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The Potential of *Vangueria madagascariensis* as a Source for Un-conventional Oil in Sudan

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Abstract

The unconventional oil in Sudan is available from *Vangueria madagascariensis* (Rubiaceae). This study was carried outtoevaluate the Oil Components of *Vangueria madagascariensis* seeds. The oil and protein contents were 40.0, 22.2% respectively in the seed. The oil was extracted using cold extraction (CE) and Soxhlet extraction (SE) methods. Fatty acids, tocopherolsandsterol were determined by GC–MS and HPLC, respectively. The major fatty acids in the seed oil extracted by CE and SE were oleic 10.5, and 10.4%, linoleic 63.1 and 63.4%, palmitic 9.7 and 9.8% and stearic 5.1 and 5.4%, respectively. The tocopherol content of CE and SE extracted oils amounted to 110.5 and 107.7 mg/100 g oil, respectively, with beta - tocopherol as the predominant tocopherol in the oil. Sterols profile shows that the major components are β-sitosterol 45.24%, campesterol 22.65% and stigmasterol 20.08% in the oil. *Vangueria madagascariensis* oil seed deserve further attention and investigations.

Keywords

Vangueria madagascariensis, Seed Oil, Extraction Method, Fatty Acids, Tocopherol, Sudan

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1. Introduction

Sudan is one of the oilseed producing countries. Recently, researchers started to be much concerned about recognition of new oil sources from large number of commonly grown wild plants in Sudan [1, 2, 3]. One ofthese wild plants is *Vangueria madagascariensis.*

The promising, unconventional and new source of oil in the Sudan is the available species of the family Rubiaceae, *Vangueria madagascariensis* (VM) J. F. Gmelin [4]. It is known locally in Sudan as Kirkir, it is widely distributed in Equatoria, Darfur and in southern Kordofan states, Kirkir plant grows as a shrub or small tree [5]. Fruits are yellowish-brown when mature, edible 4-5 seeded [6]. It has a broad range of applications in the indigenous medical system [7]. The seed contains a considerable amount of oil which has not been studied and hence not utilized in Sudan. The present investigation was carried out to assess the seed proximate analysis, physic- chemical characteristics, fatty acid composition, tocopherol and sterol composition of *Vangueria madagascariensis* seed oil obtained by two methods of extraction.

2. Materials and Methods

2.1. Plant Material and Chemicals

Vangueria madagascariensis fruits, under investigation were collected in May 2015 from Alfola Agricultural Area, Sudan. Specimens identified by taxonomist in medicinal and aromatic plant national center for research Institute (MAPRI) Khartoum, Sudan. The outer dried surrounding pith of the fruit was removed and the seeds were air-dried in an oven at 40°C to reachconstant moisture content (6.5%). The dried seeds were cracked manually, the shells carefully removed

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and thekernels thus obtained were crushed and ground in a grinding mill (Petra electric, Burgau, Germany) theground kernels with particle size of 0.5 mm were used for oil extraction. The kernels obtained were stored at 4°C until further investigations. All solvents were of analytical grade.

2.2. Approximate Analysis of Seed

2.2.1. Moisture Content

Themoisture content of the sample was determined by the air oven method according to the AOCS Official Method [8].

2.2.2. Crude Protein

The crude protein analysis of the sample was carried out and nitrogen content was determined by the semimicro- Kjeldahl digestion, distillation and titration method, as described by the Official Methods of Analysis [9].

2.2.3. Crude Fiber

The crude fiber of the sample was determined according to the AOCS official method [10].

2.2.4. Ash Content

The sample was ashed in a muffle furnace at 550°C for 3 h or more and allowed to cool and weighed following theAOCS official method [11].

2.3. Oil Extraction

Two methods were used for oil extraction

Cold solvent extraction

Soxhlet extraction

2.3.1. Cold Solvent Extraction

The powdered sample (250g) was put in conical flasks (1 L), and petroleum ether (b.p. 60–80°C) was added. Thesolvent/sample ratio was 2:1(v/w); the mixture was put in an automatic shaker (IKA, KS 501, Staufen, Germany) forabout 16 h at room temperature. The mixture was then filtered twice using glass wool. The clear filtrate was concentrated using a rotary evaporator to remove the solvent from the oil. The oil was allowed to stand in the openair at room temperature to ensure removal of all solvent from the oil. The oils obtained by cold extraction from*V. madagascariensis*(VMCE) was then kept in dark bottles and stored at 4°C for further analysis.

2.3.2. Soxhlet Extraction

The oil from the seeds of the sample was extracted exhaustively with petroleum ether (b.p. 60–80°C) analytical grade in Soxhlet extractors according to the AOCS official method [12]. The oils obtained by Soxhletextraction from *V. madagascariensis* (VMSE) was then kept in dark bottles and stored at 4°C forfurther analysis.

2.4. Oil Physicochemical Analysis

2.4.1. Physical Parameters

The AOCS official method [13] were followed for determination of the specific gravity of the oils at 60°C and refractive index of the oils at 30°C.

2.4.2. Chemical Parameters

The following chemical parameters: acid value, peroxide value, saponification value, and the amount kof unsaponifiable matter were determined according to AOCS official methods [14]

2.5. Fatty Acid Analysis

The fatty acid composition of *V. madagascariensis*oil (VMO) was determined following the ISOmethod [15].

2.6. Tocopherols (TOC)

Tocopherolswas determined according to [16].

2.7. Identificationof Sterols by GC/MS

Gas chromatography mass spectrometry was performed using a gas chromatograph-mass spectrograph (GC-MS) (trace GC 2000/finngan mat SSQ7000 mass spectrometer) fitted with electron impact (EI detector, 70 eV)) mode. The analytical column was DB-5 (5%-phenyl- methylpolysiloxane) with internal diameter (ID) 30 m X 0.25 X 0.25. Helium was used as a carrier gas at a flow rate of 1 mL/min. The temperature was programmed at 50°C for 5 min then increased to 300°C at the rate of 5°C/min. The temperature of injector was 250°C. The total run time was 53 min [17].

3. Results and Discussion

3.1. Proximate Analysis of Seeds

Table 1 shows the proximate analysis of V. *madagascariensis* seed kernels, it is clear that the sample shows high levels of protein and oil content. The oil content of *V. madagascariensis* seed kernels using cold and Soxhlet extraction methods was significantly ($P>0.05$) higher (40, 41.2%). The difference in oil content between cold extraction, and soxhlet extraction method was not significant and can be attributedto the fact that during soxhlet extraction, the high temperature employed in solvent evaporation may have caused sample heating which will letoil droplets to come out of the sample easier. There is no research data, as far as weknow, available in the oil content of *V. madagascariensis*seed.

Table 1. The proximate analysis of *Vangueria madagascariensis* seeds.

Sample	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fiber (%)	CHO (%)
VM	6.4 ± 0.1[a]	40.0 ±0.6[b]	22.2±0.3[b]	2.8± 0.1[a]	14.0±0.2[b]	14.6 ± 0.[b]

All determinations were carried out in triplicate and mean values ± standard deviations (SD) are reported. [a,b]Values with different superscript letters within a row indicate a significant difference at [P>0.05].

The oil content of *V. madagascariensis* kernels was higher than most Sudanese conventionaloilseeds (cottonseed, sunflower and groundnut) [18]. These results indicated very clearly that the seeds from this treesform a potential source of oils. Therefore, from an economic point of view, the production of oil from suchseeds could be of interest.

3.2. Oil Physicochemical Properties

The oils obtained from *V. madagascariensis* kernels was odorless, of good colour, and of good appearance. Thephysicochemical properties of the oil are represented in Table 2. From Table 2 it can be summarized that the method of extraction only significantly affects (P ≤0.05) the acid value, and nosignificant changes were observed in the refractive index, peroxide value, saponification value and specific gravity ofthe oils. Compared with the Codex standards [19] for cottonseed, sunflower, sesame and groundnut oils the *V. madagascariensis* oil showed lower values for specific gravity, refractive index and saponification values.

Table 2. Physicochemical properties of *Vangueria madagascariensis*oil.

Physicochemicalparameters	VMCE	VMSE
Oil content (% w/w)	40.0 ± 0.2[b]	41.2 ± 0.2[b]
Refractive index (40 ± 1 _C)	1.475 ± 0.003[b]	1.475 ± 0.003[b]
Acid value (mg KOH/g)	0.61 ± 0.01[c]	0.22 ± 0.01[c]
Peroxide value (mequiv O2/kg oil)	0.8 ± 0.1[a]	1.0 ± 0.2[a]
Saponification value	182.6 ± 0.1[b]	181.4 ± 0.1[b]
Specific gravity (30 ± 1 ^{0}C)	0.818 ± 0.001[b]	0.818 ± 0.001[b]

All determinations were carried out in triplicate and mean values ± standard deviations (SD) are presented. [a,b,c]Values with different superscript letters within a row indicate significant differences at [P>0.05]. VMCE *Vangueria madagascariensis* cold extraction, VMSE *Vangueria madagascariensis* Soxhlet extraction.

3.3. Fatty Acid Composition

The fatty acid composition of *Vangueria madagascariensis*oil determined by GCMS is shown in Table 3. The majorfatty acids in the oil sample were palmitic (16:0), stearic (18:0), oleic (18:1n-9), and linoleic (18:2n-6) acids, the oil wassignificantly (P ≤0.05) different in their fatty acid composition. The extraction method did not affectthe fatty acid composition of the samples. In V. *madagascariensis*oils, C18:2 was the most dominant fatty acid; it was 63.1 and 63.4% in VMCE and VMSE, respectively, followed by oleic acid which was found to 10.5 and 10.4% in VMCE and VMSE, respectively. Palmitic (C16:0) and stearic (C18:0) acids exhibited the third and fourth highestfatty acid contents in the oil, palmitic acid was 9.7%and9.8%, in VMCE and VMSE, respectively. While stearic acid was 5.1 and 5.4%, in VMCE and VMSE, respectively. The striking feature of the seed oil was the relative highlevel of polyunsaturated fatty acids (PUFA) which accounted for 71–74% of the total identified fatty acids, andthe high linoleic acid content of *V. madagascariensis* kernel oil makes it nutritionally valuable.

Table 3. Fatty acid composition (% of total) of *Vangueria madagascariensis*oil.

Fatty acid	VMCE	VMSE
10:00	3.7 ± 0.4[b]	4.1 ± 0.2[b]
14:00	0.9 ± 0.2[b]	0.8 ± 0.1[b]
16:00	9.7 ± 0.4[b]	9.8 ± 0.4[b]
18:00	5.1 ± 0.2[b]	5.4 ± 0.2[b]
18:1n-9	10.5 ± 0.5[b]	10.4 ± 0.7[b]
18:2n-6	63.1 ± 0.7[b]	63.4 ± 1.3[b]
18:3D 9,12,15	0.4 ± 0.1[a]	0.6 ± 0.1[a]
20:00	5.9 ± 0.3[b]	5.3 ± 0.2[b]
22:1 n:13	0.7 ± 0.1[a]	0.2 ± 0.1[a]
UnsaturatedFA (%)	74.3	74
Oleic/linoleicRatio	0.16	0.16

Each value is the mean ± SD of triplicate determinations. [a,b]values with different superscript letters within a row indicate significant differences at [P>0.05].

3.4. Tocopherols

The tocopherol content ofextracted *V. madagascariensis* oilis shown in Table 4. Amongthe tocopherols identified, alpha-tocopherol was 31.6 and 28.5 mg/100 g in VMCE and VMSE, respectively, and delta-tocopherol was 8.4, and 10.5, respectively. Also Beta and gamma-tocopherolswere found and beta-tocopherol wasabundant amounting to 65.7 and 63.8 mg/100 g in oilextracted using cold extraction and Soxhlet extractionmethods. The total tocopherol amount was significantlydifferent in*V madagascariensis*oils, and themethod of extraction had no significant effect on theamountof tocopherol. *V madagascariensis*oils (VMCE and VMSE) showed higher amounts of tocopherols 110.5,107.9 mg/100 g, respectively, compared to other commonoils such as sesame (33–101), groundnut (17–130) andsunflower (44–152 mg/100 g) oils [20]. In thecase of *V. madagascariensis*oils β -tocopherol was the predominantone constituting more than 59%. The other tocopherols inthe oil of the sample were below 1 mg/100 g each.

Table 4. Tocopherol content (mg/100 g)of*Vangueria madagascariensis*oils.

Sample	α-T	β-T	γ-T	δ-T	Total
VMCE	31.6 ± 0.5[b]	65.7 ± 0.6[b]	4.7 ± 0.2[b]	8.4 ± 0.3[b]	110.5 ± 0.6[c]
VMSE	28.5 ± 0.4[c]	63.8 ± 0.6[c]	5.1 ± 0.2c	10.5 ± 0.3[c]	107.9 ± 0.6[d]

All determinations were carried out in triplicate and mean values ± standard deviations (SD) are given. [a,b,c,d]Values with different superscript letters within columns indicate significant differences at [P>0.05] VMCE *Vangueria madagascariensis* cold extraction, VMSE *Vangueria madagascariensis* Soxhlet extraction.

3.5. Sterol Composition

Recently phytosterols (plant sterols) have become a focus of interest due to their serum cholesterol lowering effect and consequently their protection against cardiovascular disease [20]. Moreover, sterols comprise the bulk of the unsaponifiables in many oils. They are of interest due to their impact on health [21]. Table 5 summarizes the sterol composition of *Vangueria madagascariensis* oil determined by GC–MS, the % of sterol in sample 41.9%. Seven sterols were identified, among these the three major ones are *β*-sitosterol (45.24%), campesterol (22.65%) and stigmasterol, (20.08%) and with observation that in the oil *β*-sitosterol has the higher concentration, other sterols are present in small or minute amounts. Figure 1 illustrates the chromatogram of sterols of *V. madagascariensis* oil. Seeds ofsesame contain (8650 mg/kg) of phytosterols which slightly higher than some oilseeds commonly consumed, olive oil (2210 mg/kg) or Peanut (2200 mg/kg) [22]. In sesame free sterols constituted 54–85% of total sterols. *β*-sitosterol is the major sterol insesame with 50% [22]. Δ5-avenasterol was present in some vegetable oils in small amounts, but sesame oil contained substantially more than other oils (118–123 mg/100 g) [23].

Table 5. Sterols Composition of *Vangueria madagascariensis* (Kirkir) oil.

No.	Name	Common Name	Formula	RT	MW	Area	Area%
1	Campesterin	Campesterol	$C_{28}H_{48}O$	56.59	400	351000000	22.65
2	Stigmasta-5,20(22)-Dien-3-ol	Stigmasterol	$C_{29}H_{48}O$	56.97	412	311100000	20.08
3	Stigmast-5-En-3-ol,(3beta.,5.alpha.,24S)-	Beta sitosterol	$C_{29}H_{50}O$	58.09	414	701000000	45.24
4	Stigmasta-5,24(28)-Dien-3-ol, (3.beta.,24E)-	delta5-Avenasterol	$C_{29}H_{48}O$	58.30	412	21770000	1.405
5	Lanosta-8,24-Dien-3-ol,(3beta)-	Lanosterin	$C_{30}H_{50}O$	58.83	426	87430000	5.643
6	9,19-Cyclolanost-24-En-3-ol,(3beta)-	Cycloartenol	$C_{30}H_{52}O$	59.43	426	67040000	4.327
7	9,19-Cyclo-9.beta-lanostane-3beta.,25-diol	(+)-24-Dammarene-3β-20S-diol	$C_{30}H_{52}O$	59.67	444	10130000	0.6538

RT: Retention time in miutes.
MW: Molecular weight.
Area: Area under the peak in the sample.

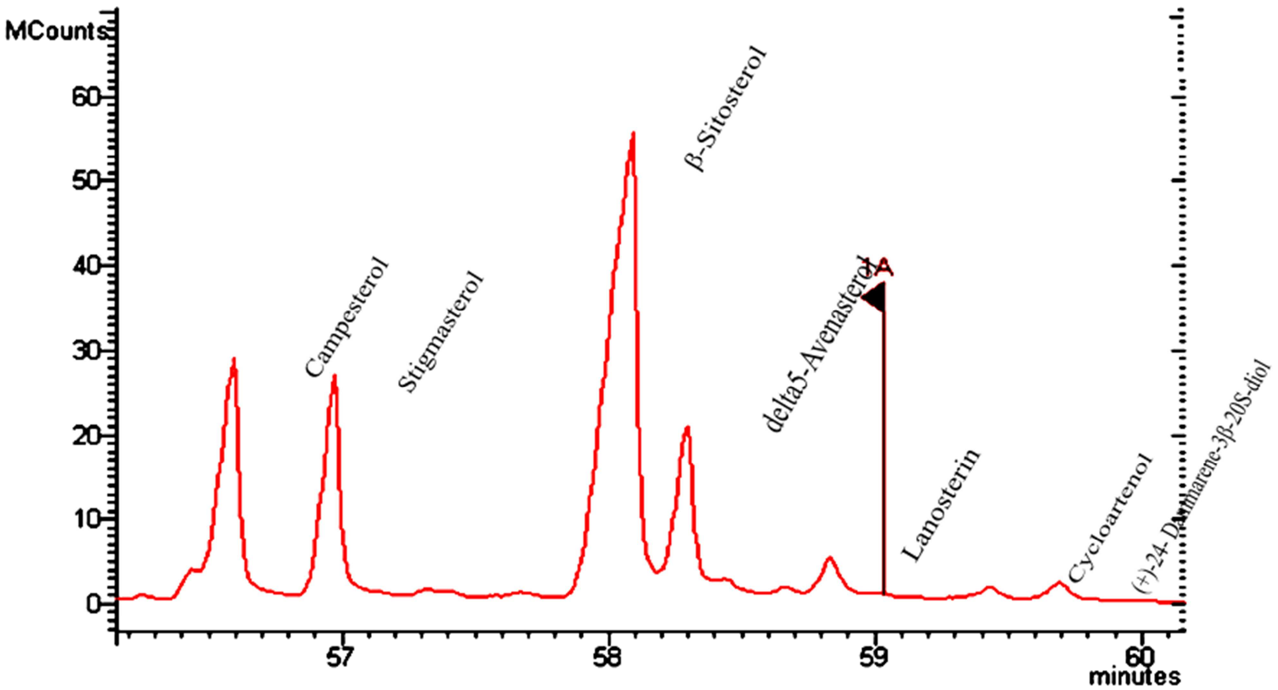

Figure 1. Chromatogram of sterols of *Vangueria madagascariensis* (kirkir) oil by GC/MS.

4. Conclusion

This study showed that *Vangueria madagascariensis* seeds are rich in protein and oil. *Vangueria madagascariensis* seed oilextracted with soxhlet (VMSE)and cold extraction (VMCE)werefound to contain high levels of unsaturated fatty acids, especially oleic (10.5, 10.4%) and linoleic (63.1, 63.4%), respectively. The oil extracts exhibited good physicochemical properties and could be useful for industrial applications. The oil proved to be good sources of both tocopheroland plant sterols.

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