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Physicochemical and Antioxidant Properties of *Carapa procera* Seed Oil from Korhogo, Côte d'Ivoire

Daouda Touré[1,2*], Monon Koné[1], Didjour Albert kambiré[1], Efui Holaly Gbekley[3], Gohi Rodrigue Gouri[3], Lanciné Traoré[4], Faustin Aka Kabran[5], Ahmont Claude Landry Kablan[1], Gustave Bedi[6] and Allico Joseph Djaman[7]

[1]Département de Biochimie-Génétique, UFR des Sciences Biologiques, Université Peleforo GON COULIBALY, BP 1328 Korhogo, Côte d'ivoire
[2]Département Santé, DEECAF (Destination Economique Afrique), Rennes, France
[3]Laboratoire de Microbiologie et de Contrôle Qualité des Denrées Alimentaires (LAMICODA), Centre de Recherche et de Formation sur les Plantes Médicinales (CERFOPLAM), Université de Lomé, Togo
[4]UFR Sciences et Technologies, Université de Man, BP 20 Man, Côte d'Ivoire
[5]Laboratoire de Constitution et Réaction de la Matière, Université Felix HOUPHOUET-BOIGNY de Cocody -Cote d'Ivoire, 22 BP 582 Abidjan 22 (Cote d'Ivoire)
[6]Ecole Normale Supérieure, Abidjan, Côte d'Ivoire
[7]Département de biochimie, Institut Pasteur de Côte d'Ivoire, 01 BP 490 Abidjan 01 (Côte d'Ivoire)

*Corresponding author (daouda-toure@upgc.edu.ci)

Abstract

Vegetable oils are gaining increasing interest due to their diverse applications in the food, pharmaceutical and cosmetics sectors. *Carapa procera* D.C (Meliaceae) is a tree found in the forests of tropical Africa. In Côte d'Ivoire, the seed oil from this plant is especially recommended for food and skin care. This study aimed to characterize the physicochemical properties, fatty acid and antioxidant activity of *C. procera* seed oil extracted by traditional methods , in order to assess its potential as alternative edible oil, with possible complementary applications in personal care products. The sample oil was supplied by a local producer from Korhogo. Analytical methods for oils were employed using pharmacopoeia assays, AOAC standard methods such as moisture content, acidity, peroxide value, saponification index, iodine value, impurity level and pH. Methyl esters were derived from the oily mixtures through an esterification process and were analyzed by GC/FID and GC/MS systems. The antioxidant activity was investigated using the DPPH radical scavenging method. *C. procera* oil is dominated by unsaturated fatty acids (59.2%, versus 38.7% saturated fatty acids). A total of fifteen free fatty acids were identified through their methyl esters. They represent 97.9% of the total chemical composition. The three main compounds are oleic acid (49.1 %), palmitic acid (28.5%) and stearic acid (8.3%). *C. procera* oil showed low antioxidant activity (IC50 > 400 µg/mL) compared with vitamin C (IC50=7.99 µg/mL). Further studies will investigate the antibacterial and anti-inflammatory properties of *C. procera* oil to better understand its potential as a functional ingredient in food system, with additional possibilities for non-food uses.

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Introduction

Vegetable oils are essential goods with a wide range of uses in the food, pharmaceutical and cosmetics industries, and their demand is still growing quickly worldwide. Due to this expansion, interest in oils derived from oleaginous has increased, especially in Africa, with has helped to the global market grow. Although the agro-industies in, West Africa, Côte d'Ivoire, is still mostly focus on palm nuts, coconuts, shea nuts and cottonseed, the industrialization of vegetal oils is becoming increasingly important. Nevertheless, the country harbors a rich diversity of underexploited oil-bearing spices that could complement or diversity the national vegetal oil sector (Koné et al., 2022; Gbamelé et al., 2020). Among these, Carapa procera D.C. exhibits potential but receives insufficient recongnition.

Carapa procera, a member of the Meliaceae family, grows naturally in the Guinean and Sudanian ecological zones of tropical Africa (Weber et al., 2010; Forget et al., 2009). Its kernels yield and oil traditionally used to cure inflammatory diseases, cattle parasitic infections, and cosmetic issues like skin and hair care (Weber et al., 2010; Dembélé et al., 2015). The chemical composition of *C. procera* oil reported in the literature

shows a high content of unsaturated fatty acids, (particularly oleic acid (37.1-48.9%) and linolenic/linoleic) along with significant levels of palmitic and stearic acid (Fish et al., 1996; Djenontin et al., 2012). Recent studies have further highlighted its anti-inflammatory (Dioum et al., 2013), antifungal (Gbamelé et al., 2020) and antioxidant (Gbamelé et al., 2020), and insect-repellent activities (Konan et al., 2003), reinforcing its potential for application in cosmetics, dermatology and natural bio-pesticides (Dembélé et al., 2015; Djenontin et al., 2012).

Compare with major African vegetable oils such us shea butter (dominated by stearic and oleic acid), coconut (rich in medium-chain saturated fats), and palm oil (containing balanced palmitic and oleic acid), *C. procera* oil presents a unique fatty-acid profile combining high unsaturation with noteworthy levels of saturated compounds (Djenontin et al., 2012). This confers potentially physicochemical properties for stability, formulation, emollience, and bioactive. However, unliked these well-established oils, *C. procera* oil remains largely under-research, particularly regarding the variability of tis composition across ecological zones, the influence of extraction practices, and the standardization required for industrial-scale valorization (Ahouansou et al., 2021 ; Koura et al., 2023). Furthermore, discrepancies in reported fatty-acid profiles, between countries (e.g Mali vs Benin) suggest that local environment and genetic factor may significantly influence oil quality, yet these aspects remain poorly understood.

Despite accumulating evidence of its chemical richness and bioactive potential, current studies on *C. procera* oil are fragmented, often limited to either compositional analysis or isolated biological activities. Very few have offered a comprehensive physicochemical characterization aligned with the requirement of cosmetic or therapeutic industries. Comparative assessments with other commercial vegetable oils are also scare, limiting understand of its competitive advantages and industrial potential.

To address this gap, the present study provides an integrated evaluation of the physicochemical characteristic, fatty acid composition and quality parameters of *C. procera* oil collected in Côte d'Ivoire. By comparing these results with published data and other oils, the study aims to clarify the biochemical specificity of *C. procera* oil and highlight its potential for industrial valorization within the cosmetic, pharmaceutical and bio-product sectors.

Methods

Materials

Mature seeds of *C. procera* oil an oleaginous species native to Côte d'Ivoire, were collected from natural populations in Korhogo (Northen, Côte d'Ivoire) during the 2019/2020 harvest season (Figure 1). The harvesting period coincides with the onset of the rainy season (late May and June).

The crude oil used in this study was supplied by a local producer's cooperative (batch number KHG 01-03-2020) and was extracted from seeds harvested in the same season using traditional methods. The oil was received without added preservatives and stored in amber glass bottle at room temperature until analyses.

Figure 1. Seeds of *C. procera* used for oil extraction

DPPH (2,2-diphenyl-1-picrylhydrazyl), Ascorbic acid (Vitamin C), Hexane, Pentane, Anhydrous sodium sulfate, polyethylene glycol, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol, Potassium hydroxide (KOH), Ethanolic iodine, Hydrochloric acid, (HCl), Sodium chloride (NaCl) was purchased from Merck (Darmstadt, Germany). Methanol, were purchased from VWR international. GC-MS calibration standards, were purchased from Supelco (Bellefonte, PA, USA). All chemicals used were of analytical grade.

Methods

Oil extraction was performed using the traditional method, in which, 500 g of *C. procera* seeds were shelled and ground into a coarse power. The powder underwent an initial drying step, followed by re-grinding. A second drying period was applied by the producer, which varied between 1 day and 4 weeks depending on seasonal conditions. The dried was toasted and subjected to final drying step. During extraction, water and local botanicals were added, and mixture was pressed. The pressed oil was the filtered to remove particulates.

To determine the pH, 2 mL of oil were solubilized in 20 mL hexane and homogenized, after which, the pH electrode was immerge in the solution to obtain the reading. The procedure followed the approach recommended for non-aqueous systems il oil quality analysis (AOCS, 1998).

Moisture content was determined according to standards gravimetric procedures recommended for edible oils (AOCS, 1998). Approximately 5 g of oil weighed into previously dried and tarred glass crucible. The sample was placed in an oven at 105°C and dried to constant weight (1-2 hours). After drying the crucible was transferred to desiccator, allowed to cool to room temperature, and weighed. Moisture content was calculated using equation content of the sample is expressed as a percentage (%).

$$\text{Moisture content}(\%) = \frac{(W_1 - W_2)\ X\ 100}{W_1}$$

Where: W_1= initial weight of the oil sample, W_2= initial weight after drying.

The acid value determination was determined according the AOAC (1997) method, with quantities the

amount of free fatty acids present in the oil. Approximately 2.0 g of oil were dissolved in a mixture of 25 mL diethyl ether. The solution was gently warmed and swirled to ensure complete dissolution.

The mixture with 0.1 N potassium hydroxide (KOH) using phenolphthalein as the indicator, until a persistent pale pink color was observed. A blank, was prepared and titrated under the same conditions.

$$Acid\ value\ (\mathrm{mg}\ KOH/g\ \mathrm{oil}) = \frac{V\ X\ N\ X\ 56.11}{m}$$

Where: V= Volume de of KOH used (mL), N = normality of KOH, 56.11= molecular weight of KOH, m= mass of oil (g).

The peroxide value determination was determined according the AOAC (1997) method. Approximately 5.0 g of oil were weighted into a flask, followed by addition of 30 mL acetic acid-chloroform (3:2 v/v). After dissolving the sample, 0.5 mL saturated KI solution was added, and the flask kept in the dark for 1 minute. Then, 30 ml distilled water was added, and the liberated iodine was titrated with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) using starch as the indicator near the endpoint. A blank, was prepared and titrated similarly.

$$Peroxide\ value\ \left(\mathrm{meq}\frac{\mathrm{O}_2}{\mathrm{kg}}\mathrm{oil}\right) = \frac{(Vsample - Vblank)\ X\ N\ X\ 1000}{m}$$

Where: V_{blank}= Volume (mL) of sodium thiosulfate used for the blank titration, V_{sample}= Volume (mL) of sodium thiosulfate used for the sample titration, N = normality (eq/L) of sodium thiosulfate, m= mass of oil (g), 1000= conversion factor used to express, the peroxide value.

The iodine value was determined using the Wijs method (AOCS, 1998). Approximately 0.3 g of oil were dissolved in 10 mL cyclohexane-glacial acid (1:1, v/v) in a stoppered flask. A volume of 25 mL Wijs reagent (iodine monochloride solution) was added, and the mixture was kept in the dark for 1 hour. After reaction, 20 mL KI solution and 150 ml distilled water were added. The liberated iodine was titrated with 0.1 N sodium thiosulfate, using Starch as indicator. A blank was titrated similarly.

$$Iodine\ value\ (\mathrm{I}_2/100\ \mathrm{g\ oil}) = \frac{(V_{\mathrm{blank}} - V_{\mathrm{sample}})\ X\ N\ X\ 12.69}{m}$$

Where: V_{blank}= Volume (mL) of sodium thiosulfate used for the blank titration, V_{sample}= Volume (mL) of sodium thiosulfate used for the sample titration, N = normality of sodium thiosulfate, 12.69= sodium thiosulfate per 100 g of oil.

The saponification value was determined using the method recommended by the AOAC (1997), which quantities the amount in milligrams of potassium hydroxide required to saponify 1g of oil. Approximately 2.0 g of oil were accurately weighed into 250 mL round bottom flask. A volume of 25 mL of 0.5 N alcoholic KOH solution was added, and flask was fitted with a reflux condenser. The mixture was heated under reflux for 60 minutes with continuous stirring to ensure complete saponification od the triglycerides. After cooling, the excess KOH was titrated with 0.5 N hydrochloric acid (HCl) using phenolphthalein as an indicator. A reagent blank, prepared and treated under the same conditions but without the oil sample, was also titrated. The saponification value was calculated using the difference between the titration volumes of blank and the sample.

$$Saponification\ value\ (\mathrm{mg\ KOH\ \ /g\ oil}) = \frac{(V_{\mathrm{blank}} - V_{\mathrm{sample}})\ X\ N\ X\ 56.11}{m}$$

Where: V_{blank}= Volume (mL) of HCl used for the blank titration, V_{sample}= Volume (mL) of HCl used for the sample titration, N = normality (eq/L) of HCl, 56.11= molecular weight (mg/nmol) of HCl.

Esterification of free fatty acids (FFA) fraction was evaluated using 40 mg sample of the previously isolated fraction, which was placed in a flask in the presence of 5 mL of a BF3/Methanol solution as derivatizing agent. The mixture was heated under reflux and stirred for 5 minutes, until the free fatty acids were completely dissolved. After cooling, the contents of the flask were transferred to a separatory funnel containing 3 mL hexane and 20 mL saturated aqueous NaCl solution. The organic phase was recovered and the aqueous phase extracted again with three times 10 mL pentane. The organic phases (hexane and pentane) were combined, dried over anhydrous MgSO4, filtered and the solvent evaporated using a rotary evaporator to obtain an extract of free fatty acid methyl esters.

Gas chromatography analyses were performed with a Clarus 500 PerkinElmer chromatograph (PerkinElmer, Courtaboeuf, France), equipped with an FID detector and two fused silica capillary columns (50 m × 0.22 mm, film thickness 0.25 µm), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The injector temperature was 250 °C and the column oven programmed was 60-220 °C at 2 °C/min. The detector (FID) was operated at 250 °C. Carrier gas: hydrogen (0.8 mL/min); division: 1/60; injected volume: 0.5 µL. Retention indices (Ir) were determined in relation to the retention times of a series of n-alkanes (C8-C29) by linear interpolation (PerkinElmer “Target Compounds” software).

Gas chromatography– Mass Spectrometry (GC-SM) analysis was carried out using a PerkinElmer TurboMass detector (quadrupole), directly coupled to a PerkinElmer Autosystem XL chromatograph (PerkinElmer, Courtaboeuf, France), equipped with a fused silica Rtx-1 (polydimethylsiloxane) capillary column (60 m × 0.22 mm, film thickness 0.25 µm). Oven temperature was programmed from 60 to 230°C at 2°/min, then maintained isothermal for 45 min; injector temperature: 250°C; ion source temperature: 250°C; carrier gas, Helium (1 mL/min); split ratio: 1:80; injection volume: 0.2 µL; ionization energy: 70 eV. Mass spectra (IE) were acquired over the 35-350 Da mass range. Compound identification was achieved by comparison of the obtained mass spectra with those contained in the libraries from the National Institute of Standards and

Technology (NIST, PC Version 2.1.2.19 of the Mass Spectral Library 2014).

The ability of carapa oil to scavenge DPPH radicals was determined according to the method of Blois (1968). In this test, the violet-colored DPPH is reduced to a yellow compound, diphenyl picryl-hydrazine, whose color intensity is inversely proportional to the reducing capacity of the antioxidants present in the medium (Sanchez-Moreno et al., 2002). Briefly, 2.5 mL of a 100 µ M methanolic solution of DPPH was mixed with 2.5 mL of carapa oil solution in methanol (containing 400 to 3.125 mg/mL of oil). The mixture was then vortexed vigorously and left for 30 min at room temperature shielded from light. The same concentration ranges were prepared with vitamin C as a positive control. A blank was run with absolute methanol alone. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

$$I(\%) = (\frac{A_{\text{blank}} - A_{\text{sanple}}}{A_{\text{blank}}})100$$

Where: A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Oil concentration providing 50% inhibition (IC_{50}) was calculated from a graph plotting percentage inhibition against oil concentration.

Results and Discussion

The physico-chemical caracteristics of carapa oil, including pH, moisture content, saponification value, iodine value, acid value and impurity level are shown in Table 1. The oil exhibited an acidic pH (5.8), a relatively high-moisture content (20%) and an impurity of 0.49. The iodine value was low (14,80 g/100 g oil) indicating a limited degree of unsaturation. In contrast, the peroxide value was high (40 meq O_2/kg oil), suggesting significant oxidation. The acid value (102.66 mg KOH/g oil), saponification (210.37 mg KOH/g oil) and ester value (107.71 mg KOH/g oil) were all considerably above the limits established by the West African Economic and Monetary Union (WAEMU) standard.

These deviations provide important insights into the oil's quality. The peroxide value is a quality criterion, enabling us to see the oxidation state of oils and to control the first stages of oxidative alteration (Naohiro and Shum, 2006). The high peroxide value reflects advanced oxidative degradation, like resulting from exposure to heat, oxygen, or sunlight during traditional extraction or storage. Such oxidation compromise self-life, promotes rancidity, and decreases nutritional and functional quality. The peroxide value obtained for *C. procera oil* (40 meq g O_2/kg oil) was higher than that of vegetable oils, which are below (20 meq g O_2/kg of oil). The very high acid value indicates extensive hydrolysis of triglycerides, leading to a large amount of free fatty acids. This condition is often associated with improper post-harvest handling particularly delayed drying, humidity, during seed storage, or microbial activity. The elevated saponification and index ester values also suggest partial breakdown of triglycerides and the presence of shorter-chain fatty acids, further conforming degradation processes.

The acid number of a fatty substance, which characterizes the purity and stability of oils, was a good means of determining its alteration by hydrolysis (Gossa et al., 2014). Carapa oil has a much higher acid number (102.66 mg KOH/g) than those found by Coulibaly et al. (2002) and Diby et al. (2021), which are 17.79 - 22.64 mg KOH/g and 14 mg KOH/g respectively.

The saponification index was related to the length of the fatty acid chains making up the oil. It characterizes the molecular weight and average length of the fatty chains, to which it was inversely proportional and provides information on the length of the fatty acid chains; it decreases as the length of these chains' increases. This index is particularly useful in the soap industry (Harper, 1977). *C. procera* vegetable oil showed an exceedingly high saponification value (210.37 mg KOH/g oil), compared with the work of Gbamelé et al. (2020), which gave saponification values of between 197.7 and 198 mg KOH/g oil. This value explains why the oil is presented in an oxidized state. The oxidizability of an oil depends largely on the nature of its constituent fatty acids. The richer an oil is in unsaturated fatty acids, the more sensitive it is to oxidation. This reaction is favored by air, light and heat. The peroxide value of carapa oil does not comply with those recommended by the Codex Alimentarius (< 10 mEq O_2/kg oil).

Overall, the results emphasize the need for improved processing and storage practices among *C. procera* oil producers. Recommend measures include rapid drying of seeds after harvest, storing seeds in dry and well-ventilated conditions, minimizing storage duration before extraction. Applying these practices would help limit hydrolysis and oxidation, thereby improving oil quality, ensuring better compliance with WAEMU, standards, and enhancing the marketability of traditionally produced *C. procera* oil.

Table 1. Physico-chemical characteristics of oil of *C. procera*

Analysis	Results	Maxima values*
pH determination	5.8	NS
Moisture content (%)	20	NS
Acid value (mg KOH/g)	102.66	≤10
Iodine value (g/100 g)	14,80	50-56
Peroxide value (meq O_2/kg)	40	≤15
Saponification value (mg KOH/g)	210.37	190-209
Ester value (mg KOH/g)	107.71	-
Rate of impurity (IA/Is)	0.49	≤ 0.05

*West African Economic and Monetary Union (WAEMU) standard
NS: Not Specified

Table 2. Fatty acids profiles of *C. procera* oil

N°	Fatty acids	Raw formula	Retention Time (min)	Ri Apol	Ri Pol	Content in vegetable oil (%)
1	Lauric acid	C12:0	52.25	1504	1801	0.3
2	Myristic acid	C14:0	63.45	1705	2008	0.3
3	Palmitoleic acid	C16:1 *n-7 cis*	72.45	1881	2243	0.2
4	Palmitic acid	C16:0	73.85	1910	2221	28.5
5	Margaric acid	C17:0	78.39	2005	2319	0.3
6	Stearic acid	C18:0	83.35	2107	2428	8.3
7	Vaccénique	C18:1 *n-7 trans*	83.47	2109	2504	1.2
8	Oleic aid	C18:1 *n-9 cis*	81.98	2080	2452	49.1
9	Elaïdique	C18:1 *n-9 trans*	82.13	2083	2454	0.7
10	Linoleic acid	C18:2 *n-6*	81.31	2066	2493	2.9
11	α-linolenic acid	C18:3 *n-3*	83.68	2113	2583	2.6
12	γ-linolenic acid	C18:3 *n-6*	84.12	2120	2596	2.3
13	Arachidic acid	C20:0	96.90	2306	2625	1.0
14	*cis*-gondoïc acid	C20:1 *n-9 cis*	94.34	2274	1642	0.1
15	*trans*-gondoïc acid	C20:1 *n-9 trans*	95.25	2286	2637	0.1
	Total fatty acid saturated					38.7
	Total fatty acid unsaturated					59.2
	Mono- saturated					51.4
	Poly- saturated					7.8
	Total identified					97.9

EO : Elution order ; retention time and percentage data on apolar column (BP-1) ; Ri : retention indices on apolar column (Ri Apol) and polar (Ri Pol).

The fatty acids composition of *C. procera* oil ispresented in Table 2. The results showed that the oil was dominated by unsaturated fatty acids (59.2%, versus 38.7% saturated fatty acids). A total of fifteen free fatty acids were identified through their methyl esters. They represent 97.9 % of the total chemical composition of the sample. The majority constituent was oleic acid (49.1%), followed by palmitic acid (28.5 %) and stearic acid (8.3%). The oil also contains significant levels of linoleic acid (2.9%) and γ-linolenic acid (2.3%), both w-6 acids, and α-linolenic acid (2.6%), a w-3 acid.

Analysis of the chemical composition of *C. procera* oil has shown that it consists mainly of oleic acid (49.1%), followed by palmitic acid (28.5%) and stearic acid (8.3%). In addition, this oil contains the essential fatty acids linoleic acid (w-6; 2.9%) and α-linolenic acid (w-3; 2.6%). γ-linolenic acid (2.3%), a w-6 acid, is also present in significant quantities. Fatty acids w-3 have a recognized protective effect on cardiovascular function. Fatty acids w-6 are considered to be good fats, as they have a positive impact on blood lipid levels, but in excess they prevent the optimal utilization of w-3 by the body. Our results are certainly close to those of Diby et al. (2021) on the chemical composition of *C. procera* oil from the Tiébissou locality, where the oil was dominated by oleic acid (51.36%), followed by palmitic acid (24.97%), stearic acid (11. 1%) and linoleic acid (10.06 %) but differed in the lower content of linoleic acid and the presence of α-linolenic and γ-linolenic acids in the Korhogo sample.

In Benin, the majority of the oil is made up of oleic acid (57.73 %), palmitic acid (20.34 %) and linoleic acid (9.71%) (Djenontin et al., 2012). *C. procera* oil from Congo has a fatty acid composition specifically dominated by oleic (37.1 - 48.9 %), palmitic (22.6 - 26.4%) and linoleic (14.4-25.4%) acids (Vieux et al., 1970 ; Miralles ., 1983). Unsaturated fatty acids predominate (59 %). This predominance of unsaturated fatty acids and the high iodine index values indicate that Korhogo oil is of the unsaturated type. Similar results were found in Tiebissou, Benin and Congo.

The antioxidant activity of *C. procera* oil are reported in Figure 1. Carapa oil showed low antioxidant activity compared with vitamin C. The oil showed a maximum inhibition of 35% of radical DPPH at the concentration of 0.4 mg/mL, i.e. an IC_{50} > 400 µg/mL, whereas the IC_{50} of vitamin C is 7.99 µg/mL.

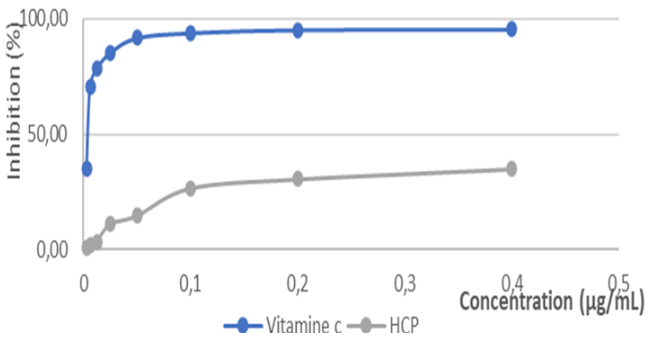

Figure 2. Antioxidant activity of *C. procera* oil

Carapa oil showed low antioxidant activity compared with vitamin C. This low antioxidant activity could be explained by the low content of antioxidant compounds in the oils. Indeed, tocopherols and tocotrienols, as well as carotenes, are known for their high antioxidant powers. This low antioxidant activity suggests that Korhogo carapa oil is low in antioxidant compounds such as tocopherols, tocotrienols and carotenes (Djenontin et al., 2012).

Like any scientific work, this study has limitations. Each analysis was performed only once, with the exception of the DPPH test. This is due to the small volume of *Carapa procera* oil supplied by traditional

artisanal extraction, which made it impossible to reproduce the experiments. This explains why it was not possible to calculate standard deviations, and the results should therefore be interpreted with caution. On the Othe hand, precise technical parameters (drying temperature et duration, grinding equipment, type and speed, pressing method, filtration system) of traditional production were not recorded by the producer. These uncontrolled variables are acknowledged as potential sources of variability affecting the oil's physicochemical and biological properties.

Conclusion

The present study provides news insights into the chemical composition and antioxidant potential of traditional *Carapa procera* oil from Côte d'Ivoire. This oil was found to be rich in unsaturated fatty acids, particularly oleic acid, together with relevant levels of palmitic and stearic acids. These results confirm the distinctive fatty-acid profile of *C. procera* oil and contribute to the existing reported in early studies.

Although its antioxidant activity was lower than of vitamin C, the oil nonetheless exhibited measurable radical-scavenging capacity, suggesting the presence of bioactive constituents that may support potential properties.

Overall, the discovery provides a scientific foundation for the future valorization of this traditional oil. Additional quality indices, antibacterial and anti-inflammatory activities, stability studies, and batch-to-bacth variability should also be included in the future research. Such information is crucial for improving access to suitable studies that would strengthen the scientific foundation for the value-adding of this traditional oil in food systems and application that promote health.

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