Original Research Article

Valorization of *Carapa procera* 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 the 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). A 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 have used a test of the effectiveness of debittering.
The in vitro antimalarial activity of bitter content, extracts, crude oil and the debittered oil on *Plasmodium falciparum* were evaluated 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_2/Kg), and iodine values (69.09 and 68.63 g I_2/100 g) was determined for crude oil and debittered oil respective antimalarial activity was evaluated. Crude oil and bitter content showed respectively significant antimalarial activity with IC_{50} 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 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.

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

1. INTRODUCTION

In the 1960s, Africa accounted for almost 20% of the oil seed trade, 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, oil requirements are steadily increasing due to the growing population [2]. Unfortunately, the oil seed 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.
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 / year) despite its abundant biodiversity that can balance the supply and demand.
However, there is a multitude of oilseed species widely distributed throughout the country, which can serve as a food supplement and a source of lipids, both in normal times and in times of scarcity, such as Carapa procera.
Cultivated in tropical and subtropical areas, 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].

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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 the 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 *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 under the number Bolema 591 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 ground to give a powder which was used 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, the 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 the oil (before and after debittering) determined 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 the reaction, several debittering times and several volumes of solvent (at different proportions) were used.

2.5 Organoleptic Sensory Analysis of the C. procera oil (OSA)

To verify the effectiveness of oil debittering, we proceeded to 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 the sensitivity of the taster to bitterness The method used is the organoleptic evaluation of virgin olive oil.
. Of the 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 nasal persistence which is the duration of the 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 retro nasal 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 presented to each taster with 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].

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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 the identification of alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids using standard procedures.The identification tests were based in part on qualitative analysis, either on the formation of insoluble complexes using precipitation reactions, or on the formation of colored complexes, using staining reactions.

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 polarities including cyclohexane, diethyl ether, isopropanol, and ethyl acetate. The various extracts obtained were placed in flasks and stored at 4 °C until they are used.
The extraction method we used is the successive maceration by four solvents with increasing polarity .

2.8 Antimalarial activity

The antimalarial study consists of evaluating the *in vitro* 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 methodology used is the maturation test of trophozoites into schizonts.
The antimalarial test was carried out at the National Institute of Biomedical Research (INRB), Kinshasa (DRC).
A rapid diagnostic 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 effect of the various extracts was evaluated by measuring tropho development after 48 hours of incubation at 37,5 °C in an atmosphere rich in CO_2 and poor in O_2. The percentage inhibition was determined in each cup compared to the standard, which was subject to the same conditions.

- Preparations 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.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical properties

Physicochemical characteristics of *Carapa procera* oil, obtained by Soxhlet extractor with n-hexane are presented in table 1. The oil content of *C. procera* (63.44 %) is higher than the results reported in the previous study (51%) [2]. Comparing the results with that 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 oil seeds such as cotton (15 – 24 %) and sunflower (25 – 40%).

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 presents 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 with the acid value standard for edible oils (max. limit 4 mg KOH/g of oil). Thereby, it could be inferred that debittering has reduced the amount of free fatty acids present in the starting crude vegetable oil.
Iodine values (IV) for both crude seed and debittered oils (69.09 g of I_2/100g and 68.63 g of I_2/100g) suggest that they are both non-siccatives. In other words, the iodine values obtained in this study indicate the presence of low unsaturated fatty acids. These results are similar to those found by Nonviho in 2015 (63.77 g of I2/100g) and Djenontin in 2012 (69 g of I_2 / 100g) [14; 1].

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Peroxide values (IP) of the crude *C. procera* oil being in conformity with the codex alimentarius for edible oils (15 meq O_2/ Kg max) [15; 16; 17], indicates as well that crude exhibits some resistance to peroxidation during the storage, unlike the debittered oil whose IP is 19.66 meq O_2/ Kg.
Saponification values (SV) of *C. procera* oil (before and after debittering), respectively 190.36 and 193.12 mg KOH/g, are comparable with the values of common vegetable oils i.e., palm oil (190-209 mg/g), groundnut oil (188-196 mg/g) and corn oil (187-196 mg/g) and justify the use of those vegetable oils by population to prepare soap [18].
The proportion of unsaponifiable content observed in the crude *C. procera* oil (20.42 %) can justify its bitter taste. It is known that unsaponifiable content consists of substances such as tocopherols, phytosterols, and β-carotene which confer certain pharmacological properties and coloration in substances that contain them [1]. Hence debittering of the crude *Carapa* oil can remove these substances and the proportion of unsaponifiable in treated Carapa oil is sufficiently reduced (6.07 %).
Concerning the density, *Carapa procera* oil before and after debittering has a density of 0.914 and 0.906 respectively, comparable values to that of olive oil which is generally in the range of 0.910 to 0.916 [18].
The viscosity of the debittered *Carapa procera* oil is higher than that of crude oil (43.39 and 34.22 cSt, respectively). This can be explained by the formation of the hydroperoxides (ROOH) in the debittered oil, which can form hydrogen bonds.

Table-1 Extraction yields, colors, and Physico-chemical properties of *C. procera* oil

Parameters (Units)	Vegetable oil before debittering	Vegetable oil after debittering	Codex alimentarius
Yield (%)	63.44 17.3	40.23	
IA (mg KOH/g)	12.72±0.44	2.72±0.36	<4.0
IP (meq O_2/Kg)	5.26±0.27	19.66±0.26	<15
SV (mg KOH/g)	190.36±1.07	193.12±1.23	188 - 253
IV (g I_2/100 g)	69.09±1.91	68.63±2.25	<100
IR	1.4683±0.0008	1.4675±0.0007	1.463-1.478
IE	177.64±1.51	190.40±1.59	186 – 187.94
Unsaponifiable (%)	20.42±6.09	6.07±0.79	≤10
Density	0.914±0.004	0.906±0.002	0.907 – 0.915
Viscosity (cSt)	34.22±0.58	43.39±1.21	
Color (code)	2	1.5	1 - 2.5

IA: Acid value IP: Peroxide value; SV: Saponification value; IV: Iodine value; IR: Refractive Index; and IE: Ester values. The values are means of three repetitions ± standard deviation (except for yield and color).

3.2 Elimination of bitter content and Sensory Organoleptic Analyzes

The liquid-liquid extraction process of the bitter content of *C. procera* was carried out with 2 different solvents: water and ethanol.

3.2.1 Extraction with water

Table 2 presents the results of organoleptic sensory analyzes done on the various tests of debittering with water (at different times) by a panel of 18 tasters.

Table 2. OSA Results of *Carapa procera* Oil for Debittering Attempts with Water as Solvent

Oil: Water		Bitterness					Retronasal persistence		Observation
Samples	Times (hour)	Ext. bit. (%)	very bit. (%)	bit. (%)	S.b (%)	Pleas. (%)	Pers. (%)	No pers. (%)	Effectivity of debittering
1 : 9	144	33	0	**50**	17	0	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.

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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 remains 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].

3.2.2 Extraction with ethanol

The second series of tests were 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	-
3 : 7	30	0	11	56	33	0	50	50	-
	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 analysis showed that the debittering was total. In addition, the perceptibility of retro nasal persistence was only noticed by 22% of the panel of tasters.

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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 of oil/solvent. These analyzes were carried out at room temperature.

3.3 Antimalarial tests

Results in Table 5 show the IC_{50} values for all extracts, debittered oil, bitter oil, and crude (bitter content) compared to quinine taken as a positive control.

Table 5. IC_{50} of different extracts

Echantillons	IC_{50} (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 (IC50 > 500 µg/mL), show some activity at concentrations below 250 µg/mL.
However, according to RITAM (Research Initiative on Traditional Antimalarial Methods), the antimalarial activity of a sample is considered effective when its IC_{50} 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_{50} = 0.30 µg/mL) greater than quinine taken as a control (0.79 µg/mL). This means that the efficiency of *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), a low value compared to that of the bitter content. Therefore, the proportion of the bitter content in it is smaller for the same volume taken.

3.4 Chemical screening on bitter content

The bitter content is the only one concerned in this analysis because it presents 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].

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4. CONCLUSION

This study aimed to eliminate the bitterness of *Carapa procera* oil by extracting the component responsible for it; carrying out physicochemical analyzes of crude oil before and after debittering and comparing 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 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 (IC50 = 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 the cytotoxic activity of bitter content to confirm its antimalarial activity. It would be interesting to separate the different constituents of the bitter content, test in synergy their antimalarial activity, and identify the compound responsible for the activity observed.

REFERENCES

[1] Nonviho G. [2015]. Chemical valorization of oleaginous biomass of Beninese origin: Lophira lanceolata and Carapa procera. Doctoral thesis, University of Abomey-Calavi, Benin.French.
[2] Mutinsumu PC. [2015]. Physico-chemical characterization: nutritional value and energy recovery of some unconventional oilseeds from the D.R. Congo, Doctoral thesis, Marien Ngouabi University, Brazzaville. English.
[3] Sidi S., Moctar S., Patrick V., Ibrahima N. [2013]. Characterization, germination and conservation of Carapa procera DC seeds. (Meliaceae), a useful species in human and animal health. Biotechnol. Agron. Soc. About. 2013;17(2):321-331.
[4] Nonviho G., Paris C., Muniglia L., Sessou P., Agbangnan D.C.P., Brosse N. and Sohounhloué D. Chemical characterization of Lophira lanceolata and Carapa procera seed oils: Analysis of Fatty Acids, Sterols, Tocopherols and Tocotrienols . Res. Diary of Chem. Science. 2014;4(9):57-62.
[5] Ngwamashi E-T. [2009]. Inventory of local woody species for reforestation for energy purposes, DRC.
[6] Mbaye D., Matara S., Gata SY., Mbeura F., Abdouc S., Birameb F., Babacarb F. Anti-inflammatory activity of the seed of Carapa procera (meliaceae). Rev. CAMES – Sciences Struct. Mast. 2013;1:2.
[7] Dembélé U., Koné Y., Témé B., Lykke A.M., Kouyaté A.M. Ethnobotanical preferences of local ligneous species exploited for the production of vegetable oil in the circle of Sikasso, Mali. Africa focus. 2016;29:49-65.
[8] ISO [1996], Animal and vegetable fats and oils. In: ISO 660: Determination of Acid Value and Acidity.
[9] ISO [1996], Animal and vegetable fats and oils. In: ISO 3961: Determination of Iodine Value.
[10] ISO [2001], Animal and vegetable fats and oils. In: ISO 3960: Determination of Peroxide Value.
[11] ISO [2002]. Animal and vegetable fats and oils. In: ISO 3657: Determination of Saponification Value.
[12] International Olive Council. [2017]. Sensory analysis of olive oil. Method of organoleptic evaluation of extra virgin olive oil with designation of origin. COI/T.20/Doc. No. 15/Rev.9.
[13] International Olive Council. [2005]. Method of organoleptic evaluation of extra virgin olive oil in designation of origin. COI/T.20/Doc. No. 22.
[14] Djenontin T., Wotto V., Avlessi F., Lozano P., Sohounhloué D., Pioch D. Composition of Azadirachta indica and Carapa procera (Meliaceae) seed oils and cakes obtained after oil extraction. Ind. Crops and Prod. 2012;38:39–45.
[15] Codex Alimentarius. [1983] Appendix V, Proposed Draft Standard for Scientifically Named Vegetable Oils.
[16] Codex Alimentarius. [1999]. Standards for Named Vegetable Oils, Codex Stan-1999.
[17] Barka A. [2016]. Evaluation of the physico-chemical nature indices of some edible frying oils and impact on consumer health. Master's thesis, University of TLEMCEN, Algeria.
[18] Benseghier K., Khamed Ous. Edible oils from Pinus pinea seed. [2014] Extraction and physico-chemical characterization. License dissertation, Kasdi Merbah University – Ouargla, Algeria.
[19] Malumba M., Fatima Z.A., Kabele N., Vanhaelen M., Taba K., Tuakashikila M., et al Oil from the fruit pulp of Raphia sese de Wild, an important source of dietary vitamin A intake , E and α--linoleic acid. Online Journal of ACASTI and CEDESURK. 2013;1:54. English.

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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...

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