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Extraction and Characterization of Vegetable Oil from 20 Accessions of *Allanblackia parviflora* Seeds in Ghana

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Abstract
The benefits derived from the forest estate in Ghana include Non-Timber Forest Products (NTFPs). NTFPs such as vegetable oil produced from seed of forest trees have been used for centuries. The oil from the seed kernel of *Allanblackia parviflora* (Allanblackia) is investigated for differences in the yield and quality between accessions. Vegetable oil from twenty (20) accessions of Allanblackia were extracted and characterized. The oil yields from the different accessions were all above 60% and no significant differences were detected amongst the accessions. The iodine value was very low indicating a very saturated oil whilst the refractive index, specific gravity and acid values were all within the range of most edible oils. The vegetable oil contains over 60% saturated fatty acids in the form of stearic acid (C18:0) and over 30% unsaturated fatty acid in the form of oleic acid (C18:1), and this may be the reason for the oil been solid at ambient room temperature.

Keywords: *Allanblackia parviflora*, vegetable oil, Non-Timber Forest product, physicochemical properties

Extraction et Caractérisation de l'huile végétale de vingt (20) accessions de graines *d'Allanblackia parviflora* au Ghana

Résumé
Les avantages tirés du domaine forestier au Ghana comprennent les Produits Forestiers non Ligneux (PFNL). Les PFNL tels que l'huile végétale produite à partir des graines d'arbres forestiers sont utilisés depuis des siècles. L'huile provenant de l'amande des graines *d'Allanblackia parviflora* (Allanblackia) est étudiée pour les différences de rendement et de qualité entre les accessions. L'huile végétale de vingt (20) accessions d'Allanblackia a été extraitée et caractérisée. Les rendements en huile des différentes accessions étaient tous supérieurs à 60% et aucune différence significative n'a été détectée entre les accessions. L'indice d'iode était très faible, ce qui indique une huile très saturée, tandis que l'indice de réfraction, la gravité spécifique et les valeurs d'acide se situaient tous dans la gamme de la plupart des huiles comestibles. L'huile végétale contient plus de 60 % d'acides gras saturés sous forme d'acide stéarique (C18:0) et plus de 30 % d'acides gras insaturés sous forme d'acide oléique (C18:1), ce qui peut expliquer pourquoi l'huile est solide à température ambiante.

Mots-clés: *Allanblackia parviflora*, huile végétale, produit forestier non ligneux, propriétés physico-chimiques.

Introduction

The tropical forest provides so many benefits to the world. These benefits include acting as carbon sink (Porter *et al.,* 2009); and providing lumber (Mmolotsi and Teklehaimanot, 2006), fuel (Korang et al., 2015), medicine (Kemigisha et al., 2018) and food (Asprilla-Perea and Díaz-Puente, 2019). Aside lumber, the rest are called Non-Timber Forest Products (NTFPs) (Mugido and Shackleton, 2019). The NTFPs are often overlooked as important service when analysing the importance of the forest. Additionally, many indigenous tree species are valuable sources of raw materials for various industries. These uses include extraction from seeds, backs, roots and leaves from trees for food (Licata *et al.,* 2018), medicine -(Lasisi et al., 2015) and fibre for pulp (Fortunati et al., 2013).

In Ghana and other countries within the sub-region, there are several forest plant species that have seeds from which oil could be produced (Tiétiambou *et al,* 2020). Most of these vegetable oils have been extracted but only a few are commercially significant. The demand for low-priced edible fats and oils has been rising steadily in most countries, including Ghana. This is partly due to the competition between the industrial requirement for oil and their use for edible purposes. It has therefore become imperative to find alternative sources of oils from unexploited and underutilized plants, which can also serve both domestic and industrial purposes.

Allanblackia parviflora (Allanblackia) belongs to the Guttiferae family, subfamily Clusiaceae (Crockett, 2015), and it is a non-timber forest product (NTFP) which can be found as wild and undomesticated tree species in the rainforests of African countries such as Nigeria, Kenya, Congo, Angola, Cameroon, Tanzania, Uganda and Ghana —' (Schmidt *et al.,* 2020; Yeboah et al., 2016). Allanblackia is an oil rich tree crop that is used for medicinal purposes and as shade tree in cocoa farms. In Ghana, it is mostly found in the forests of the Western, Ashanti and Eastern regions (Yeboah et al., 2016).

Vegetable oil is obtained from Allanblackia seed, while the bitter seedcake can be used as an animal feed (Sefah *et al.,* 2020; Sefah et al., 2019). Traditionally, oils extracted from seeds have been used locally for cooking, preparing medicines and making soap at a subsistence level. Several properties of this oil, including high melting point and better food value among others, may make it an important alternative to palm oil. The increase in demand has come from potential uses of the oil identified by the international company Unilever for use in food products like margarine, vegetable-based dairy products and ice cream " (Turck *et al.,* 2018). Allanblackia oil has the potential as a foreign exchange earner if it would be exported as non-traditional product. Hence, many African countries notably Ghana, Nigeria, Cameroon and Tanzania are collaborating with Non-Governmental Organizations in domesticating the Allanblackia tree to develop an integrated rural based enterprise to improve the livelihoods of forest fringe communities (Attipoe *et al.,* 2006). Domestication of Allanblackia for vegetable oil production requires the supply of good planting materials; investigators are still searching for accessions that produce excellent yield of oil with superior physicochemical properties. These accessions will be used as seed sources for the supply of seedlings for the domestication process.

This study was therefore undertaken to determine the vegetable oil yield and physicochemical properties of vegetable oil from different *Allanblackia parviflora* accessions in Ghana.

Materials and Methods

Sources of raw materials

Allanblackia parviflora (Allanblackia) fruits were collected from three provenances in Ghana, namely: Benso Pataho (BNP) in the Moist Evergreen Forest, Wasa Akropong Dikoto (WAD) in the Moist Semi Deciduous Rainforest and New Edubiase Anwona (NEA) in the Semi Deciduous Rainforest ecological zones. Ripe mature fruits were allowed to drop naturally from trees and the fruits collected from the forest floor. A total of 20 accessions were collected from the tree ecological zones. The list of samples is shown in Table 1.

The seeds used for the study were removed from ripe mature fruits manually through maceration. The seeds were dried in the sun for three days. The seeds were then manually dehulled to obtain the seed kernel and milled into fine powder.

Oil extraction

The milled seed kernel was placed in a Soxhlet extractor. Using petroleum ether as solvent, the extraction was performed for 16 hours. The solvent was evaporated using a rotary vacuum evaporator. The solvent traces were eliminated by drying the oil in an oven at 103 °C. The yield of Allanblackia oil was calculated using the following formula:

$$\text{Percentage yield (\%)} = \frac{M_1 - M_0}{M} * 100$$

With,

Table 1: Sample of Allanblackia

Benso Pataho (BNP)	Wasa Akropong Dikoto (WAD)	New Edubiase Anwona
NP 1	WAD 1	NEA 15
BNP 6	WAD 2	NEA 31
BNP 13	WAD 3	
BNP 14	WAD 9	
BNP 18	WAD 11	
	WAD 13	
	WAD 14	
	WAD 19	
	WAD 20	
	WAD 24	
	WAD 29	
	WAD 36	
	WAD 40	

M_1: Mass of flask containing oil, M_0: Mass of empty flask, M: Mass of milled seed kernel used

Characterization of Allanblackia seed oil

The physicochemical properties of the Allanblackia oil were determined using official methods and recommended practices of the American Oil Chemists' Society (AOCS). The specific gravity was measured at 60 / 25°C using the specific gravity bottle based on AOCS method Cc 10a-25 (AOCS, 1993), whilst the refractive index was determined at 60°C using an optical refractometer (model: Bellingham + Stanley Limited, 60/70).

Free fatty acids and acid value were determined using the AOCS method Ca 5a-40, (AOCS, 1993) and the procedure described by Kirk and Sawyer (1991), respectively. The iodine and saponification values were determined using the AOCS methods Cd 1-25, (AOCS, 1993) and Cd 3-25, (AOCS, 1993) respectively.

Triacylglycerol (TAG) was determined using High Performance Liquid Chromatography (HPLC). Non-aqueous reverse-phase HPLC separations were performed on a Dionex Ultimate 3000 model with ELSD detector. The mobile phase used was acetone: acetonitrile (63.5:36.5) and the flow rate, column temperature, detector temperature and analysis time were 1 mL/min, 30°C, 40°C and 30 minutes, respectively. Allanblackia seed oil samples (2 mL containing 0.1 mL of oil dissolved in mobile phase solvent) were injected and each TAG peak was identified by comparing with standard TAGs based on equivalent carbon number (ECN) and characteristic retention time.

Statistical analysis

The data obtained were subjected to statistical analyses using analysis of variance (ANOVA). Differences among the mean values were determined using Duncan's multiple range test.

Results and Discussion

The oil from Allanblackia seeds is the main product leading to the domestication of the tree. The seeds contain vegetable oil/fat that is solid at ambient temperatures. Table 2 shows the yield of Allanblackia oil from twenty (20) different accessions in Ghana that were investigated. The oil content from these accessions was found to range between 60.35 - 64.67%. Allanblackia tree can therefore be classified as an oleaginous plant with high content in oil matter. The oil obtained from the Allanblackia seeds by this method was comparatively higher since most commercial oil-bearing seeds have oil yields of about 30 - 40% and above(Licata et al., 2018). The range of seed oil content for these Allanblackia accessions generally exceed oils from cottonseed (Khan *et al.,* 2010), kenaf seed (Cheng *et al.,* 2016) and mango kernel (Yadav et al., 2017). The accession, WAD 9, had the lowest oil content of 60.35% and the highest oil content was 64.67 for NEA 31. Statistical analysis showed no significant differences (p>0.05) amongst the different Allanblackia accessions for oil content. The little variations in the yield of oil from different accessions of Allanblackia tree might be attributed to minimum diversity among the Allanblackia tree accessions.

Characterization of Allanblackia seed oils

The results for physicochemical parameters of

Table 2: Allanblackia seed oil yield

Sample	Oil yield (%)
BNP 1	63.33±0.094
BNP 6	62.56±0.138
BNP 13	64.29±0.156
BNP 14	63.29±0.162
BNP 18	61.65±0.022
WAD 1	63.51±0.158
WAD 2	61.41±0.099
WAD 3	62.15±0.096
WAD 9	60.35±0.083
WAD 11	63.89±0.586
WAD 13	61.37±0.113
WAD 14	63.29±0.063
WAD 19	62.35±0.031
WAD 20	64.16±0.079
WAD 24	61.35±0.130
WAD 29	63.32±0.049
WAD 36	62.30±0.066
WAD 40	61.35±0.094
NEA 15	63.31±0.069
NEA 31	64.67±0.079

Allanblackia seed oil are presented in Table 3. The refractive index of the oil for the different accessions ranged from 1.4482-1.4538. These values of refractive index of allanblackia seed oils were below what was reported by Sefah et al. (2010), but were fairly comparable to those of corn oil, olive oil, palm oil and soybean oil '(Endo, 2018). Statistical analysis showed no significant differences ($p>0.05$) amongst the Allanblackia accessions in respect of the oil refractive index.

Another physical property determined was the specific gravity of the Allanblackia oil. The specific gravity of the Allanblackia oils ranged from 0.79 - 0.89 g/cm^3. Statistical analysis showed no significant differences ($p<0.05$) in the values for densities of oils from the different accessions. These values for specific gravity of Allanblackia oils were similar to what was reported by Sefah et al (et al., 2010). However, the specific gravity of the oil was lower than that of other edible oils '(Endo, 2018).

The chemical properties investigated were

Table 3: Physicochemical characteristics of the oil samples

Sample	Refractive index	Specific gravity (60/26°C)	Acid value mg KOH/kg	Saponification value (mg KOH/g)	Iodine value (mg I_2/g)
BNP 1	1.4512±0.0003	0.868	0.50	195	55.70
BNP 6	1.4482±0.0030	0.871	0.47	197	81.19
BNP 13	1.4512±0.0014	0.866	0.48	195	55.70
BNP 14	1.4495±0.0000	0.865	0.50	195	70.02
BNP 18	1.4510±0.0000	0.872	0.48	185	57.13
WAD 1	1.4538±0.0019	0.890	0.47	200	32.79
WAD 2	1.4512±0.0003	0.867	0.46	197	55.70
WAD 3	1.4512±0.0003	0.876	0.47	200	47.11
WAD 9	1.4507±0.0006	0.876	0.50	197	59.99
WAD11	1.4515±0.0005	0.886	0.50	200	52.84
WAD13	1.4512±0.0006	0.887	0.50	198	55.70
WAD14	1.4503±0.0010	0.865	0.49	197	62.86
WAD19	1.4505±0.0005	0.867	0.50	199	61.43
WAD20	1.4523±0.0003	0.866	0.50	197	45.68
WAD24	1.4513±0.0008	0.882	0.47	195	54.27
WAD29	1.4522±0.0003	0.868	0.47	200	51.40
WAD36	1.4517±0.0006	0.873	0.49	198	55.70
WAD40	1.4503±0.0003	0.863	0.47	200	62.86
NEA 15	1.4522±0.0013	0.792	0.50	197	47.11
NEA 31	1.4505±0.0009	0.886	0.50	196	61.43

acid, iodine and saponification values. The acid value (AV) is a measure of the extent to which the triacylglycerides in the oil have been decomposed by lipase action '(Endo, 2018; Inekwe et al., 2012). The AV is often used as a general indication of the condition of edibility of the oil. Edible oil should not exceed 4 mg/g but lower than 0.1 for refined edible fats and oil '(Endo, 2018). From the results, the acid values of all the samples ranged from 0.4-0.5 mg KOH/g. The lower the acid value of oil, the fewer free fatty acids it contains which makes it less exposed to rancidity. The acid values obtained for the oils are similar to that reported by Sefah et al (et al., 2010) and were lower than AV reported for African locust seed oil, Castor seed oil, soybean, coconut and palm kernel — '–(Chioma et al., 2020; Olowokere *et al.,* 2019; Salimon et al., 2010).

Saponification value (SV) is a measure of the mean molecular weight of triacylglycerols present in the fat or oil. A higher SV is a measure of low-molecular weight triacylglycerols of edible fats and oils. Most vegetable oils such as corn, olive, rapeseed and soybean oils have an SV of about 190, whereas the SV of palm oil and coconut is more than 200 '(Endo, 2018). Fats and oils with very high the SV are rich in palmitic acid (16:0), myristic acid (14:0) and lauric acid (12:0). The SV of all the crude oil extracts was found to be in a range of 185-200 mg KOH/g. The value of the Allanblackia seed oils agrees with SV of African locust seed oil, castor seed oil, soybean, coconut and palm kernel — '–(Chioma et al., 2020; Olowokere *et al.*, 2019; Salimon *et al.,* 2010).

The iodine value (IV) obtained ranged from 32.79 to 81.19 g I_2/100 g of fat. This is a measure of the degree of unsaturation in a fat or vegetable oil and it is expressed in terms of the number of grams of iodine absorbed per 100 grams of oil sample. The wide variations in the IV results may be due to the varying amount of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in oils from the different accessions. The oil shows a relatively low iodine value due to its high content of saturated fatty acids. The iodine value obtained is relatively lower than that of commonly used seed oils such as corn oil, olive oil, palm oil and soybean oil '(Endo, 2018). The low IV makes the Allanblackia oil very stable at ambient temperature, explaining why the oil is solid at ambient temperature. The stability of Allanblackia oil at ambient conditions will make it very useful for industrial application such as oil paints manufacturing and as a dietary supplement.

Fatty acid profile of *Allanblackia* oil

Table 4 shows the results of fatty acid composition of the Allanblackia seed oils extracted from the different accessions. The Allanblackia oil contains palmitic, γ-linolenic and linoleic acid. The palmitic (C16:0), linoleic (C18:2), γ-linolenic (C18:3), stearic (C18:0) and oleic acid (C18:1) contents of Allanblackia oil has been reported to be < 2 %, < 1 %, < 1 %, 45-58% and 40-51% respectively" (Turck *et al.,* 2018). The values obtained in this investigation are within the reported ranges. The predominant fatty acids in Allanblackia oil are oleic acid and stearic acid with the latter being the majority.

The types of fatty acid present in the oil infer some physical properties to the fat/oil. The result indicates very high composition of saturated fatty acid (SFA) with a range of 61-65%, followed by mono unsaturated fatty acid (MUFA) with 34-37% and polyunsaturated fatty acid having very low range of 1-2%. The very high composition of SFA and MUFA increases the stability of the oil at ambient temperature, since the oil will be difficult to oxidize. This may also be the reason why the Allanblackia oil is very stable and solid at room temperature.

Conclusion

A. parviflora is a potential source of high-quality oil for both domestic and industrial application. The yields of vegetable oil from the different accessions investigated were all above 60% and no significant differences were detected among them. The iodine value was low indicating a high saturated fatty acid composition whilst the refractive index, specific gravity and acid values were all within range of most edible oils. It contains high content of saturated fatty acids resulting in high melting, low rate of rancidity and solid at ambient room temperature. The prominent fatty acids present in the vegetable oils are stearic acid and oleic acid.

Table 4: Fatty acid composition of Allanblackia seed oil

Sample	C16:0	C18:0	Total SFA[a]	C18:1	Total MUFA[b]	C18:2	C18:3	Total PUFA[c]
BNP 1	1.36	61.18	62.54	36.21	36.21	0.62	0.63	1.25
BNP 6	1.54	60.53	62.07	36.64	36.64	0.65	0.64	1.29
BNP 13	0.97	61.94	62.91	35.87	35.87	0.59	0.63	1.22
BNP 14	1.24	61.82	63.06	35.66	35.66	0.64	0.64	1.28
BNP 18	1.36	61.77	63.13	35.65	35.65	0.63	0.59	1.22
WAD 1	1.15	62.79	63.94	34.76	34.76	0.67	0.63	1.3
WAD 2	1.28	60.91	62.19	35.86	35.86	1.34	0.61	1.95
WAD 3	0.98	60.96	61.94	36.78	36.78	0.68	0.60	1.28
WAD 9	1.23	60.64	61.87	36.75	36.75	0.69	0.69	1.38
WAD11	1.34	61.22	62.56	36.22	36.22	0.61	0.61	1.22
WAD 13	1.15	61.17	62.32	36.35	36.35	0.66	0.67	1.33
WAD 14	1.11	61.15	62.26	36.44	36.44	0.63	0.67	1.3
WAD 19	1.11	62.45	63.56	35.16	35.16	0.64	0.64	1.28
WAD 20	1.33	61.38	62.71	35.54	35.54	0.68	1.07	1.75
WAD 24	0.89	63.35	64.24	34.54	34.54	0.55	0.67	1.22
WAD 29	0.88	61.93	62.81	35.97	35.97	0.67	0.55	1.22
WAD 36	1.42	62.55	63.97	34.78	34.78	0.63	0.62	1.25
WAD 40	1.15	61.34	62.49	36.23	36.23	0.60	0.68	1.28
NEA 15	1.31	59.96	61.27	36.78	36.78	0.61	1.34	1.95
NEA 31	1.22	61.69	62.91	35.76	35.76	0.67	0.66	1.33

[a]**SFA**: Saturated fatty acid; [b]**MUFA**: Mono unsaturated fatty acid; [c]**PUFA**: Poly unsaturated fatty acid

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Conflict of Interest
The authors declare that there is no conflict of interest regarding the publication of this paper.

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