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Oil Content and Fatty Acid Composition of Dioclea reflexa Seeds

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Abstract: *Dioclea reflexa seeds belonging to the family called Fabaceae were evaluated for its oil content and fatty acid composition chemical composition. Proximate results indicated that D. reflexa seeds contained ash (9.17±0.01%), crude fat (10.65±0.01%), fiber (9.25±0.03%), moisture (13.30±0.01%), crude protein (26.00±0.43%), carbohydrate (31.63±0.12%) and calorific value (1372.07 KJ/100g. Minerals analysis showed that D. reflexa seeds contained potassium (4.09±0.04), sodium (4.88±0.25) magnesium (0.80±0.042), zinc (2.10±0.03), iron (0.48±0.05), manganese (0.37±0.61) and calcium (7.11±0.42) all in mg/l. Physico-chemical properties of the seeds revealed that it contains acid value (2.24±0.15), free fatty acids (1.13±0.15), iodine value (115.45±1.50), peroxide value (5.60±0.20), saponification value (318.63±2.4) and ester value (316.4±2.50). Among the ten fatty acids determined in D. reflexa seeds, $C_{18:1}$ gave the highest concentration of 31.495g/100g while $C_{20:2}$ had the least value of 1.594g/100g. The results of phytochemical screening showed that D. reflexa seeds contain alkaloids, cardiac glycoside, phlobatannin and saponin. This study showed that the Dioclea reflexa seeds contained alkaloid and glycosides which are of pharmacological significance and high levels of carbohydrate, crude protein and moisture content could serve as supplementary sources of essential nutrient to man and livestock.*

Keywords*: fatty acids, phytochemical, proximate composition, mineral element*

I. Introduction

Plants are being used as valuable sources of food and medicine for the prevention of illness and maintenance of human health (Aliyu et al., 2008). Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical importance. The suitability of any oil for a particular purpose, however, is determined by its fatty acid composition. No oil from any single source has been found to be suitable for all purposes because oils from different sources generally differ in their fatty acid composition. This necessitates the search for new sources of novel oils (Dagne, 1997). In addition to nutritional, industrial or pharmaceutical uses of oil, patterns of fatty acid variation in plants have proven to be useful tools in taxonomic and phylogenetic studies. There is an increasing interest in new sources of industrial oils, in the essential fatty acids of seed oils and in toxicity aspects of edible oils. Consumers consider fatty acid composition to be one of the important parameters of nutritional quality. Particularly, consumers are concerned with the saturated/unsaturated fatty acid ratio. Lipid composition of fruits and vegetables has lately received particular attention, especially in relation to the essential fatty acids (linoleic, linolenic and arachidic acids) with emphasis on the health potential of polyunsaturated (n-3) fatty acids. It is considered that these fatty acids play a natural prevention of some other health problems (Melgarejo et al., 1995). Vegetable oils provide energy and essential linoleic and linolenic acids that are responsible for growth (Fasina et al., 2006).

Dioclea reflexa belonging to the legume family called Fabaceae is native to West-central tropical Africa, South tropical Africa and Southern America. In Nigeria it is referred to as "Agbaarin" by the Yorubas and "Ufor" by the Igbos. The objective of this work is to assay the oil content and fatty acid composition of Dioclea reflexa seeds from Nigeria. Such information is important for possible future domestication and safe use for human consumption.

II. Materials And Methods

Collection and Identification of Plant

Fresh seeds of Dioclea reflexa, were purchased from Ojo market in Ibadan and Obada market, Tapa in Ibarapa North local Government, Oyo State, Nigeria. The plant seeds was identified and authenticated at Herbarium Unit of Botany Department, University of Ibadan, Oyo state, Nigeria. The seeds were sun-dried after which they were dehulled, milled into powder and the powder kept in an air-tight polythene bags

Proximate analysis

The moisture, crude fibre, crude protein, ash, crude fat and carbohydrate of D. reflexa seeds were determined using methods of the Association of Official Analytical Chemists (AOAC, 1984). All determinations were done in triplicates. The proximate values were reported in percentage

Physico-chemical properties

The physico-chemical properties of the seed oil were determined by the standard methods. All determinations were done in triplicates. Procedures for the determination of the iodine value (Wijs'method) were those recommended by the AOAC (1984). The methods of analysis for free fatty acid, saponification, peroxide and acid values are as outlined by Ajayi and Aghanu (2011). Colour and state of the oils at room temperature were noted by visual inspection. The refractive indices of the oils were determined using an Abbe refractometer while the specific gravity which was done at room temperature was estimated by the use of a specific gravity bottle following the method of Pearson (1976).

Mineral analysis

The mineral contents of Dioclea reflexa seeds: were determined following the method of Ajayi et al. (2007). Potassium and sodium was determined using flame photometer, while calcium, magnesium, iron, zinc and manganese were determined using atomic absorption spectrophotometer as described the methods of the Association of Official Analytical Chemists (AOAC, 1990) after appropriate digestion by acids.

Phytochemical screening

Phytochemical screening of the powdered sample of Dioclea reflexa seeds was carried out by a procedure that was based on those earlier reports by Harborne (1973), Trease and Evans (1989) and Sofowora (1993). The powdered samples of Dioclea reflexa was subjected to phytochemical screening for the presence of different chemical groups of compounds such as saponins, alkaloids, terpenoids, cardiac glycosides as described in literatures of Ajayi et al. (2011). Qualitative phytochemical analyses of the selected plant seeds were determined using the method of Sofowora (1993). All determinations were done in triplicates.

Test for saponins

1 g of each powdered sample was separately boiled with 10 ml of distilled water in a water bath for 10 mins. The mixture was filtered while hot and allowed to cool. The following tests were then carried out.

(a) Demonstration of frothing: 2.5 ml of filtrate was diluted to 10 ml with distilled water and shaken vigorously for 2 mins; formation of froth which is stable for some minutes indicate the presence of saponin in the filtrate.

(b) Demonstration of emulsifying properties: 2 drops of olive oil was added to the solution obtained from diluting 2.5 ml filtrate to 10 ml with distilled water after which it was shaken vigorously for a few minutes; formation of a fairly stable emulsion indicated the presence of saponins.

Test for alkaloids

1 g of the powdered sample was separately boiled with water and acidified with 5 ml of 1 % HCl on a steam bath. The solution obtained was filtered and 2 ml of the filtrate was treated with few drops of the following reagents separately in different test tubes and observed. Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a creamy white precipitate indicated the presence of alkaloids in the extract.

Test for cardiac glycosides

5 ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

Test for terpenoids

5 ml of each extract was mixed in 2 ml of chloroform. 3 ml of concentrated H_2SO_4 was then added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids.

Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1 % aqueous hydrochloric acid was taken as evidence for the phlobatannins.

Fatty acid analysis

The methyl ester of the crude oil was prepared in University of Tuebigen, Germany following the method of Lutz et al. (1998). 5 ml of CH_3OH and 1 ml of CH_2Cl_2 were added to 0.10 g of each oil sample. Ice was used to cool the mixture and then 0.6 ml of CH_2COCl was added. 1 ml of the solution was withdrawn into a

hydrolysis tube and heated for 1 h at 110 ^{o}C. The solution obtained was again cooled with ice and discharged into a separating funnel containing 10 ml of 100 % NaCl solution. The extraction of the organics in the solution was carried out thrice with 4 ml of hexane; a rotatory evaporator was used to reduce the volume to 0.5 ml after which it was eluted on a silica gel column successively with 5 ml hexane and 4ml CH_2Cl_2. A separation was made of the CH_2Cl_2 fraction on a DB5 30 m x 0.25 mm capillary installed on a GC Chrompack 900l (model; Chrompack 900l; city: Middelburg; country: Netherlands) equipped with computer software and mosaic integration. The programming of the temperature was 35 ^{o}C for 3 min after which it was increased at 20 ^{o}C/min up to 230 ^{o}C for 5 min. the internal standard was heptadecanoic acid. The detector used was flame ionization detector.

Statistical analysis

All data generated were analyzed using descriptive statistic (Olawuyi, 1996). Statistical values that were calculated include mean and standard deviation.

III. Results And Discussion

Proximate result

The summary of the proximate composition of Dioclea reflexa seeds is presented on Table 1. The oil content of the seed is 10.65±0.01 %. This is lower than 19 % reported for soy bean (Oyenuga, 1968) but higher that of African yam bean with a fat content of 2.50 % (Edem et al., 1990). The oil might find use in cosmetic industries and in the manufacture of margarine. Total carbohydrate is high (38.59±0.12 %); it is within the range of 20.382±0.013 % and 70.123±0.020 % reported in literature for selected medicinal plants of Kyber Pakistan (Hussain et al., 2011). The seed is high in protein (26.00±0.43 %). This is slightly higher than 24.4 % of paprika seeds (El_Adawy and Taha, 2001) and 25.54±0.25 % of A. occidentale nut (Ajayi et al., 2013). This finding may focus interest on D. reflexa seeds as high protein sources in some food formulation.

Physico-chemical properties

Presented on Table 2 is the result of the physic-chemical constituents of D. reflexa seed oil. The acid value of D. reflexa seed oil (2.24 ± 0.15 mgKOH/ g oil) is lower than the one reported for T. vogelii, P. macrophylla, C. grandiflora and M. arboreus with acid values of 3.72, 5.31, 5.03 and 4.39 respectively (Minzangi et al., 2011); the low acid value makes it suitable for soap making (Ojeh, 2011). The nutritional value of a fat depends, in some respects, on the amount of free fatty acids which develop. In the tropics, where vegetable oils are the most common dietary lipids, it has been shown that the free fatty acid content of cooking oil lies within limits of 0.0-3.0 % (Onyeike and Acheru, 2002). The low level of % free fatty acid in D. reflexa seed oil is an indication that the oil might be good edible oil that may store for a long time without spoilage through oxidative rancidity. Iodine value (115.45±1.50 gI/100 g oil), saponification value (318.63±2.40 mg KOH/g oil), ester value (316.40±2.50 mg KOH/g oil) and peroxide value (5.60±0.20) are higher than the range reported by Ajayi and Aghanu (2011) for M. tenufolia seed oil. The colour of D. reflexa seeds is golden yellow and the state at room temperature is liquid.

Mineral composition

Mineral element analysis (Table 3) showed that D. reflexa seeds contained essential elements such as potassium (4.09±0.04 mg/l), sodium (4.88±0.25 mg/l) magnesium (0.80±0.04 mg/l), zinc (2.10±0.03 mg/l), iron (0.48±0.05 mg/l) and manganese (0.37±0.61 mg/l) and calcium (7.11±0.42 mg/l). Minerals are required for normal growth, activities of muscles and skeletal development (such as calcium), cellular activity and oxygen transport (copper and iron), chemical reaction in the body and intestinal absorption (magnesium), fluid balance and nerve transmission (sodium and potassium). Iron is useful in prevention of anemia and other related diseases (Oluyemi et al., 2006). Manganese plays a role in energy production and in supporting the immune system (Muhammad et al., 2011). Deficiency of these nutrients and minerals are known to affect the performance and health in both humans and livestock (Merck, 2005). Nutrients rich foods are vital for proper growth both in adults and children.

Phytochemical screening

Phytochemical screening of Dioclea reflexa as shown in (Table 4) revealed the presence of some bioactive components in the seed extract. It contains alkaloids, cardiac glycosides, saponin and phlobatannin while steroids, flavonoids and terpenoids were absent. These compounds have been shown to be active against potentially significant pathogens including those that are responsible for enteric infections Owolabi et al. (2007). Apart from their potential antibacterial activity, compounds present in this study such as alkaloids are known antimalaria agents, analgesics and can act as stimulants. Glycoside moieties such as saponins and cardiac glycosides can inhibit tumor growth, act as an antiparasitic agent, and can be used as an antidepressant.

Fatty acid composition

In the result of fatty acid composition of D. reflexa seeds (Table 5), $C_{18:1}$ had the highest value of 31.50 g/100g and $C_{20:2}$ had the least value of 1.59 g/100g. The total fatty acid was 99.10 g/100g. This value is higher than those reported for the seeds of Carapa grandiflora (81.2 g/100g), Carapa procera (82.2 g/100g), Milletia dura (78.8 g/100g), Tephrosia vogelii (61.3 g/100g), Myrianthus arboreus (63.8 g/100g) and Treculia africana (80.9 g/100g) by Minzangi et al. (2011). Oleic acid, $C_{18:1,}$ which is the dominant fatty acid of D. reflexa seed oil, is very important in nervous cell construction; it has fundamental role in cardiovascular diseases prevention (Nasri et al., 2005). The high percentage of oleic acid in the oil makes it desirable in terms of nutrition and high stability cooking and frying oil (Anwar et al., 2006). A higher intake of oleic acid is associated with decreased risk of coronary heart disease caused by high cholesterol level in blood (Corbett, 2003). The linoleic acid content of the seed oil is small, linoleic acid helps to relieve flaky or rough skin and maintain smooth moist skin (Ariffin et al., 2009). Despite the fact D. reflexa seed oil contains more of saturated fatty acids than unsaturated ones it may still have a high potential in nutrition because of its high percentage of oleic acid.

Presented on Table 6 is the monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), unsaturated fatty acid (UFA), saturated fatty acid (SAFA), oleic/linoleic, MUFA/PUFA, SAFA/UFA contents of the seed oils. The saturated/unsaturated ratio of the D. reflexa seed oil is 1.43. The MUFA/PUFA ratio of the oil is greater than 1; this shows that the oil contains more of MUFAs than PUFAs. The oil also has its oleic to linoleic acid ratio to be greater than 1. This is of great nutritional value since polyunsaturated fatty acids and their derivatives are important essential nutritive additives in mammal, especially in humans (Kamal-Eldin and Yanishlieva, 2002 and Ziboh et al., 2002).

IV. Conclusion

Conclusively, the result of this research work showed that Dioclea reflexa seeds contained appreciable amounts of phytochemicals like alkaloids, glycoside, saponin and phlobatannin which have good pharmacological effect and also carbohydrate, protein, minerals which are nutritional requirements of both humans and livestocks. Possibly, the seeds from these plants could be useful as feed supplement and as medicine to improve health and growth performance in humans and livestocks.

References

[1]. Aliyu, A. B., Musa, A. M., Oshanimi, J. A., Ibrahim, H. A., Oyewale, A. O. (2008). Phytochemical analyses and mineral elements composition of some medicinal plants of Northern Nigeria, Nigerian Journal of Pharmaceutical Sciences 7, 119-125.

[2]. Ajayi, I. A., Oderinde, R. A., Ogunkoya, B. O., Egunyomi, A., Taiwo, V. O. (2007). Chemical analysis and preliminary toxicological evaluation of Garcinia mangostana seeds and seed oil. Food Chemistry 101, 999-1004.

[3]. Ajayi, I. A., Aghanu, V. N. (2011). Chemical characterization of Monodora tenuifolia seeds from Nigeria. Seed Science and Biotechnology 5, 59-62.

[4]. Ajayi, I. A., Ajibade, O., Oderinde, R. A. (2011): Preliminary phytochemical analysis of some seeds. Research Journal of Chemical Sciences 1, 58-62.

[5]. Ajayi, I. A., Oladeji, O. S., Aghanu, V. N. (2013): Short-term toxicological evaluation of Anacardium occidentale oil in albino rats. Food Science and Quality Management 16, 8- 15.

[6]. Anwar, F., Zafar, S. N., Rashid, U. (2006). Characterization of Moringa oleifera seed oil from drought and irrigated regions of Punjab. Grasasy Aceites 57, 160-168.

[7]. Ariffin, A. A., Bakar, J., Tan, C. P., Rahman, R. A., Karim, R., Loi, C. C. (2009). Essential fatty acids of pitaya (dragon fruit) seed oil. Food Chemistry 114, 561-564.

[8]. Association of Official Analytical Chemists (AOAC) (1984). Official Methods of Analysis 14th Edition. Arlington, VA.

[9]. AOAC. 1990. Official methods of analysis, Association of Official Analytical Chemists,Washington, D.C., USA. 15th Edition, pp. 807-928.

[10]. Cobertt, P. (2003). It is time for an oil change. Opportunities for high oleic vegetables oils. Information 14, 480-481.

[11]. Dagne, K., Jonsson, A. (1997). Oil content and fatty acid composition of seeds of Guizotia cass (Compositae) Journal of Science Food Agriculture 73, 274-278.

[12]. Dubois, V., Breton, S., Linder, M., Fanni, J., Parmentier, M. (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. European Journal of Lipid Science Technology 109, 710-732.

[13]. Edem, D. O., Amugo, C. I., Eka, O. U. (1990). Chemical composition of yam beans (Sphenostylis sternocarpa). Tropical Science 30, 59–63.

[14]. El-Adawy, T. A., Taha, K. M. (2001). Characteristics and composition of different seed oils and flours. Food Chemistry 74, 47-54.

[15]. Fasina, O. O., Hallman, C. H. M. Clementsa, C. (2006). Predicting temperature- dependence viscosity of vegetable oils from fatty acid composition. Journal of American Oil Chemists Society 83, 899-903.

[16]. Fasuyi A. O., Akindahunsi A. O. (2009). Nutritional evaluation of Amaranthus cruentus leaf meal based broiler diets supplemented with cellulase/glucanase/xylanase enzymes. American Journal of Food Technology 4, 108-118

[17]. Goss, J. A. (1980). Determination of moisture in livestock feed. Feed Management, 31:32.

[18]. Harborne, J. B. (1973). Phytochemical methods, London. Chapman and Hall, Ltd. pp. 49-188.

[19]. Hussain, J., Khan, F. U., Ullah, R., Muhammad, Z., Rehman, N. U., Shinwari, Z. K., Khan, B. U., Zohaib, M., Imad-ud-din, Hussain, S. M. (2011). Nutrient evaluation and elemental analysis of four selected medicinal plants of Khyber Pakhtoon Khwa, Pakistan. Pakistan Journal of Botany 43, 427-434.

[20]. Kamal-Eldin, A., Yanishlieva, N. V. (2002). n-3 Fatty acids for human nutrition: stability Concentration. European Journal Lipid Science and Technology 104, 825-836.

[21]. Lutz, M., Esuoso, K., Kutubuddin, M., Bayer, E. (1998). Low temperature conversion of sugar cane by-products. Biomass and Bioenergy 15, 155-162

[22]. Melgarejo, C. M. F., Gee, J. M., Knight, D. J. (1994). Fatty acid profile of some Cameroonian spices. Journal of Science Food and Agriculture 66, 213–216.
[23]. MERCK. Mineral deficiencies. (2005). The Merck Veterinary Manuel, Ninth Edition. Published by Merck and Co. Inc., Whitehouse Station, N.J., USA. pp. 2320-2330.
[24]. Minzangi, K., Kaaya, A. N., Kansilime, F., Tabuti, J. R. S., Samvura, B. (2011). Oil content and physicochemical characteristics of some wild oilseed plants from Kivu region Eastern Democratic Republic of Congo. African Journal of Biotechnology, 10, 189-195.
[25]. Muhammad, A., Dangoggo, S. M., Tsafe, A. I, Itodo, A. U., Atiku, F. A. (2011). Proximate, minerals and anti-nutritional factors of Gardenia aqualla (Gauden dutse) fruit pulp. In Pakistan Journal of Nutrition 10, 577-581.
[26]. Nasri, N., Khalil, A., Fady, B., Triki, S. (2005). Fatty acid acids from seeds of Pinus pinea L.: composition and population profiling. Phytochemistry 66, 1729-1735.
[27]. Ojeh, O. (1981). Effects of refining on the physical and chemical properties of cashew kernel oil. Journal of Fats and Oils Technology 16, 513–517.
[28]. Olawuyi, J. F. (1996). Biostatistics: A foundation course in health sciences. 1st Edition. University College Hospital, Published by Tunji Alabi Printing Co. Total Garden, Ibadan, Nigeria, pp. 1-221.
[29]. Oluyemi, E. A., Akilua, A. A., Adenuya, A. A., Adebayo, M. B. (2006). Mineral contents of some commonly consumed Nigerian foods. Science Focus 11, 153-157.
[30]. Owolabi, O. J., Omogbai, E., Obasuyi, O. (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extracts of Kigelia africana (Bignoniaceae) stem bark. African Journal of Biotechnology 6, 1677-1680.
[31]. Onyeike, E. N., Acheru, G. N. (2002). Chemical composition of selected Nigerian oilseeds and physicochemical properties of the oil extracts. Food Chemistry 77, 431-437.
[32]. Pearson, D. A. (1976). Chemical Analysis of Food (7th Edn), Livingstone Churchill, Edinburgh, pp 422-523.
[33]. Sofowora, A. (1993). Medicinal plants and Traditional medicine in Africa: Spectrum Books Ltd, Ibadan, Ibadan, Nigeria, pp 289.
[34]. Trease G. E., Evans W. C. (1989). Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillian publishers.
[35]. Ziboh, V. A., Cho, Y. H., Mani, L., Xi, S. D. (2002). Biological significance of essential fattyacids/lipoxygenase-derived monohydroxy fatty acids in the skin. Archives of Pharmacology Research 25, 747-758.

Table 1: Proximate composition (%) of Dioclea reflexa seeds

Composition	Means±SD
Crude protein	26.00±0.43
Crude fat	10.65±0.01
Crude fibre	9.25±0.03
Ash	9.17±0.01
Moisture	13.30±0.01
Carbohydrate	31.63±0.12
Available Energy (KJ/100g)	1372.07

Table 2: Physico-chemical properties of Dioclea reflexa seeds

Property	Means±SD
Acid value (mgKOH/ g oil)	2.24±0.15
Free fatty acids*	1.13±0.15
Iodine value mgI/100 g oil	115.45±1.50
Peroxide value meq O_2/ kg oil	5.60±0.20
Saponification value (mgKOH/ g oil)	318.63±2.40
Ester value (mgKOH/ g oil)	316.40±2.50

* % as oleic acid

Table 3: Mineral element composition of Dioclea reflexa seeds

Minerals	Concentration (mg/l)
Sodium	4.88±0.25
Potassium	4.09±0.04
Magnesium	0.80±0.042
Calcium	7.11±0.42
Zinc	2.10±0.03
Iron	0.48±0.05
Manganese	0.37±0.61

Table 4: Result of the phytochemical screening of Dioclea reflexa seeds

Phytochemicals	Aqueous extract
Phlobatannin	+
Saponin	+
Cardiac glycosides	+
Terpenoids	-
Alkaloids	+

Table 5: Fatty acid composition of Dioclea reflexa seeds

Fatty acid	**Concentration %**
$C_{16:0}$	26.19
$C_{18:0}$	7.47
$C_{18:1}$	31.50
$C_{18:2}$	5.73
$C_{20:0}$	3.47
$C_{20:1}$	2.40
$C_{20:2}$	1.59
$C_{22:0}$	4.29
$C_{24:0}$	13.27
$C_{26:0}$	4.14
Total	100.05

Table 6: Oleic, Linoleic, MUFA, PUFA, UFA, SAFA, Oleic/Linoleic, MUFA/PUFA, SAFA/UFA contents of D. reflexa seed oil

Parameters	**D. reflexa**
MUFA[a]	33.90
PUFA[b]	7.32
UFA[c]	41.22
SAFA[d]	58.83
OLEIC/LINOLEIC	5.50
MUFA/PUFA	4.63
SAFA/UFA	1.43

[a]Monounsaturated fatty acids
[b]Polyunsaturated fatty acids
[c]Unsaturated fatty acids
[d]Saturated fatty acids