World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Chemical profile and anti-radical activity of the unsaponifiable matter of *Pseudospondias microcarpa* (A. Rich) Engl. seed oil

Sévère-Grébel BABOUONGOLO [1, 2, *], Longin Justin Clair BONAZABA MILANDOU [1], Snelle MIAKAYIZILA BAONDA [1, 2], Célestine NKOUNKOU LOUMPANGOU [1, 2], Jean Francy Istaelle KOUBAKA [1] and Adolphe Christian NGAKEGNI-LIMBILI [1]

[1] *Unité de Chimie du Végétal et de la Vie, Université Marien Ngouabi, Brazzaville, Congo.*
[2] *Faculté des Sciences Appliquées, Université Denis Sassou-Nguesso, Kintélé, Congo.*

World Journal of Advanced Research and Reviews, 2025, 28(01), 2086-2092

Publication history: Received on 19 September 2025; revised on 26 October 2025; accepted on 29 October 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.28.1.3649

Abstract

Pseudospondias microcarpa (A. Rich) Engl. is an oil-producing plant of the Anacardiaceae family that produces red fruits when ripe. Studies have already reported on the composition of this plant's vegetable oil. However, other minor compounds have not yet been chemically investigated, nor have their biological properties. The vegetable oil was extracted from the seeds of this plant and separated to obtain the unsaponifiable fraction. This fraction was analysed by CCM, UV-visible spectrophotometry and tested with the stable DPPH radical. The vegetable oil obtained, with a yield of 36%, is green in colour and fluid. The unsaponifiable fraction represents only 1.2% of the weight of the fatty substance. The chromatograms observed in visible and UV light show yellow and orange spots in visible light, blue spots at 254 nm, and purple and grey spots with sulphuric vanillin. The carotenoid content is 691.92 mg/kg of oil. These compounds are carotenes, xanthophylls, sterols and tocopherols, all of which have anti-radical activity. The DPPH reduction percentage is 94.80%, with an IC50 of 0.287 mg/ml and rapid kinetics of 15 minutes. These results show that the vegetable oil from this fruit can be used in cosmetics and for therapeutic purposes.

Keywords: Anti-Radical Activity; Unsaponifiable; *Pseudospondias microcarpa*; TLC

1. Introduction

The Congo benefits from favourable eco-geographical conditions for the development of rich and varied flora, including significant potential for food, oilseed, aromatic and medicinal plants, many of which are endemic [1, 3]. This gives it the advantage of producing new natural active ingredients with added value, such as the species *Pseudospondias microcarpa* (A. Rich) Engl, a medicinal plant whose fruits are highly prized for their fragrance and sweet, tangy taste [4]. Previous studies show that the fruit of this plant is juicy and its seeds are rich in vegetable oil. Its juice is acidic and contains sugars, amino acids, carotenoids, coumarins, tannins, flavonoids, terpenes and sterols, calcium, potassium, copper, iron and zinc [5]. The vegetable oil extracted from its seeds contains eight fatty acids, dominated by oleic acid (42.56%), palmitic acid (33.89%) and stearic acid (14.23%). Of the five sterols identified, phytosterols are mainly composed of β-sitosterol. The tocopherols identified are: α, γ and δ-tocopherol in low concentrations [6]. Other minor compounds have not yet been studied in this plant organ, nor has the antioxidant activity of the unsaponifiable fraction. For this reason, this study focuses on the anti-radical potential of the minor compounds in the unsaponifiable fraction of the vegetable oil from this plant.

* Corresponding author: Sévère-Grébel BABOUONGOLO

Copyright © 2025 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0.

2. Materials and methods

2.1. Harvesting and preparation of plant material

The plant material consisted of ripe fruits of *Pseudospondias microcarpa* (A. Rich) Engl. The ripe fruits were harvested along the Kouyou River in Owando, a town located in the Cuvette Centrale department of Congo. The botanical identity of the fruit was confirmed at the Centre d'Etude des Ressources Végétales (CERVE), where the species is registered under number 8957 on 7/08/1961. The fruits were pulped and dried at room temperature for one week. The dried endocarp was shelled by hand and the kernels were separated from the shells. The dried kernels were crushed using a wooden mortar and the resulting powder was stored in jars for extraction.

2.2. Extraction of vegetable oil from the seeds

The fat was extracted using the standard Soxhlet method (official AOAC method (1990) [7]. 30.04 g of dry seed powder (M) was placed in a cartridge and inserted into a Soxhlet apparatus topped with a condenser and supported by a 500 mL flask containing 300 mL of hexane (M1). After eight hours of heating, the flask was removed from the Soxhlet and the solvent was removed using a rotary evaporator (M2, mass of the flask containing the vegetable oil). The extraction yield was calculated using the following equation:

$$\%\ Fat = \frac{(M2 - M1)}{M}\ x\ 100$$

2.3. Extraction of unsaponifiable matter

Saponification of 5 g of oil was carried out using an ethanolic potassium hydroxide solution (1 M), followed by vortexing and heating to 75 °C in a water bath for 30 min. After cooling the mixture to room temperature, water was added to the reaction medium. The unsaponifiable fraction was extracted with diethyl ether [8]. The extraction yield was calculated using the equation:

$$\%\ Insaponifiable = \frac{Mi}{Mo}\ x\ 100$$

Where Mi is mass of unsaponifiable fraction and Mo mass of vegetable oil

2.4. Determination of the chemical profile of the unsaponifiable fraction by thin-layer chromatography

Thin-layer analytical chromatography was performed on the extract according to the methods described by Wagner et al. [9]. For this purpose, we used ready-to-use 0.25 mm Silicagel 60 F254 chromatographic plates with aluminium backing (Merck), onto which 10 µl of the extract, previously solubilised in hexane, was deposited using a micropipette. These were developed in conventional glass chambers (Camag), whose atmosphere had been previously saturated with vapours from the mobile phase (hexane/chloroform 5/3).

2.4.1. Total carotenoid assay

The total carotenoid content is determined using the method described by Rodriguez Amaya [10]. The absorbances of the solutions obtained after suitable dilution of the sample in absolute ethanol were read at 450 nm. The total carotenoid content is determined based on the linear calibration curve (y=ax+b) obtained with the β-carotene standard at different concentrations (0.50, 0.25, 0.125, 0.0625 mg/mL) under the same conditions as the sample. The total carotenoid content is expressed in mg β-carotene equivalent per gram of dry matter (mgEq βC/g DM).

2.4.2. Evaluation of antioxidant activity

Antioxidant activity was evaluated using the chemical method with DPPH reagent [11]. This is a free radical that is stable over time and widely used to evaluate the antioxidant activity of any compound. After solubilisation in absolute ethanol, the extract was deposited on silica CCM plates. After elution, the plates were sprayed with an ethanolic DPPH solution (2 mg/mL). The activity is positive when the spots are yellow on a purple background in visible light [12]. The TLC plates were prepared under the same conditions as those used for determining the chemical profile.

For each extract, a concentration range was prepared by half dilution in absolute ethanol. 1 mL of these solutions was added to 1.5 mL of an ethanolic solution of DPPH radical (0.02 mg/mL). The absorbance of the mixture was read at a wavelength of 515 nm using a UV-visible spectrophotometer every five minutes. The absorbance of the blank was

obtained by replacing the test solution with an equal volume of ethanol solution. The positive reference controls used were ascorbic acid and β-carotene.

The percentages of DPPH radical reduction are determined using the following formula:

$$\boldsymbol{R}(\%) = \frac{\boldsymbol{Ab - As}}{\boldsymbol{Ab}} \boldsymbol{x100}$$

Where R is percentage reduction of the DPPH radical; Ab absorbance of the blank; As absorbance of the sample.

The concentration and time required to reduce 50% (IC50) of radicals were determined using Excel software.

3. Results and discussion

3.1. Vegetable oil and unsaponifiable matter yield

The vegetable oil yield after eight hours of extraction is 36%. The lipid extract is fluid and green in colour at room temperature but has two phases, the upper one being more liquid and more coloured than the lower one, which is more viscous. The total lipid content (>20%) allows us to classify this plant as an oilseed [3]. In terms of fluidity, the lipid fraction is biphasic, reminiscent of crude palm oil, due to the 50/50 ratio of unsaturated to saturated fatty acids [13]. The colour, on the other hand, is reminiscent of cannabis seed oil, avocado pulp or olive seed oil (Figure 1) [14]. The unsaponifiable yield is 1.2%. The unsaponifiable content is fairly typical for vegetable oils, and its red colour is thought to be due to its high carotenoid content [14-16]. Preliminary studies by Nkounkou et al. report the same total lipid content, but do not report the unsaponifiable content [6].

Figure 1 Vegetable oil extracted from the seeds of *Pseudospondias microcarpa*

3.2. Chemical profile of the unsaponifiable fraction by TLC

Chromatograms 1, 2 and 3 below show the chemical profiles of the unsaponifiable fraction of the vegetable oil (figure 2). The orange spots in chromatogram 1 observed in visible light are characteristic of carotenoids; they are confirmed by the use of sulphuric vanillin reagents (chromatogram 3). The blue spots at 254 nm under UV light are attributable to tocopherols, and the large purple spot in chromatogram 3 characterises sterols. They are thought to be more abundant as minor compounds in this vegetable oil.

Eluent: Hexane/CHCl3 (5/3); Observation: visible light
Eluent: Hexane/CHCl3 (5/3); Observation: UV at 254 nm
Eluent: Hexane/CHCl3 (5/3); Developer: sulphuric vanillin; Observation: visible light

Figure 2 Profile chimique de la fraction insaponifiable

3.3. Total carotenoid content

The carotenoid content is 691.92 mg/kg of oil, which is similar to that of palm oil (700 mg/kg of oil) but higher than that of avocado and olive oil (31.51 mg/kg of oil) [17]. Carotenoids are known for their pro-vitamin A properties and antioxidant power. The work of Nkounkou et al reports the sterol and tocopherol content but not the total carotenoid content.

3.4. Screening and evaluation of anti-radical activity on TLC

The TLC plates in Figure 3, prepared under the same conditions as those used for the detection of compounds, show yellow spots on a purple background, characteristic of positive anti-radical activity. Comparison with plate 3 clearly shows that the carotenoids and tocopherols in the unsaponifiable fraction are more active than the sterols. These results show that the unsaponifiable fraction has strong antioxidant potential. These observations are consistent with thin-layer chromatography. In addition, the unsaponifiable fraction is more active than the two reference molecules (table I). This result can be explained by the richness of this fraction in antioxidant compounds such as tocopherols, carotenoids and phytosterols. Figure 4 shows the progressive inhibition of free radicals over time by the unsaponifiable fraction. Numerous studies report the antioxidant activity of extracts from the leaves, bark and roots of *Pseudospondias microcarpa* (A. Rich) Engl. of the blue-black fruit variety. However, no study correlates anti-radical activity with the chemical compounds present in the extract and/or fraction studied. The extracts of nutritional and therapeutic interest from the fruits of *Pseudospondias microcarpa* (A. Rich) Engl. do indeed possess antioxidant activity, the effectiveness of which depends on the nature of the chemical compounds present in the extract. This would explain the high reactivity of the unsaponifiable fraction, which is thought to be due to its high content of tocopherols, phytosterols and carotenoids, which exert a synergistic effect. This study is novel in terms of the antioxidant activity of the extract evaluated and confirms the fruit's potential as a functional food.

Eluent: Hexane/CHCl3 (5/3)
Developer: Vanillin sulphuric acid + heating Development: 1% DPPH
Observation: Visible light

Figure 3 Relationship between chemical profile and anti-radical activity

Table 1 Percentage inhibition of the extract and standards

Extracts	Concentration (mg/ml)	% reduction of DPPH	IC50 (mg/ml)
Insaponifiable	1,000	94,80	0,190
Vitamin C	1,000	97,90	0,212
β-carotene	1,000	39,76	1,309

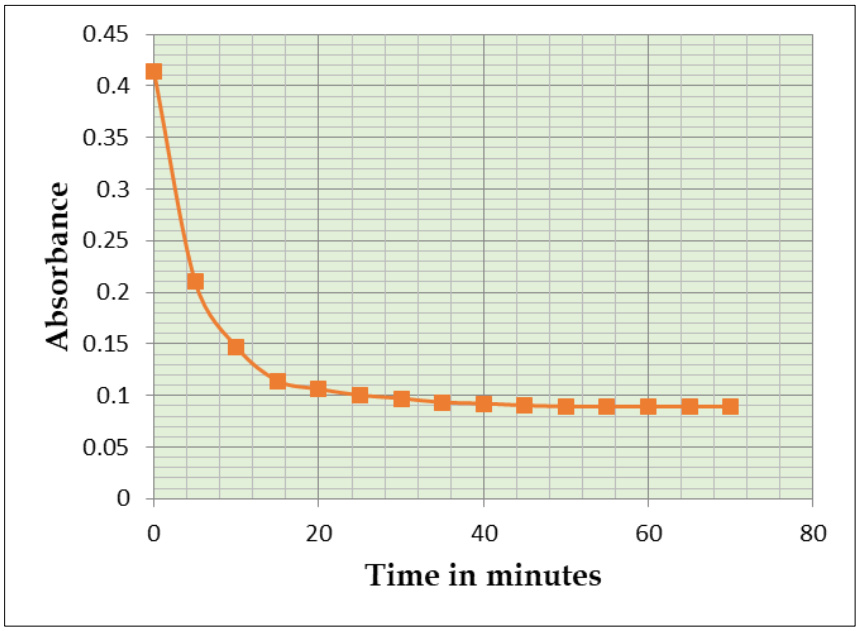

Figure 4 Kinetics of unsaponifiable matter

4. Conclusion

Studies conducted on the red-fruited variety of *Pseudospondias microcarpa* from Congo show that the species is indeed oil-bearing, its unsaponifiable matter is rich in carotenoids and sterols, and it has good antioxidant properties with rapid reduction kinetics. The results obtained prove that this oil can be used in nutrition for food supplementation, in cosmetics and in therapeutics.

Compliance with ethical standards

Acknowledgments

The authors would like to thank all members of the Plant and Life Chemistry Unit for their availability. We would also like to thank the laboratory manager for providing us with the equipment and reagents we needed.

Disclosure of conflict of interest

No conflict of interest to declare.

References

[1] Loubelo, E., (2012). Impact des produits forestiers non ligneux (PFNL) sur l'économie des ménages et la sécurité alimentaire : cas de la République du Congo. Thèse de doctorat de l'Université de Rennes 2, France. 261p. https://theses.hal.science/tel-00713758/file/2012theseLoubeloE.pdf

[2] Kimpouni, V., Lenga-Sacadura, M. Y., Chaîph Mamboueni, J., and Niamba, L. (2018). Étude de la diversité floristique des ptéridophytes à Brazzaville, Congo. VertigO-la revue électronique en sciences de l'environnement. https://doi.org/10.4000/vertigo.23458

[3] Silou, T. (2014). Corps gras non conventionnels du bassin du Congo: caractérisation, biodiversité et qualité. OCL, 21(2), D209. https://doi.org/10.1051/ocl/2013044

[4] Malela, K. E., Miabangana, E.S., Petit, J., N'zikou, J. M., and Scher, J. (2016). Enquête ethnobotanique sur les fruits comestibles de la flore spontanée de la République du Congo. Int. J. Pure App. Biosci., 4, 346-357. https://doi.org/10.18782/2320-7051.2247

[5] Sévère-Grébel, B. Lahat, N., Célestine, N., Clair, B. and Jean-Maurille, O. (2024). Nutraceutical Potentiality of Fruits of Red Variety of *Pseudospondias microcarpaI* (A. Rich) Engl. Food and Nutrition Sciences, 15, 224-234. https://doi.org/10.4236/fns.2024.153014.

[6] Nkounkou Loumpangou, C., Douniama, L. G. V., Ngakegni-Limbili, A. C, et al. (2018). caractérisation chimique des graines de *Pseudospondias microcarpa* (A. rich) engl. Annales de l'Université Marien N'GOUABI, 18(2), 21-27. https://dicames.online/jspui/handle/20.500.12177/2644

[7] Khilari, V. J., and Sharma, P.P. (2016). Determination of total lipids from five underutilized wild edible fruits in Ahmednagar disctrict, Maharashtra (India). International journal of Advanced esearch in Biological Sciences, 3(7): 14-20.

[8] AFNOR (NFT 60-205). Détermination de la teneur en matières insaponifiables.

[9] Wagner, H. Bladt, S. and H. Zgainski, E.M. (1983). Plant drug analysis. Springer-Verlag Berlin Heidelberg, 314 pages.

[10] Rodriguez-Amaya, D. B., and Kimura, M. (2004). HarvestPlus handbook for carotenoid analysis (Vol. 2, pp. 63-pp). Washington: International Food Policy Research Institute (IFPRI).

[11] Brand-Williams, W., Cuvelier, M. E., and Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 28(1), 25-30.

[12] Wang, J., Yue, Y. D., Tang, F., and Sun, J. (2012). TLC screening for antioxidant activity of extracts from fifteen bamboo species and identification of antioxidant flavone glycosides from leaves of Bambusa. textilis McClure. Molecules, 17(10), 12297-12311.

[13] Gee, P. T. (2007). Analytical characteristics of crude and refined palm oil and fractions. European journal of lipid science and technology, 109(4), 373-379.

[14] Gomez-Coca, R. B., Perez-Camino, M. C., and Moreda, W. (2015). Neutral lipids: unsaponifiable. Handbook of Food Analysis-Two Volume Set; Nollet, LML, Toldra, F., Eds, 467-468.

[15] Wolff, J.P. Analyse des lipides et separation des acides gras par CCM. Plant Food Hum Nutr 16, 113–125 (1968). https://doi.org/10.1007/BF01103869

[16] Fontanel, D. (2013). Unsaponifiable matter in plant seed oils (pp. 1-366). New York, NY, USA:: Springer.

[17] Nasri, C., Halabi, Y., Harhar, H., Mohammed, F., Bellaouchou, A., Guenbour, A., and Tabyaoui, M. (2021). Chemical characterization of oil from four Avocado varieties cultivated in Morocco. OCL, 28, 19.