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Extraction and Characterisation of *Dioclea reflexa* Hook. F. Seed Oil

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Abstract. Physicochemical analysis of the oil of *Dioclea reflexa* Hook f. seeds revealed the acid value, saponification value, iodine value, ester value and iodine number of the seeds to be 8.69 mg KOH/g, 251 mg KOH/g, 72.8 mg I/g, 242 and 27.9, respectively. The fatty acid composition determined by gas chromatography (GC) showed individual unsaturated fatty acid to be oleic acid (18:1), 0.8%, while the saturated fatty acids were palmitic acid (16:0), 10.2% and stearic acid (18:0), 21.9%. The infrared spectroscopy (IR) of the oil was also undertaken. The high saponification and iodine values of *D. reflexa* oil suggest its possible utilization in alkyd resin, shoe polish, liquid soap and shampoo production.

Keywords: *Dioclea reflexa*, physicochemical analysis, Fabaceae, fatty acid, seed oil

Introduction

Dioclea reflexa Hook. f. belongs to the family, Fabaceae. It is a hairy woody climbing shrub. It is widely spread in tropical and subtropical areas and is often considered a food crop. The seeds are arranged in pods, which are very hard and brownish in colour. *D. reflexa*, the marble vine, is highly regarded in some parts of Africa. The spherical seeds are used in games; root decoctions used to alleviate coronary pain; seed and root extracts are said to have insecticidal properties (Allen and Allen, 1981). The seeds are used to kill head lice by milling the cotyledons and mixing it with hair cream while the roasted cotyledons, are used for curing piles (Gill, 1992). The anti-microbial activity and phytochemical analysis of crude ethanolic leaf extract of *D. reflexa* has been reported. The leaf extract was reported to show broad spectrum antibacterial activity against *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Streptococcus pneumoniae*, *Escherichia coli*, *Candida albicans*, *Aspergillus flavus* and *Fusarium solani* (Ogundare and Olorunfemi, 2007). The phytochemical analysis of the leaf extract showed the presence of alkaloids, tannins, phenols and glycosides (Ogundare and Olorunfemi, 2007).

In Nigeria, the seeds of *Mucuna puriens*, another species, are used in popular medicine for prevention against the effects of snake (*Echis carinatus, Naja naja*) bite (Guerranti *et al*., 1999). Proteins inducing an immune response against the venom of *Echis carinatus* have been isolated from the cold water extract of the seeds of *Mucuna pruriens* (Guerranti *et al*., 2002). The present study was carried out to investigate the physicochemical properties and fatty acid compositions of the seed oil of *D. reflexa* Hook f.

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Materials and Methods

Plant material. *Dioclea reflexa* Hook. f. (Fabaceae) seeds were obtained from Iropora Ekiti in Ekiti State, Nigeria. They were authenticated by Mr. F. O. Omotayo of the Herbarium Section, Department of Botany, University of Ado-Ekiti, Ado-Ekiti, Ekiti State, Nigeria. The brown hard shells of the seeds were broken to remove the cotyledons.

Extraction. The cotyledons were further broken into small pieces to enhance quick extraction. The coarse powder of the seeds of *D. reflexa* was extracted with *n*-hexane for 72 h at room temperature. Extraction was further repeated with *n*-hexane. The combined extract was concentrated *in vacuo* at 40 °C to obtain the seed oil which was analyzed for iodine value, saponification value, acid value, ester value and iodine number by the methods described by British Pharmacopoeia (1988).

Acid value determination. The oil (10 g) was weighed in 250 mL conical flask. A mixture of 25 mL of 95% alcohol, 25 mL of ether and 1 mL of phenolphthalein solution was added to the conical flask containing the oil. The oil was allowed to dissolve in the solvent mixture and titrated against 0.1 M aqueous potassium hydroxide. It was shaken constantly until blue colour was observed. The process was repeated twice in exactly the same manner.

$$\text{Acid value} = (a \times 5.61)/W$$

where:

W = mass (g) of oil weighed
a = volume of 0.1 M KOH required

Saponification value. The oil (2 g) was weighed in 250 mL quick fit flask. 25 mL of 1 M alcoholic potassium hydroxide was added using burette. A reflux condenser was attached to

the quick fit flask and the mixture was refluxed for one hour on a water bath, while swirling the contents frequently. The water bath was removed under the flask and 5 mL of phenolphthalein solution was poured down the condenser. The flask was allowed to cool for 5 min under the tap water and the content was titrated with 0.5 M HCl. A blank determination was carried out with the same quantities of reagents without the oil under the same experimental conditions. The procedure was repeated in the same manner.

Saponification value = (b-a) 28.05/W

where:

W = mass (g) of oil weighed
a = volume of 0.5 M HCl required
b = volume of 0.5 M HCl required for blank

Iodine value. The oil (0.25 g) was weighed and dissolved in a conical flask containing 15 mL of tetrachloromethane. 25 mL of Wij's solution was added to the mixture. Wij's solution was prepared by dissolving 1.9 g of iodine monochloride in a litre brown bottle containing one litre of a mixture of 70 mL acetic acid and 30 mL carbon tetrachloride. The flask containing the oil and the Wij's solution was closed and swirled to mix the content. The solution was allowed to stand in the dark at about 20 °C for one hour. 20 mL of 10% aqueous potassium iodide solution and 150 mL water were added. The solution was titrated with 0.5 M sodium thiosulphate solution; small amount of starch was added as indicator. A blank determination was carried out, with the same quantities of reagents under the same conditions. The procedure was repeated in exactly the same manner.

Iodine value = $1.269(V_2-V_1)/W$

where:

W = mass (g) of oil weighed
V_1 = volume of sodium thiosulphate solution required
V_2 = volume of sodium thiosulphate solution required for blank

Ester value. Ester value was determined by subtracting acid value (AV) from saponification value (SV).

Ester value = (SV) – (AV)

Iodine ratio. The iodine ratio was determined by the ratio of the ester value (EV) to the acid value (AV).

Iodine ratio = (EV)/(AV)

Methylation of the oil for GC analysis. The oil (0.5 g) was weighed and added to 3 mL of diethylether. The oil was allowed to dissolve by shaking the mixture thoroughly. 0.2 mL of sodium methoxide was added to the mixture and the solution was centrifuged to precipitate the solid, which was then filtered and the filtrate was kept for GC analysis (Ceirwyn, 1995).

Gas chromatography of *D. reflexa* seed oil. The fatty acid composition of the methyl ester of *D. reflexa* oil was determined by Al Cambridge GC 94 at Centre Science Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria. The column length was 105 m, internal diameter was 0.53 mm and the film was 3 μm thick operating conditions of the gas chromatograph comprises of oven temperature programme which was started at 150 °C, held for 6 min and then raised at 10 °C/min to the final temperature of 250 °C. The injector temperature was 150 °C and detector FID at 300 °C. The carrier gas was helium with a flow rate of 8 mL/min with an injected volume of 1 μm.

Infrared spectroscopy analysis. The IR spectroscopy of *D. reflexa* seed oil was done using nujol mull. About 3 mg of the oil was triturated with nujol mull to give a creamy paste which was put between two sodium chloride plates for the determination (Bungard, 1983).

Results and Discussion

Table 1 presents the physicochemical properties of the seed oil of *D. reflexa*. The iodine value of *D. reflexa* oil (72.8 mg iodine/g) places it in the non-drying oil group as drying oils have an iodine value above 100 (Duel, 1951). The iodine value compares favourably with that of *Calophylum inophylum* seed oil, 67.2-70.1 mg I/g (Olaofe *et al*., 2006), *Bombcapsis glabra* seed oil, 71.0 mg I/g (Olaofe *et al*., 2006) and Khaya seed oil, 68.0 mg I/g (Okiemen, 2002).

Table 1. Physicochemical properties of *Dioclea reflexa* seed oil

Parameter	Value
Acid value (mg KOH/g)	8.69
Saponification value (mg KOH/g)	251
Iodine value (mg iodine/g)	72.8
Ester value	242
Iodine ratio	27.9

The acid value of *D. reflexa* seed oil (8.69 mg KOH/g) is greater than that of the seed oil of *Parkia biglobossa*, 2.5, and that of *Jatropha curcas*, 3.5 (Akintayo, 2004). This suggests that the oil of *D. reflexa* would require refining to make it edible, in view of the fact that acid values of edible oils should not exceed 4.00 mg KOH/g (Akintayo, 1997).

The saponification value of *D. reflexa* oil (251 mg KOH/g) was high compared to the values reported for castor oil (176-187), cod liver oil (180-190) and sesame oil (188-195) (Olaniyi and Ogungbamila, 1991). The saponification value of *D. reflexa* oil when compared with Guna melon oil (213 mg KOH/g) (Oresanya *et al*., 2000) and Samsoy oil (211 mg KOH/g) has better quality attributes most especially with reference to the stability (Kochhar, 1986). This implies that the *D. reflexa* oil is nutritionally invaluable but highly valuable for industrial purposes especially for the manufacture of pharmaceuticals, soaps, cold creams, pomades and lubricants, emulsions for insect control and fuel for diesel engines.

The ester value and iodine ratio of *D. reflexa* oil were found to be 242 mg KOH/g and 27.9, respectively (Table 1). The iodine ratio is higher than the values reported for palm kernel oil (13-17) and coconut oil (8-10) but compares very well with the iodine ratio reported for milk fat (26-50) (Lewkowitsch, 1921). The value is lower than the value reported for olive oil (79-88) and sesame oil (103-108) (Lewkowitsch, 1921). The iodine ratio of fat or oil tells the degree of unsaturation of the oil or fat. Low value of the iodine ratio of *D. reflexa* oil is supported by the result of the gas chromatography analysis of the oil with a low percentage (0.8) of unsaturated fatty acid, oleic acid (18:1), in the oil as shown in Table 2. The fatty acid composition of *D. reflexa* seed oil from the GC analysis revealed that it contains two saturated fatty acids, namely palmitic (16:0) 10.2 and stearic (18:0) 21.9. The *D. reflexa* oil contains both saturated and unsaturated fatty acids, and some other unidentified fatty acids, in varying proportions.

Table 2. Fatty acid composition of *Dioclea reflexa* seed oil

Fatty acids	Percentage
Stearic	21.9
Palmitic	10.2
Oleic	0.8
Σ Saturated	32.1

The infrared spectroscopy spectrum of the seed oil of *D. reflexa* is presented in Fig. 1. There is a broad band between 3050-3500 cm^{-1} which is associated with the overtones of the glyceride ester carbonyl absorption. The band at approximately 2924 cm^{-1} is indicative of symmetric stretching from the ubiquitous methylene group (Akintayo, *et al*., 2002). The triglyceride carbonyl stretching vibration is observed in the spectrum at approximately 1727cm^{-1}. C=C stretching mode of unconjugated olefins usually show

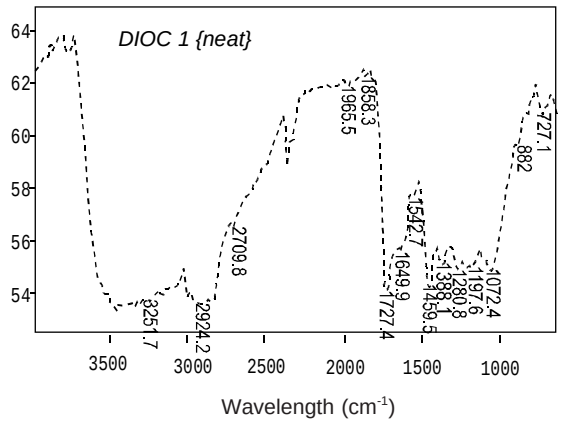

Fig. 1. Spectrum of infrared spectroscopy of *Dioclea reflexa* oil.

moderate to weak absorption bands in the region 1640-1680 cm^{-1} (Silverstein *et al*., 1979). This band was observed in the oil sample spectrum at approximately 1649 cm^{-1}. The oil sample shows a scissoring band of the bending vibration of methylene group at approximately 1459 cm^{-1}. The band at approximately 1388 cm^{-1} in the spectrum could be assigned to symmetrical bending vibration of methyl groups. Bands occur at approximately 1280 cm^{-1}, 1197 cm^{-1} and 1072 cm^{-1}. Some of these bands could be assigned to the stretching vibrations of the C-O group in esters (Silverstein *et al*., 1979). The absorption at approximately 727 cm^{-1} results from the overlapping of the methylene rocking vibration and the out of plane bending vibration of *cis*-disubstituted olefin.

References

Akintayo, E.T. 2004. Characteristics and composition of *Parkia biglobossa* and *Jatropha curcas* oils and cakes. *Bioresources Technology*, **92:** 307-311

Akintayo, E.T., Olaofe, O., Adefemi, S.O., Akintayo, C.O. 2002. Potential of fourier transform infrared spectroscopy for characterising vegetable oils. *International Journal of Chemistry*, **12:** 151-156.

Akintayo, E.T. 1997. Chemical composition and physico-chemical properties of fluted pumpkin (*Telfairia occidentalis*) seed and seed oils. *Rivista Italiana Delle Sostanze Grasse*, **74:** 13-15.

Allen, O.N., Allen, E.K. 1981. *The Leguminosae*: *A Source Book of Characteristics, Uses and Nodulation*, 812 pp., University of Wisconsin Press, Madison, USA.

British Pharmacopoeia, 1988. In 4 volumes, Her Majesty's Stationery Office, London, UK.

Bungard, L. 1983. A new spectrophotometric method for

selective determination of ampicillin, amoxicillin and cyclacillin in the presence of polymers. *Journal of Pharmaceutical and Biomedical Analysis*, **1:** 29-41.

Ceirwyn, S.J. 1995. *Analytical Chemistry of Foods*, pp. 140-141, Black Academic and Professional, London, UK.

Duel, H.J. Jr. 1951. *The Lipids: Their Chemistry and Biochemistry*, vol. **1,** Interscience Publishers Inc., New York, USA.

Gill, L.S. 1992. *Ethnomedicinal Uses of Plants in Nigeria*, 1st edition, University of Benin, Benin City, Nigeria.

Guerranti, R., Aguiyi, J.C., Leoncini, R., Pagani, R., Cinci, G., Marinello, E. 1999. Characterization of the factor responsible for the antisnake activity of *Mucuna pruriens* seeds. *Journal of Preventive Medicine and Hygiene*, **1:** 25-28.

Guerranti, R., Aguiyi, J.C., Neri, S., Leoncini, R. Pagani, R., Marinello, E. 2002. Proteins from *Mucuna pruriens* and enzymes from *Echis carinatus* venom: characterization and cross-reactions. *Journal of Biological Chemistry*, **277:** 17072-17078.

Kochhar, S.L. 1986. *Tropical Crops, Textbook of Economic Botany*, 176 pp., Macmillan, London, UK.

Lewkowitsch, J. 1921. *Chemical Technology and Analysis of Oils, Fats and Waxes*, 6th edition, pp. 419-424, Macmillan and Co. Ltd., London, UK.

Ogundare, A.O., Olorunfemi, O.B. 2007. Antimicrobial efficacy of the leaves of *Dioclea reflexa*, *Mucuna pruriens*, *Ficus asperifolia* and *Tragia spathulata. Research Journal of Microbiology*, **2:** 392-396.

Okiemen, F.E. 2002. Studies in the utilization of vegetable oil. *International Journal of Industrial Crops and Products*, **15:** 71-76.

Olaniyi, A.A., Ogungbamila, F.O. 1991. *Experimental Pharmaceutical Chemistry*, pp. 57-61, 1st edition, Shaneson C.I. Ltd., Nigeria.

Olaofe, O., Akintayo, E.T., Adeyeye, E.I., Adubiaro, H.O. 2006. Proximate composition and functional properties of Bulma cotton (*Bombcapsis glabra*) seeds. *Egyptian Journal of Food and Science*, **34:** 81-90.

Oresanya, M.O., Ebuehi, O.A.T., Aitezetmuller, K., Koleosho, O.A. 2000. Extraction and Characterisation of *Citrullus colocynthis* seed oil. *Nigerian Journal of National Production and Medicine*, **4:** 76-78.

Silverstein, R.M., Basler, G.C., Morrill, T.C. 1979. *Spectroscopic Identification of Organic Compounds*, pp. 73-79, 3rd edition, John Wiley and Sons, New York, USA.