers and cosmetics. Behenic acid gives hair conditioners and moisturizers their smoothing properties. As dietary oil, behenic acid is poorly absorbed. In spite of its low bioavailability compared with oleic acid, behenic acid is a cholesterol-raising saturated fatty acid in humans [16].

Erucic Acid (C22:1), a monosaturated omega-9 fatty acid is also known as *cis*-13-docosenoic acid and the *trans* isomer is known as brassidic acid, has a concentration of 0.22% in *Aspilia Africana*. It is prevalent in rapeseed, wallflower seed, and mustard seed, making up 40-50% of their oils, Sahasrabudhe [17]. It has many of the same uses as mineral oils, but with the advantage that it is more readily biodegradable. Its industrial applications are quite numerous. It is suitable for transmission oil, a binder for oil paint, readily forms many organic compounds, suitable for use as organic matrices that need to be polymeric, valued in tribology as a superior lubricant, useful in the manufacture of emulsions to coat photographic films and papers. A complex cocktail of many different erucic acid compounds is commonly used in color film. It is widely used to produce emollients, especially for skin and healthcare products. When used in the manufacture of plastic films in the form of erucamide, it migrates to the surfaces and so resists the sticking of each film to its neighbor. Being a hydrocarbon of high calorific value, with a very low flash point, high cetane number and good lubrication qualities, erucic acid can be a valuable component of biodiesel [18].

Erucic acid is broken down into shorter-chain fatty acids in the human liver by the long-chain acyl CoA dehydrogenase enzyme. A four-to-one mixture of erucic acid and oleic acid constitutes Lorenzo's oil; which is used to treat *adrenoleukodystrophy* (ALD). ALD is an inherited disorder that

causes adrenal gland failure, brain damage, and death. Thrombocytopenia has been seen in patients treated with Lorenzo's oil, probably related to its erucic acid content [19].

The physicochemical properties of *Aspilia africana* leaf oil is presented in Table 2. **Free fatty acid** (oleic) of *Aspilia africana* leaf oil is 8.84% which indicates that the oil is fresh and has not undergone deterioration or rancidity. *Aspilia africana* leaf oil **Saponification value** of 201.21mg KOH/g of oil (Table 2) falls within the range of the edible palm oil (195-205 mg KOH/g) but below the value for coconut oil (250-255 mg KOH/g) and *Irvingia gabonesis* (239.83 mg KOH/g) [20]. The **Peroxide value** is 16.17 meq of active Oxygen/kg oil and this value is higher than that of peanut oil (10 meq of active Oxygen/kg oil) but lower than the value of olive oil (20 meq of active Oxygen/kg oil) [20]. It then means that the oil can be stored as long as palm oil and olive oil. **Iodine value** of 79.00 (g of 1/100g of oil) suggests that the oil is non-drying and the oil contains a few unsaturated bonds in the fatty acids of the oil hence low susceptibility to oxidative rancidity, Onyeike and Acheru [22]. However, *Aspilia africana* leaf oil contains more unsaturated fatty acids (69.63%) than saturated fatty acids (30.36%). **Acid value** for *Aspilia africana* leaf oil is low (0.86mg KOH/g of oil) compared to values reported for some seed oils: castor seed oil (15 mg KOH/g of oil), coconut seed oil (12.2 mg KOH/g of oil) and *Enterrobium cyclocarpum* (5.6 mg KOH/g of oil) [21].

The mineral content of the leaf oil (Table 2) shows that potassium is the predominant element (13,800.37ppm). Potassium had been reported as the predominant mineral present in agricultural products grown in Nigeria [21,23]. The level of potassium reported in this work confirms it. Calcium (11,550.56ppm), Magnesium (3,202.53ppm), Sodium (262.09ppm) and Copper (15.46ppm) are the next elements reasonably present in leaf of *Aspilia africana*. The leaf of *Aspilia africana* can sever as both macro and micro nutrients required for normal body functioning of the body system. It can sever as a source potassium to balance the high sodium intake resulting from food additives, calcium for strong teeth and bones, copper as an antioxidant and regulation of gene expression.

CONCLUSION

Analysis of *Aspilia Africana*, (a weed usually ignored and used as fodder by grazing goats) shows it to contain essential fatty acids linoleic an omega-6 and linolenic fatty acids an omega-3 as the combined major constituents. Processing of the leaf could give valuable and essential products for various industrial applications. Lorenzo's oil could be obtained from the oleic and erucic acids while behenic acid could be sourced for the cosmetic industry. The juice will make a good elixir due to omega-6 and omega-3 while the antioxidant property could be explored due to copper. The fatty acid and physicochemical results indicate that *Aspilia Africana* is an edible leaf.

Table 1. Relative fatty acid concentration (%) of *Aspilia Africana*

S/No	**Name**	**Concentration (%)**	**Ret Time (min)**
1.	Butyric Acid (C5:0)	Trace	9.04
2.	Caproic Acid (C6:0)	Trace	10.28
3.	Caprylic Acid (C8:0)	Trace	11.12
4.	Capric Acid (C10:0)	Trace	11.25
5.	Lauric Acid (C12:0)	Trace	13.04
6.	Myristic Acid (C14:0)	0.001	14.70
7.	Palmitic Acid (C16:0)	11.77	16.46
8.	Palmitolaic Acid (C16:1)	Trace	18.16
9.	Stearic Acid (C18:0)	15.10	19.69
10.	Oleic Acid (C18:1)	18.85	21.11
11.	Linoleic Acid (C18:2)	50.28	22.61
12.	Linolenic Acid (C18:3)	0.28	23.97
13.	Arachidic Acid (C20:0)	0.54	25.11
14.	Behenic Acid (C22:0)	2.95	26.36
15.	Erucic Acid (C22:1)	0.22	27.48
16.	Lignoceric Acid (24:0)	Trace	28.53
17.	**TOTAL**	**99.99**	

Table 2. Physicochemical properties of *Aspilia Africana*

S/No	Parameter	Leaf oil value
1	Free fatty acid (oleic)	8.84%
2	Saponification	201.21mg KOH/g of oil
3	Peroxide value	16.17 MEq of active Oxygen/kg oil
4	Iodine value	79.00 (g of 1/100g of oil)
5	Acid value	0.86mg KOH/g of oil
6	K (Potassium)	13,800.37ppm
7	Ca (Calcium)	11,550.56ppm
8	Mg (Magnesium)	3,202.53ppm
9	Na (Sodium)	262.09ppm
10	Cu (Copper)	15.46ppm

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