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Chemical Profile and Radical Scavenging Activity of Extracts *Musanga cecropioides* (R. Brown) Seeds Oil from Congo

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Abstract: In Africa, many of the plant resources of high nutritional value are not yet included in the uses of local populations. This study contributes to the enhancement of the lipid potential of *Musanga cecropioides* (R. Brown) seeds. Soxhlet seed oil extraction gave a yield of 8.62%. The evaluation of the physical parameters of the oil brings it closer to that of Argan oil. The oil extracted from the seeds of *M. cecropioides* consists mainly of three major fatty acids: oleic acid (48.58 ± 0.63%), linoleic acid (26.74 ± 0.53%) and palmitic acid (15.05 ± 0.21%), the determination of which is made by CPG-MS. This fatty acid composition places *M. cecropioides* oil among oleic/linoleic oils with potentially nutritional properties. The search for minor compounds in the oily fraction shows carotenes, tocopherols and sterols on thin layer (TLC). The antioxidant activity of *M. cecropioides* oil is evaluated by the anti-free radical test on TLC and spectrophotometer using DPPH. The results obtained from the antioxidant activity of *Musanga cecropioides* oil show an important radical scavenging activity with the value of IC50 (1.539 ± 0.013 mg/mL) and a strong activity of the unsaponifiable fraction and the latter is due by the unsaponifiable fraction with an IC50 (0.073 ± 0.004 mg/ml). Analysis of the sensory and physical profile of the oil extracted from the seeds of *Musanga cecropioides* shows that it could be used in cosmetics, pharmaceuticals and food.

Keywords: *Musanga cecropioides*, Oil, Fatty Acids, Unsaponifiable, Antioxidant, DPPH

1. Introduction

Global demand for oilseeds is growing at breakneck speed, leading to all-out searches for new sources of vegetable fat supply. These form an important part of the diet in humans and contain many chemical compounds with therapeutic effects. Consumers are paying more and more attention to references to the origin and quality of the products they buy. It is in this perspective that the exploitation of the molecular heritage of plant species has continued to interest researchers, particularly chemists and biochemists [1]. In the Republic of Congo, there is considerable and varied agricultural potential due to its equato-tropical climate. However, this asset is insufficiently exploited and makes the country dependent on food imports to meet the needs of the population. This problem is observed in the field of fatty substances where the Congo only obtains the bulk of its vegetable oils from two

plants: oil palm and peanuts, although there is a diversity of oil plants in the country. The various parts (fruit, seed, almonds, etc.) have several uses [2].

It seems interesting to us for the sake of innovation to target other oilseeds of low economic importance for the moment; but nevertheless worthy of scientific and technological interest such as the *Musanga cecropioides* present in the secondary humid forests of Africa, since Sierra Leone and Liberia to Angola, passing naturally through the large forests of the Congo Basin [3]. The APG II classification (2003) places it in the order of Rosales and in the family of Urticaceae. *M. cecropioides* is known in several languages, in French (Parasolier; bois boucher), in English (Umbrella tree; African corkwood), in Spanish (Arbol paraguas) and in the dialects of Congo (Onsié; Nsenga). Musanga cecropioides is an important medicinal plant. This plant has been the subject of more precise biological studies on the bark, roots and leaves; [4–8]; But the observation of fruits and seeds has not always been very developed. The yellowish-green fruit measures 10-13cm long by 5-6cm wide with succulent flesh and small, encrusted seeds or achenes. Although edible by birds and ants, the fruit does not seem to be very popular with local populations [3, 9]. Therefore, we are interested in the seed oil of *Musanga cecropioides* (R.Br.); The presence of bioactive compounds in seed oil would lead to the enhancement of this species for notional and medicinal use.

The present study is a contribution to the valorization of the potential of the seeds of the fruits of *Musanga cecropioides*. It aims to evaluate the richness of the oil of *M. cecropioides* in chemical compounds, to compare the physicochemical properties of this oil with certain known oils such as Argan (*Argania spinosa*), Olive (*Olea europaea*), Sunflower (*Helianthus annuus*), Sesame (*Sesamum indicum*), Refined palm and to investigate the synergistic effects on biological activities such as antioxidant activity that could justify its consumption and the use of this matrix.

2. Materials and Methods

2.1. Plant Material

The fruits (figure 1, A) were harvested in September, in the locality of Owando in the northern zone of Congo-Brazzaville were cut into small pieces and then dried at room temperature (24 and 26°C) in the shade for collection. seeds (Figure 1, B), which is done by manual sorting to rid them of the species of pulp or flesh to which they are attached.

Figure 1. *Fruits and seeds of Musanga cecropioides. (A): fruits, (B): seeds.*

The seeds of the fruit of *Musanga cecropioides* constitute the raw material of this study.

2.2. Methods

2.2.1. Soxhlet Oil Extraction

The dried Musanga grains obtained by shelling the fruits were crushed to obtain a fine and homogeneous paste powder. The oil was extracted using a soxhlet method with petroleum ether as solvent for 3 hr. The oil extract was concentrated in rotary evaporator by distillation at reduced pressure and 35°C until the solved was totally removed.

2.2.2. Physico-chemical Characterization

The determinations of physicochemical parameters of seed oils for refractive index, acid value, iodine value, saponification value, unsaponifiable matter, peroxide value, free fatty acid and specific gravity are always carried out according to the methods of AOAC [10]. The two parts, fatty acids and the unsaponifiable fraction are obtained by separation using solvent extraction and their mass is calculated after drying in an oven set at 105°C [11].

2.2.3. Determination of the Fatty Acid Composition

Before analysis by gas chromatography (GC), triacylglycerols of vegetable oils and fatty acids (FAs) were transformed into their corresponding methyl esters (FAMEs) according to the procedure reported by Müller [12, 13]. Twenty milligrams of oilwere solubilized in 1 mL TBME, and then 100 µL of this solution was mixed with 50 µL TMSH solution (0.5 $mol.L^{-1}$ in methanol).

FA methyl esters (0.5 µL) from each sample were analysed by GC, using a flame ionization detector (FID) (Varian CP-Select 3900 gas chromatograph, Grenoble, France), with a fused-silica capillary column, CP Select CB (25 m × 0.32 mm, 0.50 µm film thickness). The carrier gas was helium with a flow rate of 1.2 $mL.min^{-1}$; split ratio was 1: 100. The initial oven temperature was held at 185°C for 40 min, increased at a rate of 15°C min−1 to 250°C and then held there for 10 min. A second analysis was achieved on a DB Wax column (30 m × 0.25 mm, 0.25 µm film thickness). The carrier gas was H_2 with a flow rate of 1 mL min^{-1}. The initial oven temperature was held at 150°C for 1 min, increased at a rate of 15°$C.min^{-1}$ to 200°C, followed by a rate of 2°$C.min^{-1}$ to 250°C and then held there for 15 min. The detector and injector temperatures were fixed at 250°C. FAs were identified by comparison of their retention times with those of pure reference standards.

2.2.4. Characterizations of the Unsaponifiable Faction of Oil

The compounds of the unsaponifiable fraction were separated on thin layer chromatography (TLC) plates, the plates were revealed with anisaldehyde and then heated to obtain the most intense colors. The identification of the different compounds in the unsaponifiable fraction was carried out by comparing the spots and their Rf values with the reference as described by Lercker [14].

2.2.5. Antioxidant Activity

To study the radical scavenging activity of our different fractions, we opted for the use of diphenyl picryl-hydrayl (DPPH) as a relatively stable free radical, according to the protocol described by Lesage-Messen [15].

The demonstration of the antioxidant power of *Musanga cecropioides* (vegetable oil, VO) oil and unsaponifiable extract (UF) was carried out by two chemical tests: contacting the compounds on a TLC plate by spraying with DPPH solution, and measuring the scanning activity of a powerful free radical DPPH (2,2 diphenyl-1-picrylhydrazyl) [16].

In the first method, the extracts, fractions or pure products to be tested developed on a TLC plate of silica gel GF_{254} in aluminum are dried and then revealed using a methanolic solution at 0.1 mg/mL of DDPH. In the presence of an antioxidant chemical in the analyzed sample, the DPPH is reduced and changes color from purple to light yellow. radical scavenging activities appear as a yellow-white color on a purple background.

The radical scavenging activity in the second test, performed by the DPPH scavenging method, is estimated using the following formula:

$$AAR\% = [(Abs_{517} \text{ negative control} - Abs_{517} \text{ sample})/Abs_{517} \text{ negative control})] \times 100.$$

(AAR%: anti-free radical activity; Abs_{517}: absorbance read at 517 nm).

In test tubes, 3 ml of the methanolic solution of the fraction to be tested are added to 2 ml of the methanolic solution of DPPH (0.127 mg/ml). β-carotene is used as a positive control in this experiment under the same conditions.

To overcome the influence of concentration, reactivity is estimated by the effective concentration EC50 or IC50; defined as the concentration of extract required that causes the 50% loss of DPPH activity. The IC50 is calculated graphically by linear regression of the trace graph and the percent inhibition as a function of different concentrations of the fractions tested [17].

2.3. Statistical Analysis

All experimental procedures were performed in triplicate and their mean values (± standard deviation) were given.

3. Results and Discussion

3.1. Fat Content

The oily extract of the seeds of *Musanga cecropioides* obtained by Soxhlet is liquid with an orange-yellow color and a very popular odor. Its yield is 8.62%. Relatively low yield compared to some oils unconventional, such as *Elaeis guineensis* oil, *Irvingia wombulu* oil [18], African star apple [19], and close to that of oils recognized as unconventional and classified as oleaginous, *Aframomun spulatum* (8.18%) [13] and Tamarindus indica (8%) [20].

3.2. Physico-chemical Parameters of the Oil

The results obtained from the physical analyzes of *Musanga cecropiodes* oil are shown in Table 1, these are tools for determining the quality of an oil. Density and refractive index tell us what group an oil belongs to.

The density of the oily extract of the seeds of *Musanga cecropioides* found in this study is of the order of 0.915 ± 0.001. This density value gives us the information that our oil belongs to the group of non-drying oils, since the latter is between 0.900 and 0.920 [21].

The refractive index value of *M. Cecropioides* oil (1.472 ± 0.000%). The refractive index tells us about the group to which the fatty substance belongs. It is considered a criterion for the purity of an oil. This value is proportional to the molecular weight of fatty acids as well as to their degree of unsaturation. This oil has physical characteristic values almost similar to those of argan [21] and African star apple [19] oil as listed in Table 1. This refractive index value also confirms the density result. Indeed, the refractive index value of Musanga cecropioides oil can therefore be considered as a non-drying oil.

Table 1. *Results of physicochemical parameters of Musanga cecropioides seed oil.*

Properties	Unit	*M. cecropioides* Oil	Argan oil [21]	Sésame oil [24]	African star apple oil [19]
Coulor		Orangey yellow	/	/	/
State at room temp.		Liquid	/	/	/
Density at 20°C		0.915 ± 0.001	0.917	0.91± 0.02	/
Refractive Index		1.472 ± 0.000	1.472	1.468±0.001	/
Acid value	mgKOH/g	18.70 ± 0.44	0.98 - 2.6	0.55±0.04	17.41±0.43
Peroxide value	$mEqO_2/g$	40.00 ± 1.82	/	3.79±1.02	57.74
Iodine Value	$gI_2/100g$	95.09 ± 2.03	99 – 102	135.22±0.81	29.00 ± 0.16
Indice de Saponification	mg KOH/g	241.23 ± 0.32	189 – 193	189.31±1.77	236.34
Indice d'Ester	mg/g	222.53 ± 0.33	/	0.90±0.00	/
Teneur en insaponifiable	%	2.06 ± 0.30	0.8 – 1.3	1.71±0.11	/

(/): Not available.

The acid value (AV) of *Musanga cecropioides* oil is determined to be 18.70 mg KOH/g. This value is very high compared to other oils like that of soursop oil (1.82 mg KOH/g) and close to that of African star apple (17.41 ± 0.43 mg KOH/g) [19]. The acidity of the extracted oil is a measurement that is often of great commercial importance;

Knowing that a low acidity value characterizes the purity and the stability of an oil at room temperature, this criterion is not satisfied for the oil of *M. cecropioides*, it is therefore necessary that precautions of pre-refining and conditioning are taken in order to limit a probable subsequent denaturation of this oil.

Regarding our oily extract from the seeds of *Musanga cecropioides*, the value of the peroxide value (PV) is of the order of 40.00 mEq.O_2/kg. This value remains higher than the limit established by the IOC standard for oils (20 mEq. Active O_2/Kg of oil) and is higher compared to other oil such as olive oil (17.96 - 18.3 mEq.O_2/Kg) [22], but lower than that of African star apple oil (57.74 mEq.O_2/Kg) [19]. This is a very useful criterion for assessing the early stages of oxidative deterioration of an oil. The high PI value of our oil seems to show that our sample is relatively oxidized. This oxidation could be due to the conditions of extraction and storage.

The iodine value (IV) found in this study for *Musanga cecropioides* seed oil was 95.09 g/100 g. It classifies *M. cecropioides* oil among non-drying oils, whose iodine Indexs are between (0 and 110 g/100 g) of oil. The iodine value of *M. cecropioides* seed oil is comparable to the iodine value of olive, melon, cotton, peanut and castor oil varying between 75 and 95 g/100 g [21]. This oil is therefore highly concentrated in unsaturated fatty acids, hence the liquid appearance.

The saponification value (SV) of *Musanga cecropioides* seed oil is evaluated at 241.23 mg/g. This SV is close to the saponification indices of African star apple (236.34 mg/g) and palm kernel (max = 254.00 mg/g) oils commonly used for this purpose [19]. The relatively high value of the SV indicates that the oil of *M. cecropioides* could be used as a raw material for the manufacture of foaming soaps [19].

The ester value (EV) of *Musanga cecropioides* oil is 222.53 mg KOH/g oil. This value of EV is more distant from its saponification value, ie (241.23) mg KOH/g oil. This means that this oil contains an appreciable amount of free fatty acids, which confirms the high level of acid value (18.70 mg/g). Therefore, pre-refining and conditioning precautions should be taken to limit subsequent denaturation which would lead to discoloration of the oil.

The unsaponifiable fraction content of the *Musanga cecropioides* seed oil obtained is 2.06%. This value is relatively high, compared to the results of the few conventional oils presented in Table 2 such as Sunflower oil, Olive oil, Argan oil [21, 23] and unconventional such as Sesame and of African star apple oil [23, 24]. This unsaponifiable material would add value to this oil and allow its use in cosmetics and food.

3.3. Fatty Acid Profile

The fatty acid composition of *Musanga cecropioides* seed oils is presented in Table 2, then compared to some oils. We note that three (3) main major fatty acids have been identified including oleic acid (C18:1) with a rate of 48.58%, followed by linoleic acid (C18:2) 26.74% and palmitic acid (C16:0) 15.05%. The results of this study show that the seeds of the fruits of *M. cecropioides* are a valuable source of essential unsaturated fatty acids. In addition, we note the predominance, at the level of the lipid profile, of the seeds of Musanga cecropioides, of monounsaturated fatty acids (MUFA) whose rate expressed as a percentage of total fatty acids (TFA) is 50.98%. They are predominantly represented by oleic acid followed by linoleic acid. The relative proportions of these two main acids show appropriate nutritional properties of edible oils. MUFAs are followed by polyunsaturated fatty acids (PUFAs) making up 28.29% of TFAs and which are mainly represented by linoleic acid 26.74%. The least represented class is that of saturated fatty acids (SFA) ie 20.36%, represented mainly by palmitic acid (15.05%).

Table 2. *Fatty acid composition of Musanga cecropioides seed oil expressed as% of total fatty acids.*

Fatty acids	*M. cecropioides* oil	Argan oil [27]	Sesame oil [24]
Lauric acid (C12:0)	0.19 ± 0.02	/	/
Myristic acid (C14:0)	0.33 ± 0.06	≤ 2	/
Palmitic acid (C16:0)	15.05 ± 0.21	≤ 11.5 - 15	9.4
Palmitoleic acid (C16:1n7c)	1.05 ± 0.09	≤ 0.2	/
Stearic acid (C18:0)	4.31 ± 0.16	4.3 – 7.2	5.97
Oleic acid (C18:1n9c)	48.58 ± 0.63	43 – 49.1	44.99
Elaidic acid (C18:1n9t)	0.19 ± 0.07	/	/
Linoléic acid (C18:2n6c)	26.74 ± 0.53	29.3 – 36.0	39.61
Linolénic acid (C18:3n3)	1.05 ± 0.06	≤ 0.2	/
Arachidic acid (C20:0)	0.48 ± 0.05	≤ 0.5	/
Gadoleic acid (C20:1n9c)	0.27 ± 0.08	≤ 0.5	/
Behenic acid (C22:0)	/	/	/
SFA	20.73		
MUFAs	50.98		
PUFAs	28.29		
UFA	79.27		
Ratio (UFA/SFA)	3.82		

(/): Not available. SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; UFA: Unsaturated fatty adices.

Also, it should be noted that the seed oil of *M. cecropioides* is highly unsaturated (79.27%) which justifies

its liquid aspect in accordance with the iodine value.

The high level of unsaturated fatty acids (UFA) gives the oil from the seeds of *M. cecropioides* greater stability to auto-oxidation and an important nutritional value. In addition, fatty acids (arachidic, gadoleic, myristic and lauric) were detected in low proportions.

The oxidative stability of oils will depend in particular on their UFA content and composition; but also, the content of tocopherols in the oil, which may exert an antioxidant protective action [25, 26].

The fatty acid composition of *M. cecropioides* oils is comparable to that of argan oil [27], sesame oil [24] and complies with the International Olive Council (IOC) standard [22] in terms of fatty acid composition. These two types of predominance in fatty acids allow several recovery routes: in direct food and in lipochemistry, especially in soap, in the surfactant industry, in the industrial lubricants industry and in cosmetology [28].

The oil from the seeds of *M. cecropioides* is of the oleic type. Its high percentage of oleic acid makes it more desirable in terms of nutrition, by giving it better hold and very high stability in cooking and frying [29]. The fatty acid composition shows medicinal interest in the fruits of the *Musanga cecropioides* species, according to the data in the bibliography [30].

3.4. Chemical Composition of the Unsaponifiable Fraction

The chromatogram of figure 2 revealed with anisaldehyde developed in the Chloroform / Ether-diethyl system (9/1) shows eight (8) spots of variable colors, attributable to phystosterols (Rf 0.34), triterpenes (Rf 0.50), tocopherols (Rf 0.61 and 0.66), carotenoids (Rf 0.76; 0.79 and 0.86) and hydrocarbons (Rf 0.91) [14]. The unsaponifiable fraction is richer in terpenoids (triterpenes and carotenoids). This could justify the solid appearance and the yellow coloration of this fraction.

Compounds	Colors	Spot number	Rf	Chromatogram
Hydrocarbons	Violet	1	0.91	
Carotenes	Yellow	2	0.86	
	Gray	3	0.79	
	Yellow	4	0.76	
Tocotrienols	Violet	5	0.66	
Tocopherols	Violet	6	0.61	
Triterpenes	Blue	7	0.50	
Phytosterols	Gray	8	0.34	

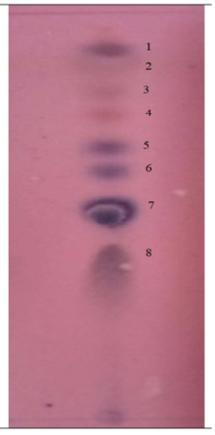

Figure 2. *Identification of minor compounds of Musanga cecropioides oil on TLC.*

The presence of carotenoids in considerable quantities would be an important pharmaco-nutritional advantage [26] and makes a contribution for *Musanga cecropioides* oil.

In addition, tocopherols and tocotrienols which are beneficial for the prevention and/or treatment of many diseases [31].

Phytosterols have generated the most interest because of their cholesterol lowering power. Besides, their effect on reducing blood cholesterol levels; the latter are also being studied for their anticancer, immunomodulatory and anti-inflammatory action [32, 33].

Indeed, this unsaponifiable fraction contains compounds from the class of carotenes, tocopherols, terpenes and sterols which are endowed with antioxidant and vitamin properties. They would protect the oils from oxidation due to oxidizing agents and the body from certain diseases [30]. It has been shown that the quality of an oil depends not only on its composition of saturated and unsaturated fatty acids, but also on its content of minor compounds belonging to the unsaponifiable fraction.

3.5. Antioxidant Activity

3.5.1. Radical Scavenging Activity of Extracts with DPPH on TLC

After development in the two solvent systems consisting of Hexane (Hex)/Ether-diethyl (Et_2O) (7/3), Chloroform ($CHCl_3$) /Ether-diethyl (Et_2O) (9/1) and revealing of the TLC plate with the DPPH solution, the yellow spots on a purple background testifies the radical scavenging activity of vegetable oil (VO) (figure 3). This activity is more marked for the unsaponifiable fraction (UF) and would be essentially attributable to carotenoids and tocopherols. We find that the Hex/Et_2O (7/3) system separates the first two compounds better than the $CHCl_3$/Et_2O (9/1) system.

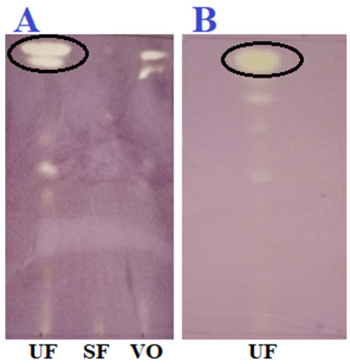

Figure 3. *TLC showing antiradical activity. UF: Unsaponifiable Fraction; FS: Saponifiable Fraction; VO: Vegetable Oil; A: elution system (Hex/Et_2O (7/3)); B: elution system ($CHCl_3$/ Et_2O (9/1)).*

3.5.2. Radical Scavenging Activity of Extracts on DPPH by Spectrophotometric Assay

From the inhibition curves, we determined the IC50 value for each fraction. The results of the IC50 obtained from these different fractions by the DPPH radical are shown in Table 3: (1.539± 0.013) and (0.073 ± 0.004) mg/mL respectively for the crude vegetable oil and the unsaponifiable fraction. β-carotene (0.039 ± 0.001 mg/mL) was used as a reference molecule.

From these data, it appears that the IC50 value of β-carotene is almost half that of the unsaponifiable fraction which in turn is much lower compared to the IC50 value of crude vegetable oil. This strong antioxidant activity could be justified by the presence of carotenoids and tocopherols, which are the majority compounds in the unsaponifiable fraction. In this study, the simultaneous presence of several families of antioxidants (carotenoids, phenolic acids and tocopherols) also works in favor of synergistic effects which can result in a mechanism of regeneration of tocopherols [15, 25].

Table 3. *IC50 values of Musanga cecrepioides of crude oil, unsaponifiable fraction and β-carotene on DPPH.*

Excerpts	IC50 (mg/mL)
M. cecropioides crude oil	1.539 ± 0.013
Unsaponifiable fraction	0.073 ± 0.004
β-carotene	0.039 ± 0.001

4. Conclusions

In view of all the data reported in the bibliography concerning the seeds of *Musanga cecropioides* fruits collected at Owando, we have devoted the present work mainly to the determination of the physicochemical characteristics and the antioxidant activity of this plant. We succeeded in optimizing the fat (oil) yield of *M. cecropioides* seeds by setting the extraction time to 3 hours and using petroleum ether as the extraction solvent. Our seeds give a low yield of around (8.62%) compared to other vegetable oils.

Regarding the physicochemical analyzes of our oil, the refractive index revealed that the latter belongs to the group of non-drying oils and can be stored for a long time. In addition, the values of the iodine and saponification indices respectively reveal that this oil is rich in unsaturated fatty acids and contains fatty acids from medium hydrocarbon chains. The value of the peroxide value does not comply with that given by the Codex Alimentarius standards, which means that there is oxidation during storage.

The analysis of the fatty acid profile by CPG/FID shows the richness of the oil in unsaturated fatty acids (79.27%) of which oleic acid (C18:1) is predominant with an average content of 48, 58% followed by linoleic acid (C18:2) 26.74%. In contrast, the content of saturated fatty acids is 20.73%, represented mainly by palmitic acid (C16:0) with a content of 15.05%.

The fatty acid composition of our oil is similar to that of argan oil in the content of (C16:0), (C18:1) and (C18:2); which gives *Musanga* oil interesting nutritional and technological properties, particularly in terms of the favorable action exerted by monounsaturated fatty acids on the evacuation of cholesterol.

Musanga cecropioides seed oil is of oleic type with a high content of oleic acid. This feature is highly desirable especially with the current trend to replace polyunsaturated vegetable oils with those that contain high levels of monounsaturated fatty acids.

A characteristic feature of the oily extract of *Musanga cecropioides* seeds is the high content of unsaponifiables (2.06%) compared to other conventional vegetable oils. This unsaponifiable extract contains a large number of bioactive chemical compounds (carotenoids, tocopherols, sterols). The study of the antioxidant activity of the two oily extracts of the seeds of custard apple showed a low activity for vegetable oil with the value of IC50 (1.539 mg/ml) and a high activity of the unsaponifiable fraction with the IC50 (0.073 mg/ml). However, the antioxidant activity of Musanga oil is lower than that of β-carotene with IC50 (0.039 mg/ml), a molecule used as a reference.

Competing Interests

Authors have declared that no competing interests exist.

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