



Characterization and Antioxidant Activity of *Myrianthus arboreus* Seed Oil Harvested from Cote d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Author KYS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KNE and GLA managed the analyzes for the study. Author NPK managed the documentary research. Authors M-BJA and BY-A made the first corrections to the manuscript. All authors have read and approved the final manuscript.

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ABSTRACT

Aims: The rich and diversified Ivorian flora abounds in countless oilseed plants that certain rural populations use for the preparation of dishes. Thus, certain physical and chemical parameters and the fatty acid composition of the fat extracted from the seeds of *Myrianthus arboreus* from four towns in Côte d'Ivoire (Agboville; Bouaflé; Daloa and Man), were determined, in order to contribute to the development of this wild plant.

Methodology and Results: Physical and chemical parameters determined by dosage comply with

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Codex Alimentarius standards. These parameters vary according to the harvest area and they show that the oil of *Myrianthus arboreus* is rich in unsaturated compounds ($lv > 90$; $Ri = 1.472 \pm 0.001$) The evaluation of the antioxidant activity, carried out by spectrophotometry by trapping the radical stable DPPH, showed a weak reduction of the stable radical DPPH by the oil of *Myrianthus arboreus* compared to vitamin C. This investigation also made it possible to determine for the first time the fatty acid composition of the oil of *Myrianthus arboreus* by GC / MS; more than 90% linoleic acid is the major acid.

Conclusion and Application: The oils analyzed showed very good contents of determined elements. Thus, the oil of *Myrianthus arboreus* could be used as well in human nutrition as in therapy. Thus, by its intrinsic characteristics, the oil from the seeds of *Myrianthus arboreus* is comparable to a conventional vegetable oil.

Keywords: *Myrianthus arboreus*; seed oil; physical and chemical characteristics; fatty acid; antioxidant.

ABBREVIATIONS

<i>M. arboreus</i>	: <i>Myrianthus arboreus</i>
An	: Acid number
Si	: Saponification index
lv	: Iodine value
Pv	: Peroxyde value
Ri	: Refractive index
MGA	: Fat from Agboville
MGB	: Fat from Bouaflé
MGD	: Fat from Daloa
MGM	: Fat from Man
DPPH	: 2,2-diphenyl-1-picrylhydrazyl

1. INTRODUCTION

In developing countries, eating habits include different nutritional strategies. Most populations rely on edible plant species, without any evidence of their nutritional values, let alone their safety. Among these unconventional species, several oilseed plants including *M. arboreus* are counted. This plant is a dioecious tree of the Cecropiaceae family. It grows in the forest zone of tropical Africa [1,2]. People use it for food, fencing, paper making [3], and in the treatment of many diseases, because of its many health benefits [4-6]. All its different and numerous operations have aroused interest in carrying out several works on almost all its parts (leaves, bark, roots) [7,8,9,10,11,12]. Some studies have been devoted to oil extracted from seeds of *M. arboreus* from Nigeria [7] and Congo [13]. In addition, biochemical analysis of fruit seeds from Côte d'Ivoire gave an average moisture content ($9.20 \pm 1.54\%$); protein ($25.37 \pm 1.97\%$); fat ($44.38 \pm 3.66\%$); in dry matter ($90.79 \pm 1.54\%$); in carbohydrates (18.67 ± 4.81) and in energy value (575.571 ± 13.43 kcal / 100g of dry matter). However, the lipid fraction of the Ivorian species

has not been studied. The purpose of this study is to determine some physical and chemical characteristics, fatty acid composition and antioxidant activity of the oil from the seeds of *M. arboreus*, harvested in different cities of Côte d'Ivoire. The aim of this scientific approach is to enhance the value of wild edible oilseeds from Côte d'Ivoire.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material consists of seeds of ripe fruits harvested from the feet of *M. arboreus*, growing in four cities in Côte d'Ivoire (Table 1).

2.2 Chemicals and Apparatus

Chemicals: Absolute ethanol; hexane; hydrochloric acid; ethyl acetate; diethyl ether.

Apparatus: Rotavapor; oven; Heating cap; Spetopotometer; GC / MS.

Glassware: bulb to the carafe; Balloons; Soxhlet.

Note: Part of the work was carried out in the Nangui Abogoua (CI) chemistry laboratory and the GC / MS analysis was conducted at the University 3 Rivers in Canada Chemistry Laboratory.

2.3 Methods

2.3.1 Oil extraction

After harvesting, the seeds were removed from the fruits (Fig. 1), dried in an oven at 40°C for

two days and then peeled. The almonds were again kiln-dried at 40°C for one day and crushed. The pastes obtained were used to extract the oil.

The oil from the seeds of *Myrianthus arboreus* from the different localities was obtained by continuous extraction with a Soxhlet for 3 hours using hexane as solvent according to French standards. Traces of hexane were removed on rotary evaporator [2].

2.3.2 Physical and physico-chemical analysis of the oil

The density, saponification, iodine, acid and peroxide indexes of the oil have been respectively determined according to NFT 60-214, NFT 60 206, NFT 60 203, NF ISO 3961 and NF T60 220 standards. The refractive index was determined using a digital display refractometer (Brand Leica AR 200 Barolworld, USA). The specific extinction coefficients K_{232} and K_{270} were measured at wavelengths 232 and 270 nm using

the Jasco 530 brand screw-type UV spectrophotometer [14].

2.3.3 Determination of unsaponifiable matter and fatty acid content of the oil

In a flask with a refrigerant, 5 g of oil was saponified with 30 ml of alcoholic (2N) potassium hydroxide (KOH). The reaction mass was boiled for 1 hour. After cooling, 100 ml distilled water was added and the aqueous phase was treated with 50 ml diethyl ether (Et₂O) three times. The organic fractions containing the unsaponifiables were collected, washed and dried on anhydrous sodium sulfate (Na₂SO₄). The solvent was removed with the rotary evaporator and the unsaponifiable fraction was weighed. To the aqueous phase, 3 ml hydrochloric acid (HCl) (5 N) was added, and the reaction mass was treated with ethyl acetate (AcOEt). The fatty acids were obtained after removal of the extractor [15].

Table 1. Information on *M. arboreus* seed collection sites

Sample	Harvesting site	Geographical location *	GPS Coordinate
MGA	Agboville	Southeast	5° 55' 41" N
	(Chief town of the Agnéby-Tiassa region)		4° 13' 01" O
MGB	Bouaflé	Central-West	6° 59' 00" N
	(Marahoué region)		5° 45' 00" O
MGD	Daloa	Central-West	6° 27' 00" N
	(Chief town of the Haut-Sassandra region)		5° 56' 00" O
MGM	Man	West	7° 24' 00" N
	(Chief town of the Tonkpi region)		7° 33' 00" O



Fig. 1. Fruits (A) and seeds (B) of *Myrianthus arboreus*

2.3.4 Determination of the fatty acid profile of the oil

The fatty acid composition of the oils was determined by analyzing their methyl esters, which were analyzed by gas chromatography on an Agilent Technologies 6890 N apparatus equipped with an internal silica capillary column (30 m × 250 μm × 0.25 μm) and coupled to a mass spectrometer (Agilent Technologies 5973). Helium was the carrier gas, used at 1 ml/min; the furnace temperature was set at 50°C for 5 min, followed by a gradient of 3°C/min to 250°C, and maintained for 15 min. The temperature of the injector and the detector was set at 230°C and 250°C respectively. Injection in split mode was done in 45 min with an ionization energy of 70 eV. The fatty acids were identified by comparing the retention times (RT) with those of the standard samples [15].

2.3.5 Evaluation of the antioxidant activity of the oil

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH/Carlo Erba) was solubilized in absolute ethanol to obtain a solution with a concentration of 0.1 mg/ml. Different concentration ranges (1; 0.5; 0.25; 0.125 and 0.062 mg/ml) of each unsaponifiable fraction were prepared in the same solvent. 1 ml of unsaponifiable matter extract and 1.5 ml of DPPH ethanolic solution were introduced into test tubes.

After shaking, the tubes were placed in a dark place for 30 min. The absorbance of the mixture was then read at 517 nm with a single-beam UV-visible spectrophotometer (HP, model 8453, 200-900 nm) against a blank consisting of 1 ml absolute ethanol and 1.5 ml DPPH. The positive

reference control is ascorbic acid (vitamin C). The percentage reduction (% I) of DPPH was calculated according to the following equation [16]:

$$I (\%) = \left(1 - \frac{A_{\text{sample}}}{A_{\text{white}}}\right) \times 100$$

Sample: absorbance of the extract; White: absorbance of the white.

This parameter was used to determine the median effective reduction concentration (EC50) of DPPH, determined graphically using Graph Pad Prism [16].

2.4 Statistical Analysis

The Statistica 7.1. software allowed for a one-factor analysis of variance (ANOVA ONE WAY). The comparisons of the means were performed by the Newman-Keuls test, the difference between the means was considered significant at the 5% threshold.

3. RESULT AND DISCUSSIONS

3.1 Extraction Yields

The yields of the extracted oil have already been published. Fat contents vary from 37.06 to 48.63 percent [2].

3.2. Oil Quality Indices

The results of the determination of the quality indices of oil extracted from the seeds of *M. arboreus* feet, found in the surrounding forests of Agboville, Bouaflé, Daloa and Man are reported in Table 2.

Table 2. Physical and physico-chemical characteristics

Characteristics	MGA	MGB	MGD	MGM
Refractive index (25°C)	1,47 ± 0,00 ^a	1,47 ± 0,00 ^a	1,47 ± 0,00 ^a	1,47 ± 0,00 ^a
Density	0,88 ± 0,01 ^a	0,88 ± 0,01 ^a	0,88 ± 0,01 ^a	0,88 ± 0,01 ^a
Saponification index	188,87 ± 0,02 ^b	189,40 ± 0,01 ^b	169,23 ± 0,03 ^a	192,61 ± 0,01 ^b
Acid number	4,34 ± 0,03 ^b	4,54 ± 0,02 ^b	2,80 ± 0,01 ^a	4,54 ± 0,02 ^b
Iodine value	105,73 ± 0,02 ^a	130,42 ± 0,01 ^b	170,62 ± 0,03 ^c	108,80 ± 0,02 ^a
Peroxide value	8,33 ± 0,01 ^{a, b}	6,66 ± 0,02 ^a	10,00 ± 0,00 ^b	8,33 ± 0,01 ^{a, b}
K ₂₇₀	0,31 ± 0,01 ^d	0,25 ± 0,02 ^c	0,11 ± 0,01 ^a	0,15 ± 0,01 ^b
K ₂₃₂	2,70 ± 0,02 ^b	2,03 ± 0,02 ^a	2,69 ± 0,02 ^b	2,87 ± 0,01 ^c

The values assigned the same letters on the same line are not significantly different at the threshold (p ≤ 0.05). MGA, MGB, MGD and MGM: oils from seeds from Agboville, Bouaflé, Daloa and Man, respectively

3.2.1 Refractive index (Ri)

This parameter provides information on the purity of the oil. Overall, as seen in Table 1, the oil of *M. arboreus* shows no variability in **Ri**, suggesting that the natural environment of the plant would not have an effect on this parameter. On the other hand, when an oil has an **Ir** between 1.471 and 1.477, it appears to be dominated by linoleic acid of the type ω -6 [17], and would be semi-siccative. The recorded **Ri** is comparable to that of some vegetable oils: cotton (1.470), peanut (1.470), palm (1.453) and jatropha (1.468) [17-18].

3.2.2 Density

The density (0.887 ± 0.01) of the oil does not seem to be influenced by the harvest site, and indicates that the oil is rich in polyunsaturated fatty acids (PUFA) [17]. It is also lower than some known edible oils: olive (0.914 - 0.918), soybean (0.919 - 0.925), sunflower (0.918 - 0.923) and rapeseed (0.916) [17].

3.2.3. Acid number (An)

An is one of the main quality indices, characterizing the degree of freshness of a fat, and is regulated. An oil is said to be virgin if **An** = 10 mg KOH/g oil [19]. Table 2 shows a significant variation in the **Ia** of the oil from seeds harvested in Daloa; this could presume that ecological factors at the harvest site would have an effect on its biosynthesis. The minimum and maximum values of **Ia** are 2.80 ± 0.01 and 4.54 ± 0.02 in MGD and MGB, MGM respectively. These values are comparable to those of some edible oils, namely coconut (4-7), palm kernel (4.7), peanut (6) and desert date (2.59) oils [20]. Overall, we find that *M. arboreus* oil is reported to be low in free fatty acids and is well suited for consumption.

3.2.4 Saponification index (Si)

Si indicates the content of fatty acids (esterified and free) in the oil. Oils with high saponification power (about 300) are recommended for soap making [21]. In general, we note from Table 1 that the **Is** of the oil studied is less than 200 mg KOH/g, but a significant fluctuation in this index is nevertheless observed at MGD and MGM. We also note that the **Si** values are comparable to those of many edible oils: soybean (189-195), groundnut (187-196), cotton (189-198), palm (195-205), olive (184-196) mg KOH/g [15].

3.2.5 Iodine value (Iv)

The results inherent to this index, highlighting a minimum value (105.73 ± 0.02 g of iodine/100g) in MGA and a maximum value (170.62 ± 0.03 g of iodine/100g) in MGD show clear variability. **Iv** of the oil studied ranged from 103.63 to 171.84 g iodine/100g. These values, which are higher than 90, make it possible to classify it as a semi-drying oil [22]. The high **Iv** values prove that *M. arboreus* oil is rich in unsaturated fatty acids (UFA), and comparable to the **Iv** values of soybean (120-143), sunflower (110-143) and sesame (104-120) oils [17].

3.2.6 Peroxide value (Pv)

Pv of an oil expresses its primary oxidation level by oxygen, at the end of which peroxides are formed. In general, looking at Table 1, we see a variation in the values of this index, except for MGA and MGM, which have similar values. On the other hand, the minimum and maximum **Pv** values obtained (6.66 - 10 meq O₂/kg oil) are in line with those required for edible oils (**Pv** \leq 10 meq O₂/kg oil) [19]. Therefore, we believe that *M. arboreus* oil would be recommendable in gastronomy.

3.2.7 Specific extinction (K) at wavelengths 232 and 270 nm

The specific extinction at 232 nm and 270 nm of an oil reflects its oxidation state [23]. The **K**₂₃₂ coefficient values for MGA, MGD and MGM (Table 1) are slightly higher than the Codex standards (**K**₂₃₂ \leq 2.5), suggesting that the oil has undergone oxidation, which would probably result from the extraction and drying time. This seems to explain the fluctuation in the values of this index at the lengths mentioned above. At 270 nm, **K** values are in accordance with international standards (**K**₂₇₀ \leq 0.25), except for MGA, which would indicate the presence of secondary oxidation products such as aldehydes and ketones. Thus, a low storability of MGA cannot be excluded [24].

3.3 Fatty Acid Profile of the Oil

The fatty acids globally identified in the oil of *M. arboreus* seeds are reported in Table 3. The fatty acid profile is the result of GC/MS analysis of methyl esters obtained by direct esterification of the oil. It was found that, in general, *M. arboreus* seed oil from four cities in Côte d'Ivoire contains a saturated fatty acid, palmitic acid (16:0), one of

the most naturally distributed fatty acids, and linoleic acid (C18:2), one of the most important unsaturated acids in the terrestrial plant and animal kingdom [25].

This high linoleic acid content (> 90%) presents the specificity of *M. arboreus* seed oil, which has been reported in the literature [26], mentioning that *M. arboreus* seeds provide the richest oil in linoleic acid (93.5%) [27]. Therefore, it is a true source of this essential acid, ω -6, which is essential for growth and physiological activity of all tissues.

From this fatty acid, the organism is able to synthesize numerous fatty acids with essential functions such as arachidonic acid (C20:4), the acid most involved in the construction of lipid membranes [27]. This unique composition of linoleic acid would confer to *M. arboreus* seed oil

a biological activity that can be used in cosmetics as well as in phytotherapy.

3.4 Unsaponifiable Matter Content

The unsaponifiable matter contents of MGA, MGB, MGD, MGM are respectively 1.40; 1.60; 1.66; 1.8%, and do not differ from those of fats generally fluctuating in the range 0.5-2% [28].

3.5 Antioxidant Activity of the Oil

The antioxidant activity of the oil was estimated by considering the capacity of its unsaponifiable fraction to reduce the stable radical of DPPH, compared to vitamin C (reference antioxidant). The results were expressed in graphical form (Fig. 2). In general, it appears that the oil reduction activity is concentration-dependent. It increases progressively with increasing concentration of the unsaponifiable fraction.

Table 3. Fatty acid composition of the oil

Oil	Fat acid	TR, min	Teneur, %
MGA	n-Hexadecanoic or palmitic acid (C16: 0)	22,57	1,38
	Linoleic acid (C18: 2)	24,54	96,65
MGB	n-Hexadecanoic or palmitic acid (C16: 0)	22,58	2,14
	Linoleic acid (C18: 2)	24,54	93,64
MGD	n-Hexadecanoic or palmitic acid (C16: 0)	22,58	2,25
	Linoleic acid (C18: 2)	24,20	94,60
	n-Hexadecanoic or palmitic acid (C16: 0)	22,57	1,80
MGM	Linoleic acid (C18: 2)	24,48	94,73

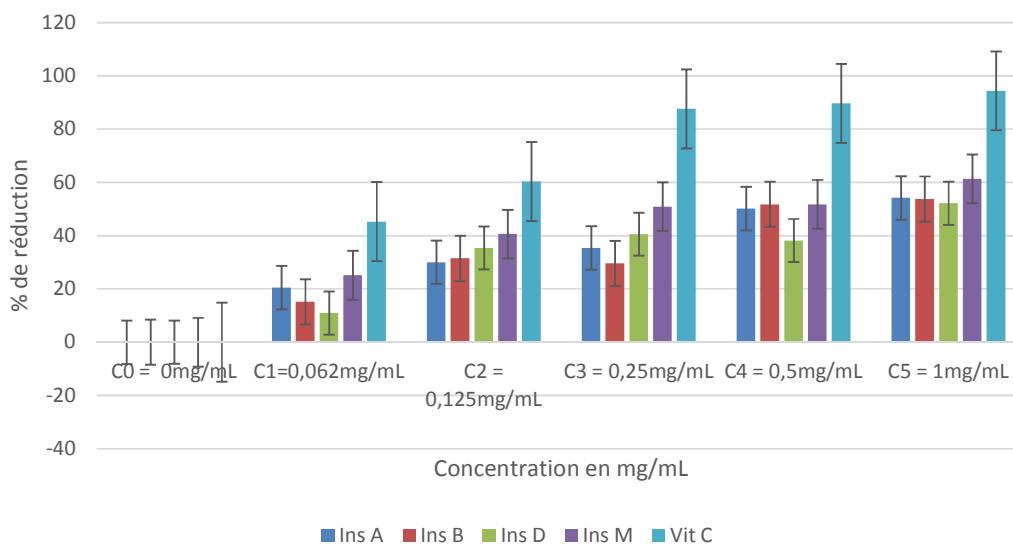


Fig. 2. Reduction percentage of DPPH by oil unsaponifiables by *M. arboreus*

Table 4. EC₅₀ of the unsaponifiable fraction of *M. arboreus* oil

Extrait	EC₅₀ (mg/ml)
MGA	0,52
MGB	0,46
MGD	0,89
MGM	0,25
Vit C	0,09

The EC₅₀ inhibitory concentration, determined graphically from the reduction percentage of DPPH, is the reliable median concentration that shows the antioxidant effectiveness of the oil. The lower the value, the more effective the oil. As a result of the above, MGM has the best antioxidant efficacy (Table 4).

4. CONCLUSION

This study we undertook determined some indicators of quality, fatty acid composition and antioxidant activity of *Myrianthus arboreus* oil from the forests surrounding four cities in Côte d'Ivoire. The results obtained are important, and attest to the nutritional importance of this oil extracted from a wild plant species. However, it would be essential to carry out a vitamin E assay and a toxicological study of the oil from the seeds of the said species. All in all, it is inappropriate to assess the quality of one vegetable oil compared to another because each has its intrinsic virtues, particularly its fatty acid profile and nutritional qualities

DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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