

## Original Research Article

# Valorization of *Carapa procera* DC. oil and evaluation *in vitro* of antimalarial activity of its bitter content

### ABSTRACT

**Aims:** To extract the bitter content of *Carapa procera* oil and to evaluate antimalarial activity of its bitter content.

**Study design:** Experimental research.

**Place and Duration of Study:** Department of Chemistry (University of Kinshasa) and National Institute for Biomedical Research, between December 2016 and June 2018.

**Methodology:** The seeds of *C. procera* were collected in the Kwilu region (Democratic Republic of Congo). Standardized method of Soxhlet was used for the extraction and determination of the oil content. The bitter content of *C. procera* oil was removed by liquid-liquid extraction. Organoleptic sensory analyzes were used as a test of effectiveness of debittering.

The *in vitro* antimalarial activity of bitter content, extracts, the crude oil and the debittered oil on *Plasmodium falciparum* were evaluated in according to the method of maturation test of trophozoites into schizonts.

**Results:** Quality indexes such as : acid values (12,72 and 2,72 mg KOH/g), saponification values (190,36 and 193,12 mg KOH/g), peroxide values (5,26 and 19,66 meq O<sub>2</sub>/Kg) and iodine values (69,09 and 68,63 g I<sub>2</sub>/100 g) was determined for crude oil and debittered oil respectively.

Antimalarial activity was evaluated. Crude oil and bitter content showed respectively significant antimalarial activity with IC<sub>50</sub> values of 3,44 and 0,30 µg/mL.

**Conclusion:** The elimination of the bitter content does not affect the quality of *C. procera* oil. These results were for the most and part, in accordance with the codex alimentarius for edible oils. Concerning the antimalarial activity, according to RITAM, *C. procera* crude oil could present probable antimalarial activity against *P. falciparum* (3,44 µg/mL), low value compared to that of the bitter content.

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**Keywords:** *Carapa procera*, debittering, Organoleptic Sensory Analysis, bitter content, antimalarial activity.

## 1. INTRODUCTION

~~In the 1960s~~, Africa accounted for almost 20% of oilseed trade In the 1960s. Nowadays, it is less than 2% because of the unavailability of commodities and the instability of their price more and more exorbitant [1]. In addition, requirements are steadily increasing due to the growing population [2]. Unfortunately, the oilseed sectors in Africa are mainly fueled by palm and groundnut oils which sometimes have supply deficits [1]. The Democratic Republic of Congo (DRC) is not spared by this phenomenon.

Comment [K1]: Which commodities??

Indeed, edible oils in DRC are mainly supplied by palm nuts (conventional oilseeds) [2]. To cope with the increasing demand for lipid-type food, the DRC is currently importing large quantities of vegetable oil (50,000 to 60,000 t) despite its abundant biodiversity that can balance the supply and demand.

Comment [K2]: What is the global picture? (state, the global, africa, DRC)

However, there is a multitude of oilseed species widely distributed throughout the country, which can serve as supplement and a source of lipids, both in normal times and in times of scarcity, such as *Carapa procera*.

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Comment [K4]: Is it only DRC??

Comment [K5]: Citation???

Comment [K6]: Source?

~~Carapa procera is c~~Cultivated in tropical and subtropical areas. ~~It is, Carapa procera~~ is a multi-purpose plant in the Meliaceae family. It is a widespread species in wooded savannahs in Africa and Latin America [3 ; 4]. It grows in primary forests in general, thus requiring a tropical climate [5].

The oil extracted from its seeds is mainly used for therapeutic and cosmetic purposes [6]. However, this oil does not have a food use due to its unbearable bitterness [7]. Thus, the elimination of its bitter content should be a matter of major concern as it could enhance additional supply of edible oil ~~variety~~. In addition, studies have shown that the bitterness of these seeds is due to the presence of some triterpenes endowed with febrifuge and insecticidal activities, and some limonoids with proven anti-inflammatory and anti-parasitic activities [4 ; 6]. Therefore, this study aims to ~~debittere~~debitter *Carapa procera* oil for its consumption by extracting its bitter content, and also to valorize this bitter content by evaluating its *in vitro* antimalarial activity.

## 2. MATERIAL AND METHODS

### 2.1 Plant Collection and Authentication

The seeds of *Carapa procera* were collected in the city of Bandundu (Democratic Republic of Congo), and ~~authenticated~~authenticated at the INERA herbarium in the Department of Biology, Faculty of Sciences, University of Kinshasa. Seeds were stored for 5 days at room temperature in dark.

Seed kernels were extracted from hulls manually, dried in an oven at 50 °C for 6 days and grinded to give a powder which was used ~~de~~ for subsequent ~~work~~.

### 2.2 Oil extraction

The standard method of Soxhlet (NF EN ISO 659) was used for the extraction and determination of the oil content. n-hexane, organic solvent used in this study, containing the dissolved lipids is then evaporated under vacuum on a rotary evaporator to recover the oil.

After evaporation under vacuum (45-60 °C), the oil was placed in an oven for 5 days at 40 °C to remove residual solvent.

### 2.3 Physico-Chemical properties of oil

Physico-chemical properties of oil (before and after debittering) determined ~~were-were~~: acid, peroxide, iodine, ester and saponification values, determined according to International Organization for Standardization (ISO) [8 ; 9 ; 10 ; 11], and, unsaponifiable content, refractive index, color, density and viscosity. For each parameter, experiments were performed in ~~triplicate~~.

### 2.4 Extraction of bitter content

The debittering process of *C.procera* oil was done by liquid-liquid extraction. Organoleptic sensory analyzes were used as a test of effectiveness debittering.

In order to evaluate the kinetics of reaction, several debittering times and several volumes of solvent (at different proportions) were used.

### 2.5 Organoleptic Sensory Analyzes of the *C. procera* oil (OSA)

The effectiveness of debittering of the *C. procera* oil, was assured according to OSA by a panel of tasters. The exclusion criteria of tasters focused on the results of the OSA card count (see questionnaire in appendix). Based on sensitivity of taster to bitterness.

From twenty individuals who agreed to be part of the panel of tasters, ~~eighteen~~ were selected because they have no tolerance for bitter taste or food-grade allergy.

The sensory evaluation focused on two olfactory-taste parameters: bitterness, which is the characteristic taste of *Carapa procera* oil comparable to that of quinine ; and the ~~retro~~retro nasal persistence which is the duration of the ~~retro~~retro nasal sensations that persist after expelling the mouthful of oil from the oral cavity.

Thus, the following descriptors were used :

- For bitterness: extremely bitter ; very bitter ; bitter ; slightly bitter ; pleasant.
- For the retronasal olfaction: persistent and non-persistent. The tasting took place in a very ventilated room, and the oil samples were presented in small new vials. The tasters took turns to taste with a different spoon per sample and per individual. In order to avoid certain effects of contrast that could provoke the immediate tasting of other samples, it was

**Comment [K7]:** It is not succint. Description is scanty.

**Comment [K8]:** Be specific. Give examples.

**Comment [K9]:** How is it used traditionally?? Give proper description of the plant (Taxonomy and Description, Traditional Medicinal Uses and Chemical Constituents)

**Comment [K10]:** Is it safe to use?

**Comment [K11]:** Provide information on it in vitro and in vivo assay coupled with toxicity studies.

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**Comment [K12]:** How was it stored afterwards??

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**Comment [K15]:** Ethical clearance?????

**Comment [K16]:** How did you know???

presented to each taster apple slices and a glass of water at room temperature [12]. The tasting sessions were carried out between 10 AM and 12 noon as it is proved that periods of optimal acuity for the taste and the smell exist much more during the day [13].

## 2.6 Phytochemical tests

Different secondary metabolites in bitter extract responsible for the antimalarial activity were evaluated on the aqueous extracts and the organic extracts respectively for the water-soluble and liposoluble components of the bitter content. The aqueous and organic extracts were subjected to phytochemical investigation for identification of alkaloids, flavonoids, tannins, saponins, steroids and terpenoids using standard procedures.

## 2.7 Increasing polarity extraction

Bulk content used as a stock solution for this extraction was obtained by dissolving 10 mL of bitter content in 40 mL of ethanol.

This process was achieved by using four solvents of different polarity including: cyclohexane, diethyl ether, isopropanol and, ethyl acetate.

The various extracts obtained, placed in flasks, were stored at 4 °C until they are used.

## 2.8 Antimalarial activity

The antiplasmodial study consist in evaluating *in vitro* the activity of bitter content (the crude), bitter content extracts, the crude oil and, the debittered oil on *Plasmodium falciparum* at various concentrations in order to compare their activities with that of an antimalarial reference.

The antimalarial test was carried out at the National Institute of Biomedical Research (INRB), Kinshasa (DRC).

A rapid diagnosis test was realized for *Plasmodium* species identification. The parasite density was determined by counting the number of infected erythrocytes. 5 mL of venous blood was collected from donors in a tube coated with EDTA (Greiner Labortechnik). Samples with mono-infection due to *Plasmodium falciparum* and a parasite density between 1% and 2% were used for the *in vitro* antimalarial tests.

The methodology used is the test maturation of trophozoites into schizonts.

The effect of the various extracts was evaluated by measuring trophozoite development after 48 hours of incubation at 37,5 °C in an atmosphere rich on CO<sub>2</sub> and poor on O<sub>2</sub>. The percentage inhibition was determined in each cup compared to the standard, which was subject to the same conditions.

> Preparation of the stock solutions

The stock solutions were prepared from 8 samples of which the first 4 resulted from the extraction with increasing polarity, the remaining 4 being respectively the totum (bitter content), debittered oil, crude oil and quinine. Ethanol (96%) was used as a diluting solvent. For each of these stock solutions, the starting concentration was 1000 µg/mL.

> Dilution of the stock solutions

The dilutions were made in order to obtain a concentration range from 1000 to 0,49 µg/mL.

Comment [K17]: State the conc.

Comment [K18]: State specific method employed

Comment [K19]: Please this is not well presented

## 3. RESULTS AND DISCUSSION

### 3.1 Physicochemical properties

Physicochemical characteristics of *Carapa procera* oil, obtained by Soxhlet extractor with n-hexane are presented in Table 1.

Oil content of *C. procera* (63,44 %) is higher than the results reported in previous study (51%) [2]. Comparing our results with those of Djenontin [14], the yield turns out to be almost the same (61,5 %) and lower than the one found by Nonviho (74,76%) [1]. This high oil content makes *Carapa procera* seeds an interesting commodity for commercial exploitation, since this oil content exceeds that of some conventional oilseeds such as cotton (15 – 24 %) and sunflower (25 – 40%).

Comment [K20]: Explain statistically.

Comment [K21]: Recast

The crude oil of *C. procera* has an acid value (IA) of 12,72 mg KOH/g of oil. As for the debittered oil, it presented a much lower acid value (2,72 mg KOH/g of oil). In reference to the Codex Alimentarius, debittered oil has a value that complies



1 : 9	144	33	0	50	17	0	50	50	-
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Legend :

Ext. bit. : Extremely bitter ; very bit. : Very bitter ; Bit. : Bitter ; S.b. : Slightly bitter ; Pleas. : Pleasant (Not bitter) ; Pers. : Persistence ; No pers. : No persistence ; - : No debittering.

The organoleptic analysis of *Carapa* oil was carried out after 144 hours (6 days) rest time of the mixture oil-water in proportions 1 : 9 (V/V). Compared with the organoleptic analysis of the crude oil, which is extremely bitter, the observation is that the debittering was not very effective, because the perceptibility of the bitterness remain slightly intense. However, it is possible that the bitter content of *Carapa* oil doesn't contain hydrophilic properties contrary to the work done on the seeds of *Raphia sese* where the debittering was perfectly realized with the water [19].

Comment [K24]: Recast. Not well presented

### 3.2.2 Extraction with ethanol

The second series of tests was performed by mixing crude oil and ethanol. For this purpose, 4 different proportions were carried out during a certain time interval (up to 6 days for the 1:1, V/V proportion).

Tables 3 and 4 present the results of organoleptic sensory analyzes carried out respectively on the debittering tests for the proportions 1:1; 3:7 and for the proportions 2:8; 1:9 (at different times) by a panel of 18 tasters.

**Table 3. OSA Results of *Carapa procera* Oil for Debittering Attempts with Ethanol for proportions 1:1 and 3:7 as Solvent**

Oil : Ethanol	Bitterness						Retronasal persistence		Observation
Samples	Times (hour)	Ext. bit. (%)	very bit. (%)	bit. (%)	S.b (%)	Pleas. (%)	Pers. (%)	No pers. (%)	Effectivity of debittering
1 : 1	144	43	29	14	14	0	89	11	-
	30	0	11	56	33	0	50	50	-
3 : 7	48	0	25	13	37	25	50	50	-
	72	0	0	17	50	33	50	50	-

Legend :

Ext. bit. : Extremely bitter ; very bit. : Very bitter ; Bit. : Bitter ; S.b. : Slightly bitter ; Pleas. : Pleasant (Not bitter) ; Pers. : Persistence ; No pers. : No persistence ; - : No debittering.

**Table 4. OSA Results of *Carapa procera* Oil for Debittering Attempts with Ethanol for proportions 2:8 and 1:9 as Solvent**

Oil : Ethanol	Bitterness						Retronasal persistence		Observation
Samples	Times (hour)	Ext. bit. (%)	very bit. (%)	bit. (%)	S.b (%)	Pleas. (%)	Pers. (%)	No pers. (%)	Effectivity of debittering
2 : 8	3	0	29	43	0	28	62	38	-
	6	0	17	33	33	17	67	33	-
	24	0	0	38	62	0	70	30	-
	30	0	17	33	33	17	37	63	-
	48	0	0	16	67	17	78	22	-
	72	0	0	20	20	60	71	29	+
1 : 9	3	0	0	0	33	67	33	67	+
	6	0	0	0	0	100	28	72	++

Legend :

Ext. bit. : Extremely bitter ; very bit. : Very bitter ; Bit. : Bitter ; S.b. : Slightly bitter ; Pleas. : Pleasant (Not bitter) ; Pers. : Persistence ; No pers. : No persistence ; - : No debittering ; + : Slight debittering ; ++ : Total debittering.

These results (Tables 3 and 4) show that the 1:9 ratios (oil/ethanol, V/V) is the most effective as approved by the tasting panel, because after 3 hours of settling, the bitterness of crude oil had considerably blurred. After 6 hours of rest, the organoleptic analyzes showed that the debittering was total. In addition, the perceptibility of retronasal persistence was only noticed by 22% of the panel of tasters.

On the other hand, for other proportions (1:1, 3:7), the attempts were not effective. Concerning the proportion 2:8, the dezamerisation was partial after 72 hours.

These results indicate that the kinetics of debittering strongly depend on the proportion oil/solvent. These analyzes were carried out at room temperature.

Comment [K25]: What is this???

### 3.3 Antimalarial tests

Results in Table 5 show the IC<sub>50</sub> values for all extracts, debittered oil, bitter oil and crude (bitter content) compared to quinine taken as a positive control.

Table 5. IC<sub>50</sub> of different extracts

Echantillons	IC <sub>50</sub> (μ g/mL)
Crude (Bitter content)	0,30
Cyclohexane extract	7,47
Diethyl ether extract	154,29
Isopropanol extract	< 500
Ethyl acetate extract	207,76
Debittered oil	130,19
Bitter oil	3,44
Quinine	0,79

From these results, it appears that all extracts, except the isopropanol extract (IC<sub>50</sub> > 500 μg/mL), show some activity at concentrations below 250 μg/mL.

However, according to RITAM (Research Initiative on Traditional Antimalarial Methods), antimalarial activity of a sample is considered effective when its IC<sub>50</sub> is less than 5 μg/mL.

In view of the results, the bitter content isolated from crude oil of *Carapa procera* (crude) has a probable antimalarial activity drug (IC<sub>50</sub> = 0,30 μg/mL) greater than quinine taken as a control (0,79 μg/mL). This may mean that this efficiency on *P. falciparum* could be due to a positive synergy of several compounds. Still according to RITAM, *Carapa procera* crude oil could present probable antimalarial activity against *P. falciparum* (3,44 μg/mL), low value compared to that of the bitter content. We just understand that the proportion of the bitter content in it is therefore smaller for the same volume taken.

Comment [K26]: Not well presented

### 3.4 Chemical screening on bitter content

The bitter content is the only one to be concerned by this analysis because it presented the most important antimalarial activity.

Table 6 presents the results of the chemical screening of the bitter content in *Carapa procera* oil.

Table 6. Results of the chemical screening of the bitter content in *Carapa procera* oil

Secondary metabolites	Results
Alkaloids	-
Flavonoids	-
Saponins	-
Tannins	-
Triterpenes and steroids	+++

+++ = abundantly present; ++ = moderately present; + = present; - = absent.

According to these results, the bitter content of *Carapa procera* crude oil would be constituted on triterpenes and steroids. Similar results of Mbaye et al [6].

#### 4. CONCLUSION

This study aimed to eliminate the bitterness of *Carapa procera* oil by extracting the component responsible for it; to carry out physicochemical analyzes of crude oil before and after debittering and compare these parameters to those recommended by the official codex alimentarius standard. It was also a question of testing the antimalarial activity of the bitter content extracted from crude oil.

Chemical analyzes have shown that the seeds of *C. procera* have a very high oil content (63.44%), which should motivate their commercial exploitation.

The results of the physicochemical parameters showed that the crude oil of *C. procera* did not have properties so different from debittered oil, except for the peroxide values, where there is a significant difference between the two oils. Consequently, the elimination of the bitter content almost does not affect the quality of this vegetable oil. These results were, for the most part, in accordance with the official standard codex alimentarius for edible oils.

From the evaluation of in vitro antimalarial activities, it comes out that the bitter content, consisting of triterpenoids and steroids, and crude oil could present very good antimalarial activities (IC<sub>50</sub> = 0.30 and 3.44 µg/mL respectively) unlike the debittered oil and the different extracts of bitter content, which prove to be inactive against *P. falciparum*.

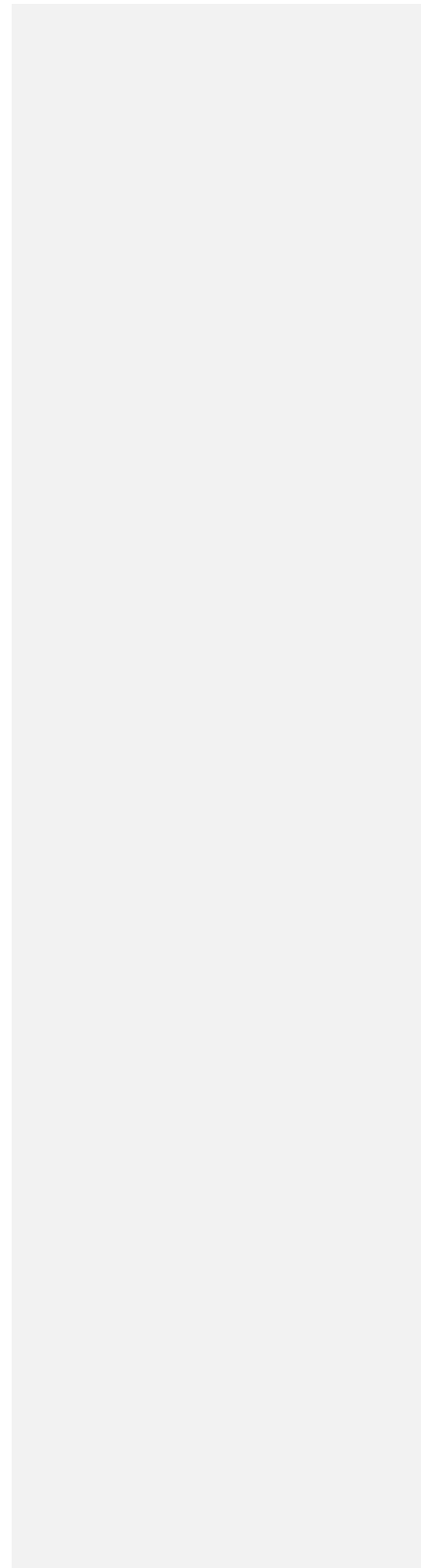
Therefore, it would be necessary to evaluate cytotoxic activity of bitter content to confirm its antimalarial activity. It would be interesting to separate the different constituents of the bitter content, to test in synergy their antimalarial activity and identify the compound responsible for the activity observed.

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UNDER PEER REVIEW



**APPENDIX**

SENSORY PROFILE SHEET (*Carapa procera oil*)

1) NAME AND POST-NAME :

2) SEX :

3) AGE :

4) PROFESSION :

A) DO YOU HAVE SOME TOLERANCE FOR ALL THAT IS BITTER ?

YES

NO

B) WHAT ARE YOU ALLERGIC TO ?

C) HOW DO YOU FIND THIS PRODUCT?

EXTREMELY BITTER (BAD, UNPLEASANT)

VERY BITTER

SLIGHTLY BITTER

BITTER

PLEASANT (NOT BITTER)

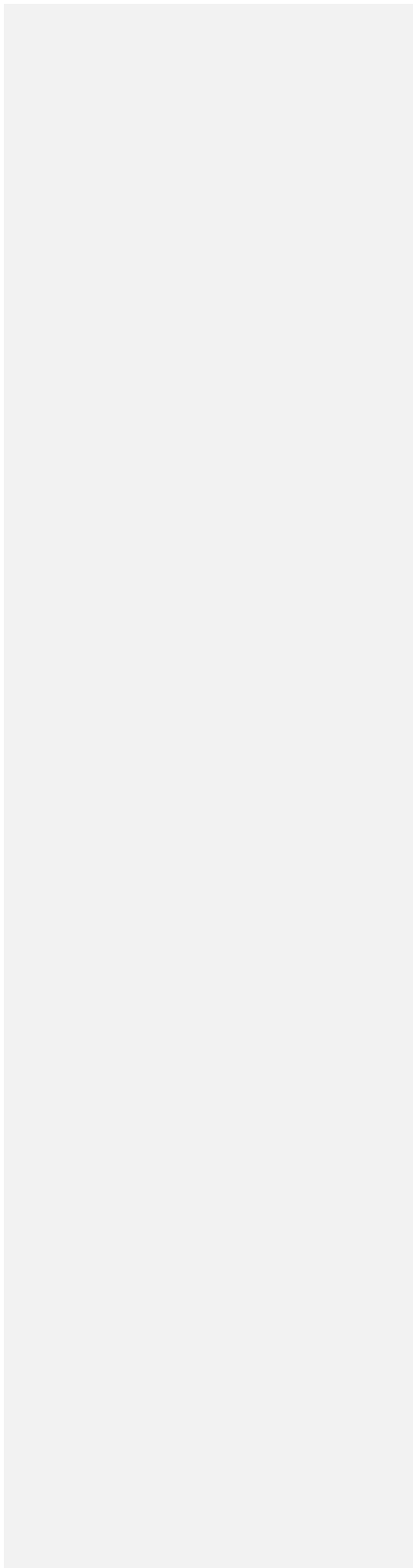
D) HAVE YOU EXPERIENCED RETRONASAL PERSISTENCE OF THIS PRODUCT ?

YES

NO

SIGNATURE OF THE TASTER

KINSHASA, ... /... /20...



UNDER PEER REVIEW

