CLASSICAL, GC-MS AND FT-IR CHARACTERIZATION OF *AZANZA GARCKEANA* SEED OIL

Myina, O. M.*; Abdullahi, A. A. and Ogundiran, O. A.

Department of Chemical Sciences, Taraba State University, Jalingo, Nigeria.

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Abstract: The pharmaceutical and wellness industries have recourse to nature for guidance, inspiration and as a source of novel compounds to produce new consumer products. Seed oils provide renewable sources of high-value fatty acids for these industries. This work was carried out to establish the oil content of *Azanza garckeana* (*A. garckeana*) seeds (from Tula village, Kaltungo Local Government Area of Gombe State, Nigeria) and the composition of the oil. Soxhlet extraction of *A. garckeana* seed oil (AGSO) was carried out at 75 °C for 3 h using petroleum ether (60-80 °C) and 42.2 % oil yield was obtained. Thus, *A. garckeana* seed is a renewable source of seed oil comparable to palm kernel. The AGSO was analyzed for moisture/volatiles (4.0 %), specific gravity (0.926 at 25 °C), refractive index (1.4694 at 25 °C), saponification value (189.0 mg/g), iodine value (80.0 mg I_2/g), peroxide value (5.6 meq/kg), acid value (0.56 mg/g) and free fatty acid (0.28 mg/g) using classical standard (AOCS) methods. GC-MS analysis of theAGSO revealed its major fatty acids as: linoleic acid (30.0 %), palmitic acid (13.0 %), oleic acid (2.5 %), stearic acid (1.0 %), sterculic acid (0.25 %) and myristic acid (0.19 %). Other major components of the AGSO are *n*-hexane (43.6 %) and 5-bromo-2,4-bis(methylthio)-pyrimidine (7.8 %). By these results, AGSO is a nondrying oil with omega-6 polyunsaturated fatty acids (PUFA) dominating its fatty acid profile. This suggests that AGSO has the potential to lower risk of coronary heart disease (CHD), reduce total and low-density lipoprotein (LDL) cholesterol as well as correct insulin sensitivity and blood pressure, if used as a dietary replacement for saturated fatty acids. AGSO also presents as a veritable renewable source for *n*-hexane and a potentially bioactive pyrimidine derivative. Thus, AGSO has the potential of a natural source for nutraceuticals and vital daily supplements or therapeutic remedy in nutrition and/or healthcare.

Keywords: *Azanza garckeana*, seed oil, omega-6, polyunsaturated, fatty acids, nondrying oil

INTRODUCTION

For centuries, seed oils have been used by rural communities as food, medicine, for cosmetic applications and as fuel. Recently, there has been a renewed interest in these non-timber forest products (NTFPs) specifically for use in cosmetic formulations as a result of immense consumer pressure for innovative products. The demands of the healthcare professional industry for nutraceuticals and vital supplements for human health maintenance have led many researches to focus on finding nutraceuticals with plant origins [1]; highlighting the role of metabolites (primary and secondary) of edible plants and herbal medicine in the development of nutrient supplements for human maintenance.

The world production of seed oils in year 2006 was reported [2] as 127 million tons, presenting an increase of about 50 million tons compared to ten (10) years earlier. Within same period, annual production of animal fats (tallow, lard and butter) was approximately 22 million tons, with fish oil put at about one (1) million tons. These oils, together with seed oils, represent the world's natural oil supply. It is estimated that about 14 % of these fats and oils are used chemically and 6 % used as feed material.

Majority of seed oils are produced from four (4) crops: oil palm, soybeans, rapeseeds and sunflower seeds, which together accounts for approximately 79 % of the total production [2]. The seed oils of domesticated seed crops are major agricultural commodities that are used primarily for nutritional applications. In recent years, however, the production of biofuels and chemical feedstocks usage of these oils have put a sustained pressure on these primary

applications of seed oils for nutrition. This pressure is driven partly by sustained increase in cost of petroleum products, increased concerns about the adverse environmental impact of fossil fuel usage and the need to develop renewable domestic sources of fuel and industrial raw materials. Arising from these is also the need to develop sustainable alternative sources of nutritionally important fatty acids such as those typically derived from seed oils. Such new areas of use for seed oils presents very high pressure on the oil market generally, as well as survival of the foods, chemical, cosmetics and pharmaceutical industries.

Seed oils provide renewable sources of high-value fatty acids for chemical and health-related industries. The value and application of oils are determined largely by their fatty acid composition. There is a rich diversity of fatty acids present in the world's flora, many of which have potential usage in industries to produce high-value products. With few exceptions such as the waxes of Jojoba oil, seed oils consist, almost entirely, of triacylglycerol (TAG) esters containing three fatty acids (FAs) with chain length of $C_8 - C_{24}$, and C_{18} being the most common [3]. The pharmaceutical and wellness industries turn to nature for guidance, inspiration and as a source of novel compounds to produce new consumer products.

Information on African seed oils is gradually receiving sustained attention of researches with emphasis mostly on their cosmetic applications. Vermaak *et al.* [4] reported on the cosmetic applications of African seed oils of commercial importance: *Adansonia digitata* (Baobab), *Citrullus lanatus* (Khalahari melon), *Schinziophyton rautanenii* (Manketti/Mungongo), *Sclerocarya birrea* (Marula), *Trichilia emetica* (Mafura butter) and *Ximenia americana* (Sour plum).

Azanza garckeana (*A. garckeana*), known locally as *Goron Tula* (Hausa, Nigeria) is a valuable edible indigenous African fruit tree species. In Nigeria, it is found in Tula area of Kaltungo Local Government Area (LGA) of Gombe State. *A. garckeana* grows naturally in semi-arid areas and is an incidental component of many farming systems. The sweet, slimy flesh of the young fruit of *A. garckeana* is edible while the matured hairy fruit exudes edible pulp. A root decoction is drunk to relieve painful menstruation and to treat coughs and chest pains. An infusion of a mixture of the roots and leaves is dripped into the ear to treat earache or administered orally as an antiemetic [5].

Jacob *et al.* [6] reported on the proximate composition of *A. garckeana* fruits, showing carbohydrate content of 49 – 56 %; ascorbic acid 5.71 – 6.17 mg/g; fat content 0.0541 – 0.0543 %; and starch as well as selected metals (Fe, Mg, Ca & Mn). The physicochemical composition of *A. garckeana* seed has been reported [7]. The seed contains tannins (0.22 %), saponins (1.72 %), alkaloids (3.7 %), flavonoids (1.0 %), phenols (2.6 %), cyanogenic glycosides (0.33 %) and carotenoids (3.4 %). These antinutrients are very vital due to their multipurpose applications: medicinal, nutritional and genetical applications. There has not been any report on the quantification and characterization of *A. garckeana* seed oil (AGSO). This paper is, therefore, aimed at extraction and characterization of AGSO.

MATERIALS AND METHODS

Sample Collection and Preparation

A. garckeana fruits were collected from Mallam Garba farm in Tula village, Kaltungo LGA, Gombe State, Nigeria, and brought to the Research Laboratory, Department of Chemical Sciences, Taraba State University. The identity of the samples was confirmed as *A. garckeana* fruits by appropriate colleagues from the Department of Biological Sciences. The seeds were removed and sun-dried for 72 h, deshelled and sundried for 48 h. The dried deshelled seeds were pulverized into powder using porcelain mortar and pestle. The pulverized sample was stored in polyethene bags for analyses.

Oil Extraction

Extraction of AGSO was carried out according to standard methods [8] for oil extraction from plants. A portion (20 g) of the *A. garckeana* seed powder was weighed and quantitatively transferred into the thimble of a Soxhlet apparatus. Petroleum ether (60-80 ^{o}C, 150 mL) was measured into the flask, assembled and refluxed for 3 h at 75 ^{o}C. The assemblage was dismantled and the solvent removed using rotary evaporator. The extracted oil was weighed to a constant weight and the percentage yield calculated from equation (1).

$$\% \text{ Yield} = \frac{\text{wt. of oil obtained (g)}}{\text{wt. of powdered sample (g)}} \times 100 \quad (1)$$

Oil Analyses

Analyses for moisture, specific gravity, refractive index, iodine value, saponification value, peroxide value, acid value and free fatty acid of the AGSO extracted was carried out according to classical standard methods [8].

GC-MS Analysis

The GC-MS analysis of the extracted AGSO was carried out using Gas Chromatography (GC) machine (Agilent Technologies 7890 B) coupled to a mass selective detector (MSD, Agilent Technologies 5977A) equipped with a nonpolar Agilent HP-5MS (5 % phenyl methyl polysiloxane). Identification of components in the oil was based on the comparison of their mass spectra and retention time with data from literature and by computer matching with NIST and Willey Library as well as comparison of fragmentation pattern of the mass spectra data with those reported in literature.

FT-IR Analysis

Fourier transform infrared spectrophotometer (Agilent Technologies) was used for identification of active functional groups of the AGSO extracted.

RESULTS AND DISCUSSION

Average values for the results of the oil content and physicochemical determinations were as presented in Table 1 while major components of the extracted AGSO are presented in Table 2. The GC-MS chromatogram of the extracted AGSO and the corresponding spectra for the major components of the oil as well as the FT-IR spectrum of the oil are presented in Figures 1, 2 and 3, respectively.

Table 1: Percentage Yield and Physicochemical Characteristics of AGSO.

Parameters	Results
Yield (%)	42.2±0.02
Moisture (%)	4.0±0.01
Specific Gravity (25 °C)	0.926±0.01
Refractive Index (25 °C)	1.4694±0.02
Saponification Value (mL KOH/ g)	189.0±0.35
Iodine Value (mg I_2/g)	80.0±0.04
Peroxide Value (meq/kg)	5.6±0.00
Acid Value (mg/L)	0.56±0.01
Free Fatty Acid (mg/L)	0.28±0.01

Table 1 shows that *A. garckeana* seeds contain 42.2 % oil. This oil content level is higher than 25 % palm oil in a bunch reported for oil palm [9], and 20-22 % oil for Soybeans [10]. The percentage yield, however, compares well with the 42 % oil reported [11] for palm kernel. This result shows that *A. garckeana* seeds are a viable source of seed oil. Moisture content of oils is an important parameter in assessing quality of an oil sample. High moisture content (>10 %) indicates ease of spoilage and rancidity, and eventually short shelf-life due to instability during storage. The moisture (and volatile matter) content of the AGSO extracted is 4.0 % (Table 1). This value is lower than 6.5 % moisture reported [11] for palm kernel oil (PKO). The low moisture content suggests stability of AGSO during storage.

Iodine value (IV) of oils point to degree of unsaturated (double binding) molecular structure. IV increases with unsaturation and consequent susceptibility to oxidation. This parameter has a direct impact on the processing, shelf-life and suitable applications for fat-based products. It is also of a crucial interest for lubricants and fuel industries. High IV is associated with good quality edible oil [12]. Table 1 presents IV of 80.0 mg I_2/g for the AGSO extracted. This value is higher than 49-55 mg I_2/g and 14-21 mg I_2/g reported for palm oil [13] and PKO [14, 13] respectively; and lower than 120-139 mg I_2/g reported [15] for soybean oil. The IV of AGSO, however, compares favorably with that of castor oil (81-89 mg I_2/g) and jojoba oil (80-85 mg I_2/g). This result show that AGSO is a rich source of

polyunsaturated fatty acids (PUFA) known to be beneficial to health and helps in regulating (lowering) blood cholesterol level and high blood pressure. Classification of oils by IV categorizes AGSO as a non-drying oil (IV < 125 mg I_2/g).

Free fatty acids (FFA), acid value and peroxide value are critical parameters considered in quality assessment of fats and oils' nutritional and industrial applications. FFA in oil samples arise from hydrolysis of the triglyceride molecules caused by high moisture levels. This leads to rancidity of the oil, which renders its refining process less efficient. Acid value is indicative of the amount of FFA present in the oil. Low FFA and acid value indicates virginity of the oil, and suggests its edibility and good shelf-life without spoilage through oxidative rancidity [16]. The FFA and acid value of the AGSO extracted is 0.28 mg/L and 0.56 mg/L respectively (Table 1). Peroxide value quantifies the peroxides and hydroperoxides present in the oil sample. It is also indicative of oxidative rancidity of the oil. Good quality oil must have a peroxide value less than 10 meq/kg [17]. The peroxide value of AGSO extracted is 5.6 meq/kg, meaning its good quality oil. The saponification value (189 mg/g) of the AGSO suggests it could be used for soapmaking.

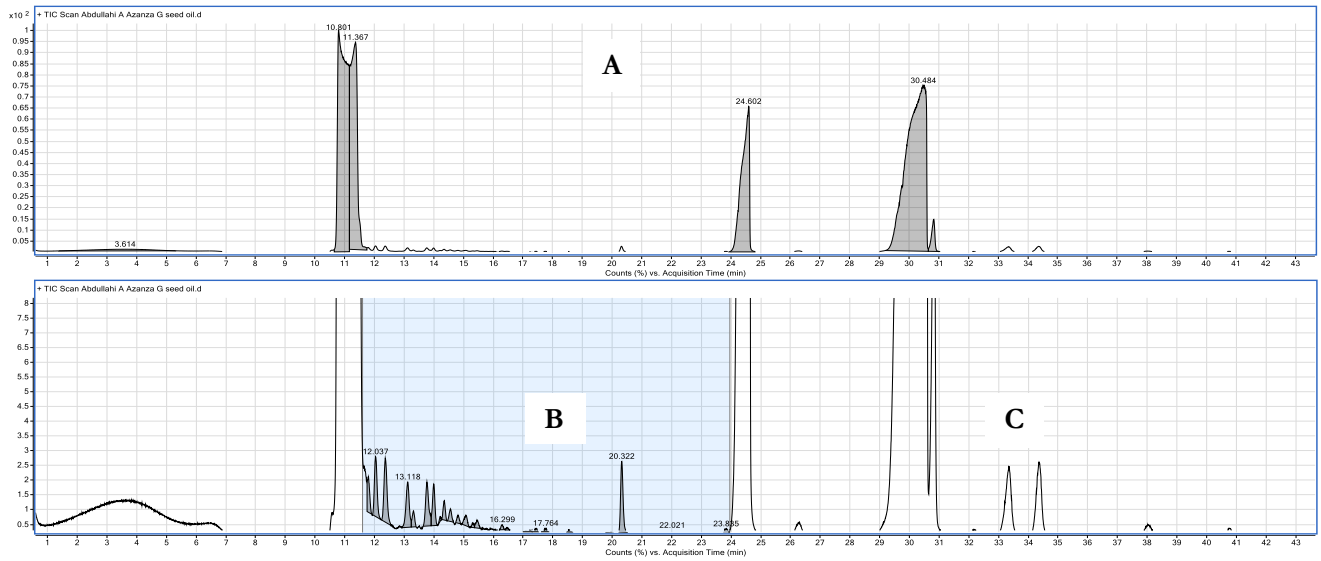

Figure 1: GC-MS Chromatogram of the Extracted AGSO: Normal Chromatogram (A) and Zoomed Chromatogram (B & C)

The AGSO chromatogram (Figure 1) marked A, is the normal size chromatogram while that marked B and C is the zoomed chromatogram to reveal more about the peaks in the marked regions. The chemical components of the oil associated with the peaks of the chromatogram are presented in Table 2 in order of increasing retention time i.e., decreasing volatility. The chromatograms and structures of these respective components are presented in Figures 2a–2n. Of the components identified, fatty acids top the composition.

Among functional food ingredients having noteworthy effects on human health promotion, fatty acids and phytosterols are the most important compounds in terms of nutritional value. Unsaturated fatty acids (UFA) have beneficial impact on incidence of cardiovascular disease and diabetes. There is strong evidence that replacing saturated fatty acids with UFA lowers the risk of coronary heart disease (CHD) very effectively [18]. The composition AGSO is dominated by UFA, with linoleic acid (9, 12-Octadecadienoic acid), a polyunsaturated fatty acid (PUFA) – an omega-6 PUFA – taking the lead (30.0 %). Oleic acid (9-Octadecenoic acid (Z)), a monounsaturated fatty acid (MUFA) is at 2.5 % in composition of the oil. This suggests that AGSO is a good source of UFA (especially PUFA) and consequently, a heart-friendly oil. Replacement of saturated fatty acids with linoleic acid, in particular, reduces total and low-density lipoprotein (LDL) cholesterol as well as improves insulin sensitivity and blood pressure [19-21]. Dietary linoleic acid intake is inversely associated with CHD risk in a dose-response manner [22]. In cosmetics, linoleic acid is the most used fatty acid as it moisturizes the skin, aids in the healing process of dermatoses and sunburns, and it's used for the treatment of *Acne vulgaris* [4]. AGSO may, therefore, be beneficial in acne lesion reduction as the anti-inflammatory effects have been shown to inhibit *Propionibacterium acnes*. The second dominant fatty acid contained in this oil is palmitic acid (Hexadecanoic acid), a

saturated fatty acid (13.0 %). Other fatty acids in the composition of this oil are stearic acid (Octadecanoic acid, 1.0 %), sterculic acid (2-Octylcyclopropene-1-octanoic acid, 0.25 %) and myristic acid (tetradecanoic acid, 0.19 %).

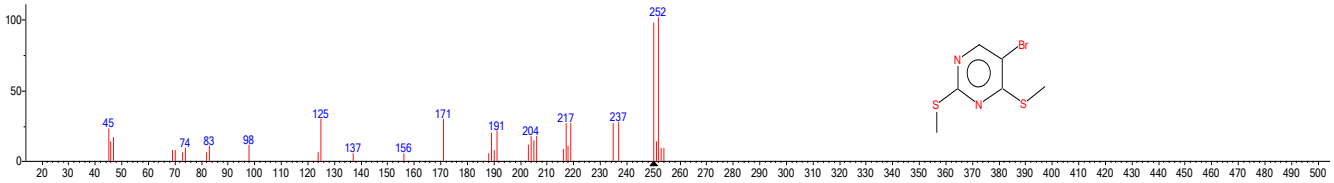

Figure 2a: GC-MS Chromatogram of 5-bromo-2,4-bis(methylthio)- Pyrimidine

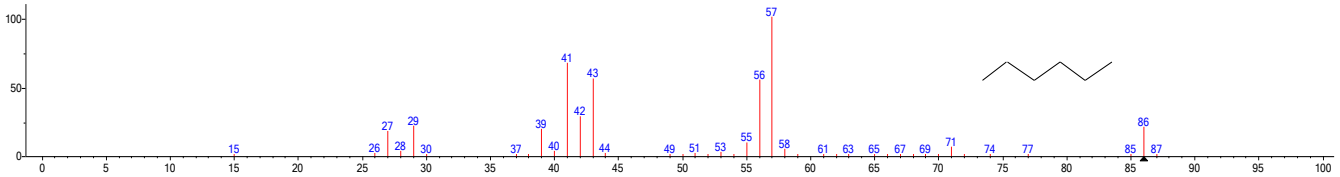

Figure 2b: GC-MS Chromatogram of *n*-Hexane

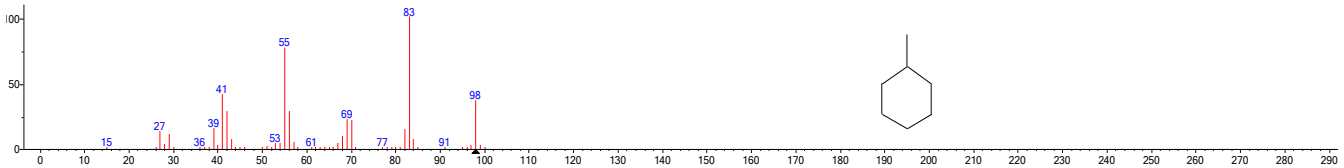

Figure 2c: GC-MS Chromatogram of Methyl Cyclohexane

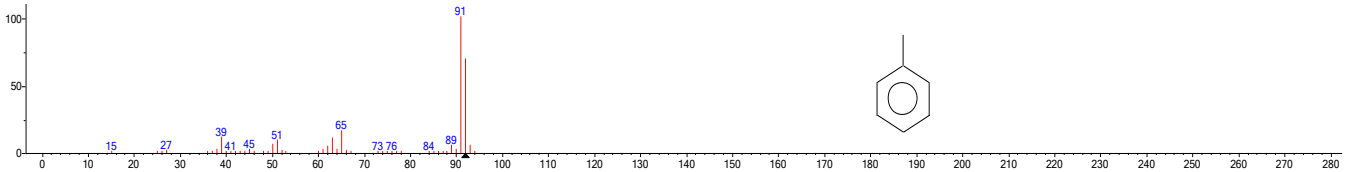

Figure 2d: GC-MS Chromatogram of Toluene

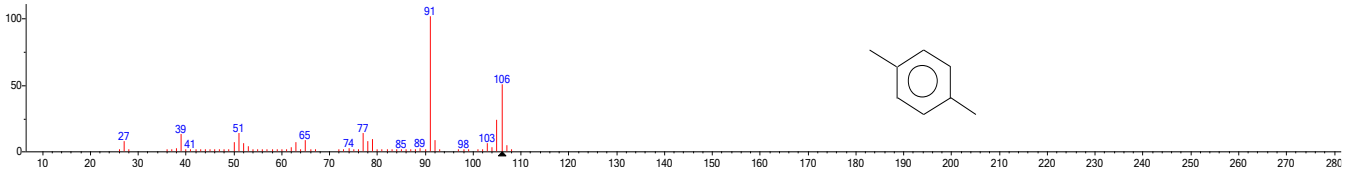

Figure 2e: GC-MS Chromatogram of *p*-Xylene

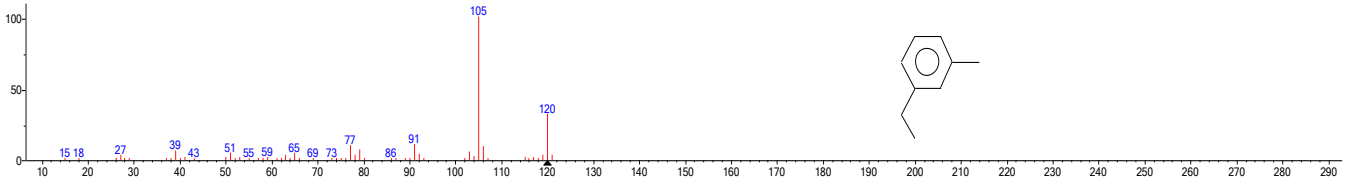

Figure 2f: GC-MS Chromatogram of 1-Ethyl-3-Methyl Benzene

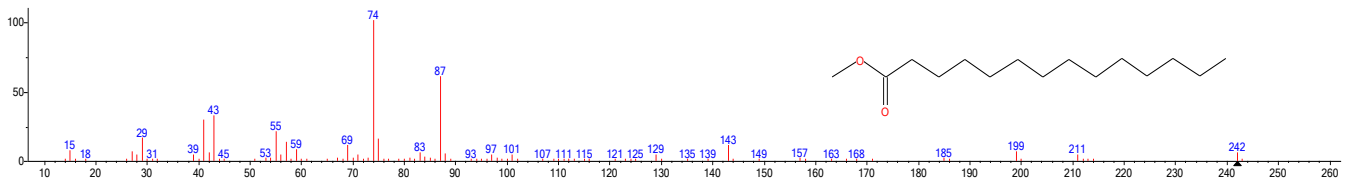

Figure 2g: GC-MS Chromatogram ofTetradecanoic Acid, Methyl Ester

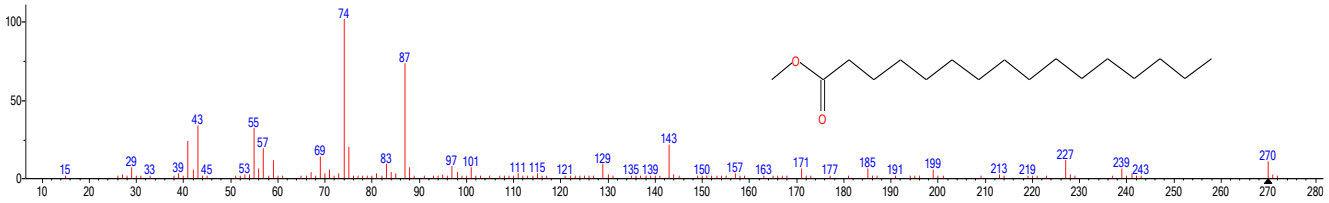

Figure 2h: GC-MS Chromatogram ofHexadecanoic Acid, Methyl Ester

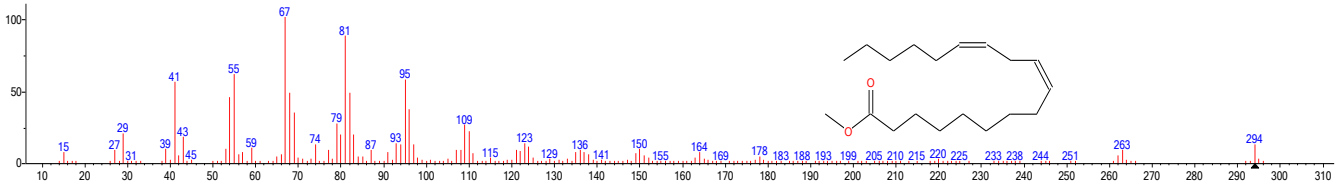

Figure 2i: GC-MS Chromatogram of 9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester

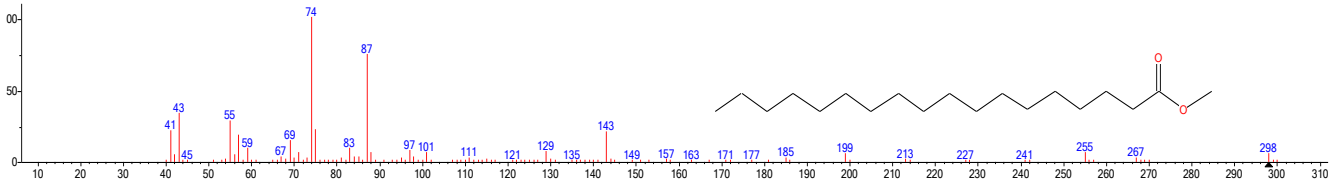

Figure 2j: GC-MS Chromatogram ofOctadecanoic Acid, Methyl Ester

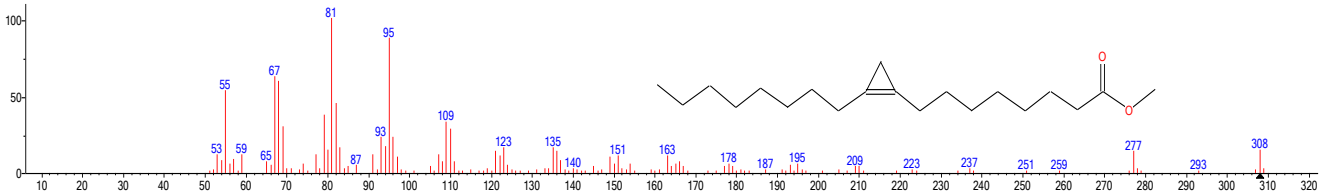

Figure 2k: GC-MS Chromatogram of 2-Octylcyclopropene-1-Octanoic Acid, Methyl Ester

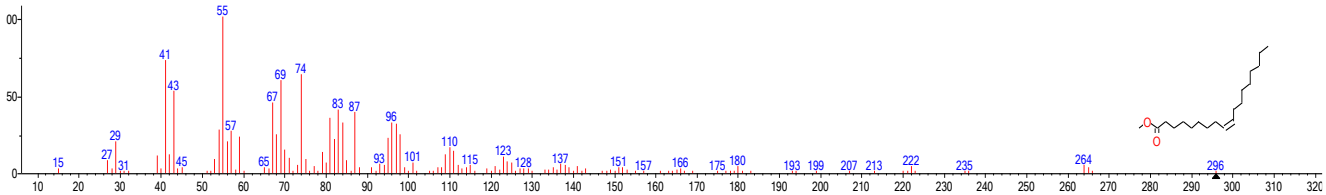

Figure 2n: Chromatogram of 9-Octadecenoic Acid (Z)-, Methyl Ester

Table 2: Composition of AGSO

S/No.	Name of Compound	Retention Time (min)	Molecular Formula	Quantity (%)
1.	5-bromo-2,4-bis(methylthio)-pyrimidine	3.614	$C_6H_7BrN_2S_2$	7.81
2.	*n*-Hexane	11.084	C_6H_{14}	43.64
3.	Methylcyclohexane	12.037	C_7H_{14}	0.29
4.	Toluene	12.363	C_7H_8	0.34
5.	*p*-Xylene	13.118	C_8H_{10}	0.25
6.	1-Ethyl-3-methylbenzene	13.879	C_9H_{12}	0.19
7.	Tetradecanoic acid (myristic acid)	20.322	$C_{14}H_{28}O_2$	0.19
8.	Hexadecenoic acid (palmitic acid)	24.602	$C_{16}H_{32}O_2$	13.00
9.	9,12-Octadecadienoic acid (linoleic acid)	30.484	$C_{18}H_{32}O_2$	30.00
10.	Octadecanoic acid (stearic acid)	30.833	$C_{18}H_{36}O_2$	1.00

11.	2-Octylcylopropene-1-octanoic acid (sterculic acid)	33.357	$C_{20}H_{36}O_2$	0.25
12.	9-Octadecenoic acid (Z) (oleic acid)	34.364	$C_{18}H_{34}O_2$	2.52
13.	Total saturated fatty acids			13.19
14.	Total unsaturated fatty acids			33.77

Other major components of the oil are 5-bromo-2,4-bis(methylthio)- pyrimidine (7.8 %) and *n*-Hexane (43.6 %). Pyrimidine derivatives and *N*-phenyl-2- derivatives having pyrimidine moiety in their core structures have been reported [23] to demonstrate potent inhibitory activity against SARS-CoV 3CL^{Pro}. Also reported is the potent inhibitory activity of metal (especially zinc) conjugated compounds against SARS-CoV 3CL^{Pro}. These suggest AGSO as a sustainable alternative source for a pyrimidine derivative, which could be employed in biogenic synthesis of enhanced small molecule chemotherapy for severe acute respiratory syndrome – coronavirus (SARS-CoV). This is in the works in our laboratory. The level of *n*-hexane presents this oil as a veritable source of an important member of alkanes, and by extension, hydrocarbons. Alkanes such as hexadecane, octadecane and eicosane were also reported [18] in the composition of *Crotaegus aronia* (Yellow azarole), *Cydonia oblonga* (Quince), *Entada rheedi* (Snuff box sea bean), *Lallemantia royleana* (Balangu), *Phalaris minor* (Small canary grass), *Quercus brantii* (Persian oak) and *Scutellaria laterflora* (Blue skullcap) seed oils. Chacon Fernandez *et al.* [24] also reported presence of hexane in *Tamarindus indica* (tamarind) seed oil. Other hydrocarbons in the AGSO composition are toluene (0.34 %), methyl cyclohexane (0.29 %), p-xylene (0.25 %) and 1-ethyl-3-methylbenzene (0.19 %).

Other compounds detected with peak areas lower than the quantification limit includes: 4-methyl-1-hexene,(S)-Spiro[4.4]nona-1,6-diene, trans-3-Caren-2-ol; Carveol; 4-ethyl-1,2-dimethyl benzene; 8,10-octadecadiynoic acid; 7,9-octadecadiynoic acid; 13,16-octadecadiynoic acid; 1,2-dihydro-2-naphthalenol; 3-[(1-phenylethyl-2-propynyl) oxy] butanoic acid; (7R,8R) -ethyl-8-hydroxy-trans-bicyclo [4.3.0] -3-nonene-7-carboxylate; 6-methy l-4-[(4-methylphenyl) sulfonyl]-5-heptenoic acid; 7-Hexadecenoic acid; 13-Tetradecynoic acid; 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-cyclopropane octanoic acid; 3-pentadecyl-3-chloropropionate and tridecanoic acid.

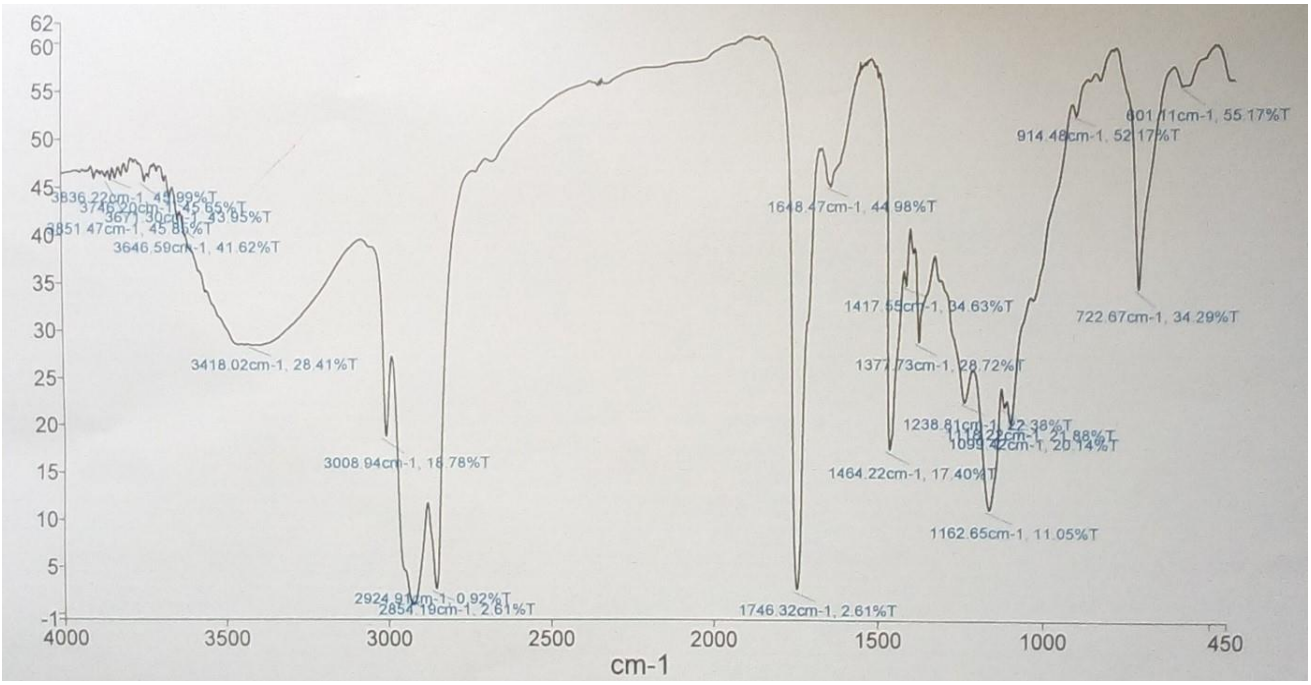

Figure 3: FT-IR Spectrum of the Extracted AGSO

In the FTIR spectrum of AGSO (Figure 3), absorptions in the region of high frequencies (4000 – 3500 cm^{-1}) corresponds to hydroxyl groups, including water (residual moisture in the sample) is identified. The strong signal at 1746.32 cm^{-1} corresponds to the carbonyl groups due to ester bonds of fatty acids and glycerol of the triglycerols of the oil, including free fatty acid carbonyl bonds from free fatty acids present in the oil. Bending vibrations of methyl

and methylene hydrogen are seen at 1464.22 cm^{-1} and 1377.73 cm^{-1}. Vibrations of the C–O bonds are seen at 1238.81, 1162.65 and 1099.42 cm^{-1}. The farther region of low frequencies (750 – 450 cm^{-1}) is considered the area where a group of signals due to absorption of characteristic groups of lipid compounds including benzene derivatives appear [25, 24].

CONCLUSION

This investigation has demonstrated that *A. garckeana* seed is a veritable renewable source of seed oil comparable to palm kernel. The oil is the nondrying type with polyunsaturated fatty acids dominating its fatty acid profile. Other than fatty acids, the oil also contain a good percentage of hexane and a fair amount of a potentially bioactive pyrimidine derivative. The *A. garckeana* seed oil, therefore, has the potential of a natural source for nutraceuticals and vital daily supplement or therapeutic remedy in nutrition and/or healthcare.

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