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Physico-chemical properties, fatty acid and tocopherol composition of oils from some Sudanese oil bearing sources

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RESUMEN

Propiedades fisicoquímicas, ácidos grasos y tocoferoles de aceites obtenidos de plantas y semillas de Sudán.

Se analizan siete aceites procedentes de plantas y semillas de Sudán: *Albizia lebbek* Oil (ALO), *Cassia obtusifolia* Oil (COO), *Cassia occidentalis* Oil (CCO), *Abutilon pannosum* Oil (APO), *Mitracarpus villous* Oil (MVO), *Ipomea indica* Oil (IIO) y *Sesamum alatum* Oil (SAO). Las semillas se recolectaron en el estado de Ghibaish, North Kordofan. Además del contenido en aceite (12.8, 7.0, 3.2, 7.1, 11.5, 8.8, y 21.3 %, respectivamente), se determinaron los ácidos grasos mediante cromatografía de gases y los tocoferoles mediante HPLC. Los aceites contenían, respectivamente, 16.4, 20.0, 14.3, 16.8, 19.4, 19.9 y 10.9 % de ácido palmitito, 21.0, 24.4, 22.1, 10.9, 27.7, 34.0, 45.1 % de ácido oleico, 43.8, 38.2, 45.0, 63.9, 34.6, 33.5 y 36.3 % de ácido linoleico y trazas de ácido linolenico. El contenido en tocoferol total fue de 85.6, 48.2, 32.7, 163.5, 52.5, 30.9, y 26.4 mg/100 g oil, respectivamente. Alfa- tocopherol fue el predominante en los aceites de *Albizia lebbek, Cassia obtusifolia), Cassia occidentalis,* y *Abutilon pannosum,* mientras γ-tocopherol fue el mayoritario en los aceites de was the predominant tocopherol in the oil of *Mitracarpus villous, Ipomea indica* y *Sesamum alatum.*

PALABRAS CLAVE: Abutilon pannosum – Ácidos grasos – Albizia lebbek – Cassia obtusifolia – Cassia occidentalis – Ipomea indica – Mitracarpus villous – Sesamum alatum – Tocoferoles.

SUMMARY

Physico-chemical properties, fatty acid and tocopherol composition of oils from some Sudanese oil bearing sources.

Seven oils, obtained from seven Sudanese oil bearing sources that are *Albizia lebbek* Oil (ALO), *Cassia obtusifolia* Oil (COO), *Cassia occidentalis* Oil (CCO), *Abutilon pannosum* Oil (APO), *Mitracarpus villous* Oil (MVO), *Ipomea indica* Oil (IIO) and *Sesamum alatum* Oil (SAO), were investigated. The seeds were collected in Sudan from Ghibaish, North Kordofan state. In addition to the oil content, the fatty acid was determined by capillary gas chromatography and the tocopherols were evaluated by high-performance liquid chromatography. The oil content of the seven seeds amounted to 12.8, 7.0, 3.2, 7.1, 11.5, 8.8, and 21.3 %, respectively. The oils contained 16.4, 20.0, 14.3, 16.8, 19.4, 19.9 and 10.9 % palmitic acid and 21.0, 24.4, 22.1, 10.9, 27.7, 34.0, 45.1 % oleic acid, and 43.8, 38.2, 45.0, 63.9, 34.6, 33.5, 36.3 % linoleic acid and traces of linolenic acid, respectively. The tocopherol content of these oils amounted to 85.6, 48.2, 32.7, 163.5, 52.5, 30.9, and 26.4 mg/100 g oil, respectively. Alpha tocopherol was the predominant tocopherol in the oil of *Albizia lebbek, Cassia obtusifolia), Cassia occidentalis, and Abutilon pannosum,* while Gamma tocopherol was the predominant tocopherol in the oil of *Mitracarpus villous, Ipomea indica and Sesamum alatum.*

KEY-WORDS: Abutilon pannosum – Albizia lebbek – Cassia obtusifolia – Cassia occidentalis – Fatty acid – Ipomea indica – Mitracarpus villous – Sesamum alatum – Tocopherol.

1. INTRODUCTION

Albizia lebbek (L Benth.), family Mimosaceae is a fast growing nitrogen-fixing, heavy shade tree, recommended for reforestation and firewood plantations. It is native to tropical Africa, Asia, and northern Australia, widely planted and naturalized throughout the tropics (NAS 1980). Bark containing saponin can be used in making soap, and containing tannin, can be used for tanning. According to Hartwell (1967-1971) the tree is used in folk remedies for abdominal tumors, cough, eye ailments, flu, and lung ailments. The seed oil is used for leprosy, the powdered seed to scrofulous swellings. Seeds have yielded 5.3-6.8 % fixed oil or fat, the endosperm 11 %. The oil contains 9.6 % stearic, 10.9 % arachidic, 39.3 % oleic, and 32.9 % linoleic acid (Watt and Breyer-Brandwijk, 1962). Bark contains 5-15 % tannin (leaves contain ca 4 %) and saponins. The saponin from the seed yields oleanolic acid and albizziagenin (Mitchell and Rook, 1979). Fernandez *et al*, (1996) reported the use of *Albizia lebbek* seed pods for water softening by the adsorption of calcium at 25 °C and they mentioned that the adsorption increased with the pH value. The seeds of *Albizia lebbek* contain 33.60 % crude protein and 3.13% crude fat, while the pods contain 17.86 % crude protein and 2.6 % crude fat. Prohibitive levels of toxic compounds were not detected in any of the plant parts analyzed (Roskoski *et al* 1980).

Cassia obtusifolia family Leguminosae (Fabaceae) is widely distributed in Africa and the Americas. In the Sudan it is found mostly on the

clay plains of the central rain lands and in the southern regions. The plant has a compound, pinnate leaf, composed of three pairs of leaflets. Flowers are yellow to orange and the pod is slender, slightly curved and may reach 20cm long. The plant has a slender stem and may grow up to 2.0 m high. The plant commonly known in Sudan as Kawal (Dirar 1993). The fruit is a slender pod up to 18 cm long, 5 mm wide, 4-angled in cross section and usually curved downward. The pods are green and turn brown as the seeds mature, the seeds are 4-6 mm (Hall *et al*, 2006). Cassia obtusifolia and its seeds, common contaminants of agricultural commodities, are toxic to cattle and poultry. Toxicity has been attributed to anthraquinones which are major constituents of *Cassia obtusifolia* (Kenneth and Lucas 1991). *Cassia obtusifolia* leaves, seeds, and root are used medicinally, primarily in Asia. It is believed to possess a laxative effect, as well as to be beneficial for the eyes. The plant's seeds are a source of cassia gum, a food additive usually used as a thickener. As a folk remedy, the seeds are often roasted, then boiled in water to produce a tea. Roasted and ground seeds have also been used as a substitute for coffee (www.hptt wikipedia.com)

Cassia occidentalis belonging to family Caesalpinaceae and commonly known in Sudan as Soreib. The pods are 10-13 cm long and up to 0.8 cm in diameter containing dark olive green seeds. The *C. occidentalis* seeds are rich source of galactomannan (Gupta *et al 2005*). In India, it has been reported to be used as antidote of poison, blood purifier, expectorant, anti-inflammatory agent and a remedy for the treatment of liver diseases. The composition of the seed oil has also been described (Akhtar *et al 1988*)

Abutilon pannosum (family Malvaceae) is an erect, 1.5-3 m tall perennial herb to shrub leaves 4-13 cm across, broadly ovate, 7-9 nerved. Flowers appearing in racemes or panicles in terminal branches by the reduction of leaves; pedicel 1-3.5 cm long, uniformly hairy, articulate from below the middle to near the apex. Fruit globose, 10-15 mm. Seeds are three in each mericarp, 2-2.5 mm long, 2 mm broad. The seeds of *Abutilon pannosum* contained 13.4 % oil, and 23.0 % protein. The respective seed oil had iodine values of 118.4 and saponification value of 194.3, acid value 1.9 with refractive index of 1.4652 at 30 °C. The fatty acid composition (wt %), as determined by gas liquid chromatography was: palmitic 21.3, stearic 2.8, oleic 10.2; linoleic 60.7, malvalic 2.2, and dihydrocum sterculic 1.3 (Kittur *et al, 1982*)

Mitracarpus villosus family Rubiaceae a climbing plant with red flowers, the leaves 3-5 cm long, reproduces by seeds. M. villosus is a common weed in upland areas from the forest to the savanna zones. It is widespread in Sudan. Ethanolic extracts of *Mitracarpus villosus* leaves showed antifungal activities (Irobi and Daramola 1993). The crude extracts also showed antibacterial activities using agar diffusion and tube dilution techniques against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Streptococcus faecalis* (Irobi and Daramola 1994).

Ipomea indica belongs to the *Convolvulaceae* which has over 600 species ranging from annuals, herbs, climbers, shrubs and trees. They occupy tropical and warm temperate regions but predominantly from the Americas. Some species contain alkaloids and other drugs, including strong purgatives, others have hallucigenic properties. *Ipomoea indica* is a perennial with heart-shaped leaves and with flowers produced daily from a dense clustered inflorescence. There are others which are annuals and widely grown as decorative plants. *Ipomoea* 'Heavenly Blue' is probably the favorite with sky-blue flowers and, when grown in pots in a cool conservatory, gives endless pleasure with its huge disc-like flowers throughout the summer, Ipomoea indica is described as a herbaceous climber but it does not die down to the ground. Instead new growths appear from the main stems each spring, but, in an ambient temperature, growth continues all year round. The flowers appear from the leaf axils. Twining perennial vine with stems to more than 7 m long. Plants hairy. Leaves with blade ovate in outline, 4-17 cm long, 3-16 cm wide, base heart-shaped, margins entire to deeply 3-lobed; leaf stalk 2-18 cm long. Capsule globe-shaped, about 10 mm wide, with 3 chambers (http://www.weeds.org.au).

Sesamum alatum belonging to family Pedaliaceae is a tall erect herb of cultivated land with winged seed. Seed oil content was 28.9. Three new saponins, alatoside, with a 18, 19-secours-12-ene skeleton were isolated from the aerial parts. In addition, verbascoside and two cyclohexylethanol derivatives, rengyol (2a) and isorengyol (3a), were isolated and identified (Potterat *et al*, 2004).

2. MATERIALS AND METHODS

2.1. Materials

All solvents used were of analytical grade. Petroleum ether, *n-heptane*, ethanol and diethyl ether, carbon tetrachloride were obtained from Merck (Darmstadt, Germany). Seeds of *Albizia lebbek, Cassia obtusifolia, Cassia occidentalis*, *Abutilon pannosum*, *Mitracarpus villous, Ipomea indica* and *Sesamum alatum* were collected from Ghibaish, North Kordofan State, Sudan. Seeds were dried, crushed and ground by a grinding mill (Petra electric, Burgau, Germany).

2.2. Methods

2.2.1. Oil extraction

The oil was extracted from the ground seeds by extraction with petroleum ether in a Soxhlet apparatus for 6 hr following the AOCS official methods (1993). The obtained oils were stored at 4 °C until further investigation.

2.2.2. Physico-chemical properties

The following physicochemical properties of African oils were determined following the AOCS Official methods (1993) as given below.

Refractive index. Official method Cc7-25 was followed to determine the refractive index of the oils at 30 °C.

*Specific gravity***.** Official method Cc 10 a-25 was followed for determination of specific gravity of oils at 60 °C.

*Free fatty acids***.** Free fatty acids (FFA%) were determined using the AOCS official method Ca 5a-4. In brief, 3-10 grams of the sample were dissolved in 50 ml of a mixture consisting of ethanol and diethyl ether (1:1, v/v) neutralized with 0.1 N KOH against phenolphthalein indicator. Then the solution was titrated with a 0.1 sodium hydroxide solution.

Fatty acid composition (FA). The fatty acid composition of seven African oils was determined following the ISO draft standard ISO/FIDS 5509 (1997). In brief, one drop of the oil was dissolved in 1 mL of *n*-heptane, 50 µl 2M sodium methanolate in methanol were added, and the closed tube was agitated vigorously for 1 min. After addition of 100 µL of water, the tube was centrifuged at 4500 g for 10 min. and the lower aqueous phase was removed. After that 50 µL 1 M HCl were added to the heptane phase, the two phases were shortly mixed and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure, Merck, Darmstadt, Germany) were added, and after centrifugation at 4500 g for 10 min the top n-heptane phase was transferred into a vial and injected in a Varian 5890 gas chromatograph with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 µm). The temperature programme was: from 155 °C heated to 220 °C (1.5 °C/min.), 10 min isotherm; injector 250 °C, detector 250°C; carrier gas 1.07 mL/min hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1 µL. The integration software computed the peak areas and percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalization.

Tocopherols (TOC). For determination of tocopherols a solution of 250 mg of studied samples oil in 25 mL *n*-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with an L-6000 pump, a Merck-Hitachi F-1000 Fluorescence Spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm) and a D-2500 integration system; 20 µL of the samples were injected by a Merck 655-A40 Autosampler onto a Diol phase HPLC column 25 cm x 4.6 mm ID (Merck, Darmstadt, Germany) using a flow rate of 1.3 mL/min. The mobile phase used was *n*-heptane/tert, butyl methyl ether (99+1, v/v) (Balz *et al*., 1992).

2.2.3. Statistical Analysis

The analyses were performed with three replicates. The mean values and standard deviation (mean ± SD) were calculated and tested using Duncan's test ($P < 0.05$). Statistical analysis of variance (ANOVA) was performed on all values using the statistical program Statgrafics® Statistical Graphics System version 4.0 (Statgraphics®1985-1989).

3. RESULTS AND DISCUSSION

3.1. Oil content

The oil content of studied samples of Albizia *lebbek, Cassia obtusifolia, Cassia occidentalis*, *Abutilon pannosum*, *Mitracarpus villous*, *Ipomea indica* and *Sesamum alatum* was 12.8, 7.0, 3.2, 7.1, 11.5, 8.8, and 21.3 respectively, as shown in Table 1. With wild sesame as the highest in oil content, but it is less than 28.9 % which was reported by Potterat *et al*., (2004). *Albizia lebbek* seed oil was higher than that reported by Watt and Breyer-Brandwijk (1962) who reported only 5-6 %. The oil content of studied samples was found significantly ($P < 0.05$) different (except *Cassia obtusifolia* and *Abutilon pannosum*). On the whole, the oil content of the seeds is very low compared with that of common oil seeds. Therefore, from an economical point of view, the production of oil from such seeds could not be interesting unless some genetic modifications could be applied.

Table 1
The oil content of seven oil-bearing seeds

Oil-bearing seeds	Family	Oil content [%]
Ipomea indica	Convolvulaceae	8.8
Albizia lebbek	Mimosaceae	12.8
Cassia obtusifolia	Leguminosae	7.0
Cassia occidentalis	Leguminosae	3.2
Abutilon pannosum	Malvaceae	7.1
Sesamum alatum	Pedaliaceae	21.3
Mitracarpus villous.s	Rubiaceae	11.5

mean value ± standard deviation (n = 3).

3.2. Physico-chemical properties

Some of the chemical and physical properties of the crude oils of ALO, COO, CCO, APO, MVO, IIO and SAO from Ghibaish, North Kordofan state, Sudan samples are presented in Table 1. The seven samples were similar in most of their physicochemical properties except unsaponifiable values were higher in APO and ALO samples. The other five oils were low in unsaponifiable matter. The seven samples had relatively similar specific gravity values, and had average values for refractive index and free fatty acids.

Table 2
Chemical and physical properties of oils from *Albizia lebbek, Cassia obtusifolia, Cassia occidentalis*, *Abutilon pannosum*, *Mitracarpus villous*, *Ipomea indica* and *Sesamum alatum*

Physico-chemical Parameters	ALO	COO	CCO	APO	MVO	IIO	SAO
Oil content (%)	12.8± 0.4	7.0± 0.3	3.2± 0.2	7.1± 0.3	11.5± 0.3	8.8± 0.4	21.3± 0.5
FFA (%)	1.5± 0.2	2.2± 0.2	2.0± 0.1	1.2± 0.2	1.1± 0.1	2.4± 0.2	1.5± 0.3
Unsaponifiables (%)	1.41± 0.2	0.81 ± 0.2	0.80±0.2	1.81 ± 0.2	0.85 ± 0.2	0.81 ±0.2	0.78 ±0.2
Refractive index (30 °C)	1.4750± 0.1	1.4740± 0.1	1.4743 ± 0.1	1.4752± 0.1	1.4685 ± 0.1	1.4741 ± 0.1	1.4762 ± 0.1
Specific gravity (60 °C)	0.9220 ± 0.05	0.9215 ± 0.04	0.9222 ± 0.03	0.9225 ± 0.05	0.9224 ± 0.05	0.9211 ± 0.03	0.9223 ± 0.03

mean value ± standard deviation (n = 3). ALO, *Albizia lebbek;* COO, *Cassia obtusifolia*; CCO, *Cassia occidentalis;* APO, *Abutilon pannosum*; MVO, *Mitracarpus villous*; IIO, *Ipomea indica*; SAO, *Sesamum alatum*.

3.3. Fatty Acids

The fatty acid composition of *Albizia lebbek ALO*, *Cassia obtusifolia* COO, *Cassia occidentalis* CCO, *Abutilon pannosum* APO, *Mitracarpus villous* MVO, *Ipomea indica* IIO and *Sesamum alatum* SAO is shown in Table 3. Fourteen fatty acids were identified among which linoleic acid (18:2Δ6) contributed 43.8, 38.2, 45.0, 63.9, and 43.6 % to the total in ALO, COO, CCO, APO, and MVO respectively, followed by oleic acid (18:1Δ9) at 33.5 and 36.3 % in (IIO) and (SAO) respectively. Palmitic acid (16:0) at 16.4, 20.0, 14.3, 16.8, 19.4, 19.9, 10.9% respectively, and stearic acid (18:0) 5.2, 9.6, 8.2, 2.4, 9.8, 7.6 and 5.2 % respectively. The remaining fatty acids contributed only few percentages to the total fatty acids present.

3.4. Tocopherols

The content of tocopherols in freshly extracted oil of *Albizia lebbek ALO*, *Cassia obtusifolia* COO, *Cassia occidentalis* CCO, *Abutilon pannosum* APO,

Table 3
Fatty acid composition (% of total) of seven oil-bearing seeds

Fatty acid	ALO	COO	CCO	APO	MVO	IIO	SAO
14:0	0.3±0.01	0.1±0.01	0.1±0.03	0.2±0.01	0.2±0.01	0.2±0.01	0.0±0.0
16:0	16.4±0.2	20.0±0.3	14.3±0.2	16.8±0.2	19.4±0.3	19.9±0.2	10.9±0.4
16:1	0.7±0.01	0.6±0.12	0.3±0.1	0.3±0.01	0.3±0.01	0.4±0.1	0.3±0.02
17:0	0.2±0.01	0.1±0.01	0.1±0.02	0.1±0.01	0.1±0.02	0.1±0.01	0.1±0.01
18:0	5.2±0.2	9.6±0.12	8.2±0.2	2.4±0.2	9.8±0.2	7.6±0.3	5.2±0.3
18:1Δ9	21.0±0.3	24.4±0.3	22.1±0.4	10.9±0.3	27.7±0.4	34.0±0.4	45.1±0.4
18:1Δ11	3.6±0.01	1.4±0.02	n.d*	0.9±0.2	0.4±0.1	n.d	0.0±0.1
18:2	43.8±0.1	38.2±0.1	45.0±0.3	63.9±0.1	34.6±0.3	33.5±0.5	36.3±0.4
18:3	4.3±0.01	1.0±0.01	1.5±0.1	0.6±0.1	n.d	0.4±0.01	0.8±0.01
20:0	1.1±0.1	1.9±0.01	2.6±0.01	0.6±0.1	2.2±0.1	0.6±0.01	0.7±0.02
20:1	0.5±0.02	0.3±0.01	n.d	0.1±0.01	2.1±0.1	0.3±0.02	n.d
22:	0.2±0.01	0.1±0.01	0.2±0.01	n.d	0.1±0.2	0.1±0.01	0.0
24:0	0.6±0.01	0.5±0.01	n.d	0.2±0.01	n.d	0.3±0.01	0.1±0.01
24:1	0.2±0.01	0.1±0.01	n.d	n.d	n.d	n.d	0.1±0.0

mean value ± standard deviation (n = 3); n.d not detected. For other abbreviations, see table 2.

Table 4
Summary of the important fatty acid parameters of oil-bearing seeds

Oil	PUFA (g/100g)	TSFA (g/100g)	TUSFA (g/100g)	Ratio Unsaturated/saturated
ALO	48.1	18.4	74.7	4.06
COO	39.2	32.3	66.0	2.04
CCO	46.5	25.5	68.9	2.70
APO	64.5	20.1	76.7	3.80
MVO	34.6	31.8	65.1	2.04
IIO	33.9	28.9	68.6	2.37
SAO	37.1	17.0	82.6	4.85

mean value ± standard deviation (n = 3); PUFA, Polyunsaturated fatty acids; TSFA, Total saturated fatty acids. TUSFA; Total unsaturated fatty acids. For other abbreviations, see Table 2

Table 5
Tocopherol content (mg/100g) and composition of oil-bearing seeds

Oil	α-T	α-T3	β-T	γ-T	P8	γ-T3	δ-T	δ-T3	Total
Albizia lebbek	48.2±0.1	0.0±0.1	12.1±0.3	22.7±0.3	2.7±0.1	0.0±0.1	0.0±0.1	0.0±0.1	85.6±0.4
Cassia obtusifolia	35.0±0.4	0.0±0.1	0.6±0.1	9.1±0.2	3.3±0.1	0.0±0.1	0.2±0.1	0.0±0.1	48.2±0.2
Cassia occidentalis	20.4±0.3	0.0±0.1	0.9±0.2	3.1±0.1	8.0±0.4	0.0±0.1	0.3±0.2	0.0±0.1	32.7±0.1
Abutilon pannosum	125.7±0.5	0.7±0.2	3.5±0.1	29.0±0.4	2.9±0.2	1.2±0.2	0.4±0.1	0.2±0.1	163.5±0.4
Mitracarpus villous.s	1.5±0.1	0.2±0.1	1.6±0.1	45.9±0.3	1.2±0.1	0.9±0.3	0.8±0.1	0.4±0.2	52.5±0.2
Ipomea indica	0.1±0.1	0.2±0.1	1.2±0.1	24.2±0.2	1.7±0.1	1.6±0.1	1.8±0.1	0.0±0.1	30.9±0.1
Sesamum alatum	1.9±0.1	0.2±0.1	1.4±0.1	21.4±0.1	1.0±0.1	0.3±0.1	0.2±0.2	0.0±0.1	26.4±0.2

mean value ± standard deviation (n = 3); T = tocopherol ,T3 = tocotrienol, P8 = plastochromanol. For other abbreviations, see Table 2.

Mitracarpus villous MVO , *Ipomea indica* IIO and *Sesamum alatum* SAO is shown in Table 4. Among the tocopherols identified, α-tocopherol was most abundant accounting for 56.3, 72.6, 62.4, 76.9 % of the total (85.6, 48.2, 32.7, 163.5 mg/100 g) in ALO , COO , CCO , and APO while γ-tocopherol was most abundant accounting for 87.4, 78.3, 81.0 % of the total (52.5, 30.9, 26.4 mg/100 g) in MVO, IIO and SAO respectively. Oil of SAO had low amounts of tocopherols (26.4 mg/100 g,) compared with other common oils such as sesame oil, groundnut oil or sunflower oil, in which the amount of tocopherols was between 27.9 and 97.6 mg/100 g. The amount of tocopherols found in ALO, COO, CCO, APO, MVO, and IIO was comparable to the other oils.

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