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Physicochemical Analysis and Characterization of the Lipid Fraction from Côte D'ivoire *Myrianthus Arboreus* (Cecropiaceae) Seeds

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ABSTRACT

The physicochemical characteristics and chemical composition of the liquid fat matter from Côte d'Ivoire Myrianthus arboreus seeds have been determined. The contents in fat matter and in unsaponifiables are the order of 46 and 1.66%, respectively. The acid value (2.805 mg KOH/g), iodine value (171.84 g iodine/100 g of oil), peroxide value (10 meq/kg of oil), saponification value (171.105 mg KOH/g), ester value (168.30 mg KOH/g), refraction value (1.4615) and the lower calorific value (40372.34 kJ/kg) have been determined according to the international standards. They revealed the studied oil quality. After the oil esterification, GC/MS analysis showed the predominance of unsaturated fatty acids (95.58%) consisting predominantly of linoleic acid C18:2 (93.20%). Thus, Myrianthus arboreus nutritional, cosmetic and therapeutic potential advantages have been highlighted.

Keywords: Physicochemical characteristics, *Myrianthus arboreus*, Liquid fat matter, Fatty acid, Côte d'Ivoire

INTRODUCTION

The plant oils are traditional food products at high energy values. They are present in all biological tissues and are essential fatty acids (linoleic, linolenic and arachidonic acids) source. Also, they contain essential nutrients, vitamins, phospholipids and sterols that provide their anti-oxidant, anti-inflammatory, healing; and other properties which contribute to the regulation of metabolism. The main source of the plant oils is the oleaginous. On present hour, there are the plants whose oils have not been studied. Indeed, the African flora for example, contains many oil-bearing plants that could provide oil in balance for human food, health, the soap factory, chemistry and even for energy production [1]. Among these non-conventional oleaginous plants whose fat matter is under exploited in Côte d'Ivoire, we have *Myrianthus arboreus* from Cecropiaceae family. It is a dioecious tropical tree up to 15 m high with stilt roots and spreading branches. Its leaves in spirals are very large and its fruits, 10-15 cm diameter, contain seeds, 12 mm long [2,3]. This plant is known under various Ivorian and Nigerian vernacular appellations respectively: "Tikriti" (Bété), "wougnan" (Abbey), "djin" (Akyé), "pissia" (Dida) "Angaman" (Baoulé) and "oujoujou "(Ibo). *Myrianthus arboreus* is widely distributed in the tropical regions of West Africa (Côte d'Ivoire, Guinea, and Nigeria), Central Africa (Angola, Cameroon) and East Africa (Ethiopia, Tanzania). This plant stretches along the rivers in primary or secondary forests [3,4]. According to ethnobotanical investigations achieved at rural populations of Central-West of Côte d'Ivoire, *Myrianthus arboreus* is one of the plants most used for their vital needs [5]. Indeed, all its parts are variously used in Africa. In West Africa, the leaves are commonly consumed as vegetable soup [6,7].

In traditional medicine, they are widely used to treat jaundice, dysentery, vomiting, fever, heart disease, stomach pain, dysmenorrhea, pregnancy complications, infant hernia, boils, diabetes, bronchitis, toothache, throat and head [8-11].

The crushed roots are used as an antitussive [12]. This multitude of *M. arboreus* medicinal uses attracted the attention of researchers to carry out extensive work on its chemical composition and pharmacological activities. Thus, the Cameroonian [13] and Nigerien researchers [14] showed that the vegetables-leaves contain meaningful abundance of secondary metabolites (alkaloids, anthocyanins, carotenoids, flavonoids, saponins, tannins) and oxalates. The vegetables-leaves physicochemical and nutritional properties have been evaluated in several works [7,15,16] of which it demonstrated that *M. arboreus* leaves are not only rich in minerals (Ca, Mg, K, Fe, Zn, P) but also contain substantial quantities in vitamin C and polyphenols. Besides, they present good nutritional and antioxidant profile. Otherwise a survey showed that the considerable anti-oxidant capacity of *M. arboreus* roots excerpts would be linked to the phenolic compounds presence [17]. Several phytocompounds have been extracted from the plant organs. Triterpenes, myriantic acid and pentacyclic triterpenic diacids notably, have been isolated from its leaves [18-20] characterized peptidic alkaloids obtained from the aforesaid plant leaves extracts. Two triterpenic acids (tormentic and euscaphic acids) have been isolated from its roots [21]. The leaves and bark antibacterial properties [9,22], leaves powerful antinociceptive properties) [23] and bark antiplasmodial effect have been shown [24,25]. With regard to the plant almonds, a survey put in evidence the composition in fatty acids of the Congolese species oil [26]. However, the chemical composition of the oil extracted from the Ivorian species seeds has never been studied to our knowledge. To fill this deficit, the present work aims to contribute to the sources diversification of lipids through the under exploited non-conventional oleaginous study of the Ivorian flora. Thus, the Ivorian species chemical study has been conducted. Specifically, some physicochemical parameters of the oil extracted from this species seeds, have been determined and its unsaponifiable fraction GC/MS analysis has been achieved.

MATERIALS AND METHOD

Plant material

M. arboreus seeds come from fruits harvested 2014/07/25 in Daloa, a city of West-Central of Côte d'Ivoire (High-Sassandra Region). After species authentication the by botanists, seeds have been cleaned, shelled, kept at the steam room to 50°C for 7 days, then reduced into flour by milling.

Fat matter extraction

M. arboreus liquid fat matter (LFM) has been extracted hexane using a Soxhlet apparatus during 3 h [27]. The oil has been gotten by distillation of the solvent with a rotary evaporator (Buchi, Rotavapor® R-300).

LFM some physical and physicochemical characteristics determination

Saponification value (Is) and acid value (Ia) have been determined according to the literature [28,29]. Iodine value (Ii) and peroxide value (Ip) have been calculated respectively [30,31]. Ester value (Ie) has been calculated on the basis of analytical data following the formula Ie=Is-Ia. The calorific value (PC) has been determined using the formula:

PC=47645–4.187Ii–38.31Is (kJ/kg) [32,33].

The refractive index (Ir) has been determined according to ISO 6320 standard at 20°C with a refractometer (type thermostatically Abbe/ATAGO Tliquid NAR-1).

LFM analysis

Unsaponifiables extraction

LFM (5 g) was dissolved in 2 N ethanolic KOH (50 mL). The mixture was refluxed on a hot plate for 1 h. After cooling, distilled water was added (100 mL). The unsaponifiables were extracted by pentane (4 × 50 mL) and then washed with distilled water until neutral pH. The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure [27]. Unsaponifiable fraction was dried, weighed and analyzed by GC/MS. The unsaponifiable matter has been evaluated according to the formula:

$$\mathrm{Ins}(\%)=\left(\frac{m_1}{m_2}\right)\times 100$$

Ins: unsaponifiable

m1: mass of the unsaponifiables (g)

m2: mass of sample (g)

Fatty acids methyl esters preparation

After the unsaponifiable components extraction, soaps have been decomposed by addition of HCl (5 N) (1 mL). The fatty acids were extracted with AcOEt (3 × 25 mL). The solvent was evaporated in vacuum to provide concentrated fatty acids (3.99 g). Thereafter, their methylation has been performed by addition 1 mL of hydrochloric MeOH (2 N) boiled on a hot plate [34]. After cooling the mixture, 20 mL of distilled water were added and the methyl esters have been extracted with AcOEt (25 mL). After elimination of the solvent under reduced pressure, the obtained fraction (1.82 g) has been analyzed by GC/MS.

Unsaponifiable fraction and methylated fatty acids GC/MS analysis

The analysis of the unsaponifiable fraction (1 mg) dissolved in 2 mL of pyridine/acetic anhydride mixture (50/50, v/v) and methylated fatty acids (1 mg) dissolved in hexane (2 mL) was conducted by means of a GC/MS spectrometer SHIMADZU brand, model QP2010SE column with a Zebron ZB-5 ms 20 m long, with an inside diameter (0.18 mm) and a film thickness of the stationary phase (0.18 um). Helium was used as carrier gas at a linear speed (0.9 mL/s). The oven temperature program was from 50-280°C to 4°C/min and maintained at 250°C for 15 min. The injector temperature set at 250°C and the detector at 280°C. The injection was carried out in split mode/30. The parameters of the mass spectrometer for the electron impact ionization are the source temperature (230°C), the electron energy (70 eV), the scan speed (50 scans/s) and the acquisition speed (10.000 u.m.a/s). The compounds identification has been obtained while comparing the retention times with those of authentic compounds and spectral data of the spectrometer internal libraries.

RESULTS AND DISCUSSION

LFM and unsaponifiables yields

M. arboreus seeds provided a yellow LFM with an aroma similar to that of peanut. The extracted LFM yield is 46.6 ± 0.78%, comparable to those of certain edible oils [peanut (45-50%), palm kernel (48%)] [35] and to the one of non-conventional oil *Canariium schweinfurthii* Engl. (45%) [36]. LFM Unsaponifiables have been obtained with 1.66 ± 0.02% yield. This value is the average of insaponifiables yields (0.2-2%) encountered in the literature [37]; what denotes that *M. arboreus* is rich in unsaponifiables.

Physical and physicochemical characteristics

M. arboreus different physical and physicochemical characteristics have been presented in Table 1.

Table 1: LFM physical and physicochemical characteristics

Characteristics	Mean ± Standard deviation
Refractive index at 20.8°C	1.4615 ± 0.005
Average molecular weight (g/mol)	328.54 ± 9.987
Lower calorific value (kJ/kg)	40372.34 ± 201.16
Ia (mg KOH/g of oil)	2.805 ± 0.00
Is (mg KOH/g of oil)	171.105 ± 5.12
Ip (méq O_2/kg of oil)	10 ± 0.00
Ii (g iodine/100 g of oil)	171.84 ± 4.35
Ie (mg KOH/g of oil)	168.30 ± 5.12

LFM presents an acceptable content in free fatty acids because Ia=2.805 mg of KOH/g agrees to the Food Codex norms (<4 mg of KOH/g) [38]. This value reflects the LFM stability and purity. Moreover, we note that LFM Ia is less than some conventional oils [Coco ($4 \leq Ia \leq 7$); Palmist (Ia=4.7); Peanut FM (Ia=6)] [39].

LFM Is is weak with regard to those oils fluently used in soap factory [Coco ($246 \leq Is \leq 268$); Palmist ($240 \leq Is \leq 257$)]. So, it is founded to affirm that LFM little advisable like input in soap factory [40]. LFM Ip (limited value Food Codex) indicates that it is not safe from oxidation. However, this physicochemical parameter is an indicator of quality, not of safety. An Ip high value of oil indicates a meaningful presence of oxidation products whose admissible maximum concentration is not fixed. Indeed, one of the best edible oil is the one of virgin extra olive ($10 \leq Ip \leq 20$) [41]. LFM Ii permits to classify it among siccative oils (Ii>130). Its high value reveals the notable presence of esters unsaturated fatty acids in liquid fat. While Comparing LFM Ii liquid fat of *M. arboreus* to the iodine values of Colza oil ($94 \leq Ii \leq 120$), Soy ($120 \leq Ii \leq 143$) and Sesame ($118 \leq Ii \leq 120$), rich in essential fatty acids [42], we show its nutritional potentialities. Moreover, some ulterior studies will assess its oxidative stability and mineral content. The

lower calorific power superior to 35000 kJ/kg (minimal value for a calorific oil) shows that *M. arboreus* oil is a source of energy because 1 g could bring 9,7 kcal (40, 37 kJ) to the organism.

LFM composition in fatty acids

Myrianthus arboreus organic composition in fatty acid obtained by GC/MS is presented in Table 2. LFM mainly constituted of unsaturated fatty acids (95%) with an abundance of linoleic acid (93.2%). What is not in opposition with the assertion mentioned in the literature [43], according to which *M. arboreus* seeds provide the most known oil rich in linoleic acid (93.5%). Besides, the Table 2 indicates that among the saturated fatty acids (4.42%) contained in LFM, palmitic (2.16%) and stearic (1.56%) acids are respectively majority.

Table 2: Contents in main fatty acids present in LFM

Fatty acid	Retention time (min)	Content (%)
palmitoleic acid	10.976	0.08
Palmitic acid	**11.080**	**2.16**
Linoleic acid, Methyl ester	**11.974**	**75.04**
Stearic acid	**12.110**	**1.56**
Linoleic acid, Ethyl ester	**12.263**	**18.16**
nonadecylic acid	12.392	0.43
linolenic acid	12.938	0.17
11,14- cis-eicosadienoic acid	12.993	0.18
cis-11- eicosenoic acid	13.023	0.17

Of these results, we note that *M. arboreus* oil is particularly rich in linoleic acid (C 18:2). Therefore, it constitutes the real source of this essential acid, essential to the growth and physiological activity of all tissues. Indeed, linoleic acid plays an important role in the ceramides synthesis. It is known that acetylceramides, including linoleic acid is one of its main components, play a leading role in the lipid barrier formation of the skin. They bind adjacent lipid layers with each other so that a multilayer solid forms. In the case of a deficiency in linoleic acid, oleic acid or other acids take its place in acetylceramides. In consequent, the construction of extensive and continuous lipid layers becomes impossible. Not only the permeability of the lipid barrier is modified, but also the keratinocytes normal differentiation interrupts itself. The lack of linoleic acid in the body is manifested clinically by dryness, scaling, itching and redness [44,45]. This unique composition of LFM in linoleic acid would give him an exploitable biological activity both in cosmetics and in herbal medicine. It is well to note that the fatty acid composition of *Myrianthus arboreus* oil is comparable to the one of the therapeutic oil of Nigella (black cumin), which contains 84% of unsaturated fatty acids of which 56% linoleic acid [46]. It is also known that the black cumin oil is used due to its antibacterial and antiseptic properties. We further note the presence of eicosadienoic and eicosenoic acids (0.35%) in LFM, which are the precursors of prostaglandins. These last are responsible for many vital processes: formation and growth of cells, heart rate, pressure and blood coagulation, fertility and stimulation of anti-infectious reactions in the immune system. Linoleic acid (93.2%) in the liquid fat matter *Myrianthus arboreus* from Côte d'Ivoire is higher than linoleic acid content (77.5%) in the oil of the species of Congo. On the other hand, the contents in palmitic (2.16%) and stearic (1.56%) acids in the Ivorian species are lower than the content in palmitic (3.9%) and stearic (2.8%) acids in the Congolese species oil [26].

Unsaponifiable fraction composition

LFM content in unsaponifiables is the order 1.66 ± 0.02%. The aforesaid fraction GC/MS spectrum is presented (Table 3).

M. arboreus unsaponifiable fraction is dominated by oil (33%), followed hydrocarbon alcohols (4.03%), phytosterols (3.48%) and terpenes (3.39%). The principal hydrocarbon is stigmastan-3,5-diene (26.29%). The origin of this hydrocarbon is the dehydration of β-sitosterol [47]. Often, vegetable oils are characterized by the presence of stigmasta-3,5-diene that can arise during the GC analysis because of high temperatures and the reducing environment in the chromatographic system [48,49]. *M. arboreus* oil unsaponifiable fraction which consists of important quantity of phytosterols will certainly require a survey more deepened, since the components ratio can detect different types of activity and physiological effects.

Table 3: Compounds identified in the unsaponifiable fraction

Compounds	Retention time (min)	Content (%)
β-Myrcene	4.76	1.11
3,7-Dimethylocta-1,3,6-triene (ocimene)	5.28	0.61
Diethylethylphosphonate	5.61	1.83
2,5-Dihydro-2,2,4-trimethylfurane	6.56	0.04
3,7-Dimethylocta-1,6-dien-3-yl-2-aminobenzoate	6.91	4.08
Epoxy-α-terpinylacetate	7.01	0.06
Tetradec-1-ene	7.92	0.06
4,5,9,10-Dehydroisolongifolene	8.78	0.18
α-Calacorene	8.98	0.07
Butyl-2-ethylhexyloxalate	9.16	0.39
(Z)-Heptadec-3-ene	9.19	0.76
Dodecan-1-ylacetate	9.28	0.64
Caryophyllene oxyde	9.43	0.32
Isoelemicin	9.54	1.10
Nonadec-1-ene	10.34	1.05
6,10,14-trimethylpentadecan-2-one	10.60	2.03
Eicosane	10.95	0.46
Palmityl acetate	11.45	5.15
N-[4-Bromo-n-butyl]-piperidin-2-one	11.50	0.13
11-Methylheptacosane	11.68	0.31
Methyl-(E, Z)-octadeca-10,12-dienoate	11.86	0.57
Acide linoléique	12.08	1.98
Ethyloctadeca-9,12-dienoate	12.19	2.32
Ethyloctadec-9-enoate	12.21	0.87
(E)-Octadec-8-enylacetate	12.29	0.42
1-Octadecanethiol	12.34	0.45
Heneicosane	12.37	0.41
Eicosyl acetate	12.42	5.06
3,7,11,15-tetramethylhexadec-2-en-1-ol (phytol)	12.45	4.03
Tetratetracontane	12.71	0.18
Hexatriacontane	12.77	0.98
Squalene	17.07	2.50
2,4-Dibromo-6-phénylphenol	17.63	0.45
Stigmast-5-én-3-yl-(Z)-octac-9-enoate	18.34	0.21
(3β)-Cholesta-4,6-dien-3-ol	19.48	0.11
(3β)-20-Méthylpregn-5-en-3-ylacetate	19.92	0.05
(3β)-(Acétyloxy)cholest-5-en-24-one	22.89	0.95
Stigmasterol acetate	23.17	1.33
Stigmastan-3,5-diene	24.01	26.29
3,5,7-Pregnatrien-20-one	24.13	3.65
(3β)-9,19-Cyclolanost-24-en-3-ol	24.52	0.57
(3β, 9β)-24-Méthylène-9,19-cyclolanostan-3-ylacetate	25.25	0.26

CONCLUSION

The main objective of this study was the physical and chemical analysis and the characterization of the lipid fraction seeds from *Myrianthus arboreus* (Cecropiaceae) of Côte d'Ivoire. The results showed that the liquid fat matter extracted from this plant presents a nutritional, cosmetic and therapeutic interest. The seeds of *M. arboreus* are rich in fat matter; making this plant a promising source of oil. The profile of the fatty acids permits to classify the liquid fat matter of *M. arboreus* among oils with a high content of essential fatty acids. The established physicochemical parameters of

the liquid fat matter highlight its food potential. The presence of bioactive compounds in the unsaponifiable fraction obtained from the liquid fat matter could permit the use of this oil in the treatment of some pathology. To the look of its richness in linoleic acid, the liquid fat matter of *M. arboreus* would find use in the formulation of cosmetic emulsion.

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