

Antimicrobial, Anti-inflammatory, and Chemical Evaluation of *Buchholzia coriacea* Seed (Wonderful Kola)

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Abstract: This study investigated the phytochemical content, proximate analysis, acute toxicity test, anti-microbial, and anti-inflammatory effect of *Buchholzia coriacea* (wonderful kola) seed fractions using standard methods. The antimicrobial activity of the n-hexane, methanol and aqueous extracts of *B. coriacea* seeds against *Escherichia coli*, *staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* was determined using the agar well diffusion method. The proximate composition shows that freshly dried *B. coriacea* seeds consist of 13% moisture, 88.19% total solid, 0.45% crude fat, 3.92% ash, 1.96% nitrogen, 12.8% protein, 69.8% carbohydrate and 3.5% crude fibre. The acute toxicity study showed that the seed is safe; as no death was recorded. In the assay for anti-inflammatory activity, the results showed the aqueous extract to be the most active fractions. The preliminary antimicrobial evaluation, revealed that at the concentrations analysed (6.25-100 mg/mL), the inhibition zone diameters (IZDs) produced by the aqueous extracts against the test isolates ranged from 0-18 mm; the methanol extract recorded IZDs that ranged from 0-15 mm; and the n-hexane extract recorded IZDs that ranged from 0-7 mm. The antimicrobial results of the extracts of *B. coriacea* showed that the aqueous extract recorded the best antibacterial activity, while the methanol extract showed the best antifungal activity. It can be concluded that the aqueous extract recorded more pharmacological activities than the methanol and n-hexane extracts of *B. coriacea* seeds and this confirms the common use of aqueous decoctions of this plant seeds in South-Eastern Nigeria traditional medicine practice. Analysis of the seed oil, revealed the significant presence of Estra-1, 3, 5 [10]-trien-17 β -ol (35.26%), Oleic acid (6.49%), 1-(+)-Ascorbic acid-2,6-dihexadecanoate (5.98%), Docosanoic acid (2.85%) with other palmitic acid derivatives.

Keywords: *Buchholzia Coriacea*, Antimicrobial, Methanolic Extracts, Aqueous Extracts, Anti-Inflammatory, Nigeria

1. Introduction

The use of plants as food as well as for herbal remedies against diseases in Africa has become an accepted practice. In 1991, WHO as it concerned with herbal drug's standardization emphasized on the need and importance of determining proximate and micro nutrients composition of the herbal plants. [1-2]

Buchholzia coriacea Engler. belonging to the family

Capparacea (common name-magic/wonderful kola, English name-Musk tree) is an evergreen under storey tree of low land, rainforest up to 20m high and can be found in Guinea up to west Cameroon [3]. The bark slash of the seed is deep red and the sap excludes with a violently spicy pungent smell which causes sneezing [3]. The plant parts commonly eaten are the seeds which are either cooked or eaten raw [4]. Literature reveals that the seed is made into a pulp for inhalation or into snuff to relieve headache, sinusitis,

bronchitis, ophthalmias, pleurisy, kidney pains and nasal congestion in Ivory Coast; to treat small pox and skin-itch in Gabon and ear ache in Ghana. In Nigeria, the Edos boil and eat the fruit after storage for a few days [5]. The seed decoction is usually made in lime or local gin, sometimes a hot water decoction is usually made by boiling in water all for the treatment of diabetes mellitus, hypertension, rheumatism, cold, cough and catarrh by some herbalist in our locality (Anambra state Nigeria) (personal communication). Wonderful kola has the ability to stop migraine headache on the forehead within 5-10mins, it is also regarded as brain food to promote memory.

Plants have basic nutritional importance by their content of proteins, carbohydrates, fats and oils, minerals, vitamins, and water responsible for growth and development in humans and animals. Proximate and nutritive analysis of edible plant and vegetables play a crucial role in the assessing their nutritional significance [6].

The presence of bioactive plant constituents often called Phytochemicals have been considered of crucial nutritional importance in the prevention of diseases [7]. Secondary metabolites may be applied in nutrition and as Pharmacologically-active agents [8]. Knowledge of the chemical constituents of plants is desirable and relevance in discovering the actual value of folkloric or ethno-remedies.

The compilation of useful drugs derived from medicinal plant is impressive; these includes; heart drugs, analgesics, anaesthetics, anti-biotic, anti-cancer, anti-parasitic compounds, anti-inflammatory drugs, oral contraceptive hormones, as well as laxative diuretics [9].

The traditional use of *Buchholzia coriacea* in Nigeria and Africa at large as food and medicine necessitates the chemical investigation of this seed; by assessing the chemical component, Phytochemicals, nutritive values, acute toxicity, antimicrobial and anti-inflammatory activity of its aqueous, methanolic extracts and fractions.

2. Materials and Methods

2.1. Plant Materials

The seeds of *Buchholzia coriacea* were collected from Anambra State in Nigeria and authenticated by a taxonomist, Dr E. I Mbaekwe of the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra state Nigeria. The

Voucher, specimen (*Capparaceae*/PCG/474/A/047) was deposited at the herbarium of the department of Pharmacognosy faculty of pharmaceutical sciences, Nnamdi Azikiwe University Awka Anambra state Nigeria. The seeds were washed with clean water, air-dried at room temperature and crushed into coarse powder. The samples was packed in air tight plastic container and stored in a refrigerator for further analysis.

2.2. Chemicals

All reagents and materials used for this work were of analytical grade. Solvents were from Sigma Chemicals CO.,

USA. Also the reagents used, were all freshly prepared.

2.3. Extraction and Fractionation

About 500g of the seed powder were extracted with hexane using cold maceration for 3 days which was then filtered and the filtrate concentrated. The residues were air dried and successively extracted with chloroform and methanol. The solvent was removed by the use of rotary evaporator at 40°C under pressure. Absolute ethanol was used for an extraction from a fresh powder (50g) to obtain ethanolic extract for acute toxicity test. Also the water extract was obtained by dissolving the seed powder (100g) in clean water for only 24hrs and was dried using a freeze dryer. All samples were stored at 4°C in sample containers prior to use.

2.4. Phytochemical Tests

In this study, the preliminary screening involves detailed phytochemical screening of the crude extracts and fractions of the seed powder. The different qualitative and quantitative chemical tests were performed in order to establish the chemical composition of the extracts and fractions. The phytoconstituents determinations were carried out using standard methods as described. [10-12].

2.5. Proximate Analysis

The proximate analysis of *Buchholzia coriacea* seed, to establish its nutritional profile based on the following parameters; Moisture content, total solid, crude fat, ash content, percentage nitrogen, crude protein, crude fibre, carbohydrate and caloric value. These parameters were determined using [13] standard method. The caloric value of the sample was estimated using certain to multiply the value of crude protein, lipid and carbohydrate respectively and taking the sum of the product [14]. The AOAC methods of various parameters are as follows: Crude protein 955.04 (2.4.03), crude fibre 962.09 (4.6.01), moisture. 934.01 (4.1.03), ash 942.05 (4.1.10), Crude fat 920.39 (4.5.01) and carbohydrate by difference.

2.6. Antimicrobial Screening

Test Organisms

Clinical isolates of *Escherichia coli*, *staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, were used in this research.

Culture Media and Reagents

Culture media used were Nutrient agar, Nutrient broth, Mueller Hinton agar, Sabouraud Dextrose agar, Sabouraud Dextrose broth (Oxoid Limited, England), Reagents used include McFarland 0.5 turbidity standard (prepared from barium chloride, sulfuric acid and water), Methanol and N-Hexane (SIGMA-ALDRICH Inc., Germany), sodium chloride (BDH Chemicals, England), Dimethyl sulfoxide

(DMSO), distilled water, etc.

Antimicrobial Assay

Preparation of Stock Solutions

For the preliminary antimicrobial screening of the extracts of *B. coriacea* seeds, stock solution of the various plant extracts were prepared by dissolving 500mg of the extracts in 5mL of distilled water (for the aqueous extract) and DMSO (for methanol and n-hexane extracts) to obtain a final concentration of 100mg/mL. These were transferred to a screw capped bottle and stored at 4°C.

2.7. Preliminary Screening of Plant Extract for Antibacterial and Antifungal Activity

The agar well diffusion assay method described by Perez and his co-workers [15] was used to evaluate the antibacterial and antifungal activities of the aqueous, methanol and n-hexane extracts of *B. coriacea* seeds against the test microorganisms. Dilutions of 50, 25, 12.5, and 6.25 were prepared from the 100mg/mL stock solution of the plant extracts in a 2-fold dilution process. Twenty (20) mL of molten Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (for bacterial and fungal isolates respectively) were poured into sterile Petri dishes (90 mm) and allowed to set. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were swabbed aseptically on the agar plates and holes (6mm) were made in the agar plates using a sterile metal cork-borer. Twenty (20μL) of the various dilutions of the plant extracts and controls were put in each hole under aseptic condition, kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium and incubated accordingly. Ampicillin (30μg/mL) and fluconazole (50μg/mL) were used as positive controls in the antibacterial and antifungal evaluations respectively; while distilled water (aqueous extract) and DMSO (methanol and n-hexane extracts) were used as the negative controls. The MHA plates were then incubated at 37°C for 24 hours, and the SDA plates were incubated at room temperature (25-27°C) for 2-3days. The inhibition zones diameters (IZDs) were measured and recorded. The size of the cork borer (6mm) was deducted from the values recorded for the IZDs to get the actual diameter. This procedure was conducted in duplicate and the mean IZDs calculated and recorded.

Animals:

Male and female Swiss albino rats (100-200g) and mice (20-27g) were obtained from the breed of animal house of the department of Pharmacology and toxicology, Nnamdi Azikiwe University, Awka. They were kept in cages under standard laboratory conditions and fed with pellets from Guinea feed Nigeria Ltd and water. The animals were fasted for 24hrs prior to experimentation with free access to only tap water.

Ethical Clearance:

According to the international guidelines for handling of Laboratory animals [16] and the relevant University ethical committee on laboratory use; all animal experiments were conducted in accordance with the approved institutional and

national guidelines for the care and use of animals.

2.8. Acute Toxicity Study

Thirty Swiss albino mice and rats each, divided into six groups of five animals per group was used for this study. Group 1-5 received oral administration of 100, 1000, 2000, 4000, 5000 mg/kg doses of the extract after six hours fasting for mice and overnight fasting for rats respectively. The control group (6) received oral administration of 10mg/kg of normal saline. All the animals were observed for obvious toxic symptoms and mortality from 24hrs post administration of the extract LD50 was estimated using probity analysis [17].

2.9. Acute Inflammation: Egg Albumen Induced Paw Edema in Rat

The test was carried out as described by Osadebe and Okoye [18]. The animals (n=6 per group) were fasted for 5 hours and deprived of water only during the experiment. They were given intraperitoneal (i.p) injection of the crude extract and fractions solubilised in 10% Tween 80 at doses of 100, 200, and 300mg/kg. Control animals received 0.4ml of 10% Tween 80 or 100mg/kg aspirin (i.p); all substances were administered 30 minutes before the subplanta injection of the phlogistic agent (0.1ml of fresh undiluted egg albumen). Paw volumes were measured by water displacement method at 0, 1, 2, 3, and 4 hours after induction of edema. The anti-inflammatory effect was calculated at each time of observation as percentage inhibition of edema in the animals treated with the substances compared with the vehicle treated animals. The percentage inhibition of edema was calculated as described by Perez, [19] using the formula

$$\% \text{ inhibition} = \frac{V_0 - V_t}{V_0} \times \frac{100}{1}$$

V_0 = the volume of edema of the control (vehicle treated) group at time t

V_t = the volume of edema at corresponding time of the treated rats

2.10. GC-MS Analysis

The GC-MS analysis of the petroleum ether oil extract was performed using Agilent 5973N GC-system coupled to Agilent 6890N, equipped with a cross-linked 5% PH-ME siloxane HP5-MS capillary column (30m x 0.25mm, film thickness of 0.25μm). Helium was the carrier gas at a flow rate of 2ml/min. The injector was operated at 280°C and the column temperature 60-275°C at 4°C /min; while the injected volume is 2μL, with split ratio of 1:50. The mass detector tub was operated in EI mode. Identification of the components in the oil was based on comparison of their mass spectra with NIST Library and in comparison of their retention indices with literature.

3. Results and Discussions

The phytochemical compositions of different fractions and

the powdered seed are presented in the in table 1. The presence of secondary metabolites such as alkaloids, saponins, tannins, steroids, and cardiac glycosides may contribute to its medicinal value. These phenolic compounds

were found to reside more on the water extract and methanolic fraction; which suggests that this seed is likely going to contain more of polar compounds.

Table 1. Extracts and fractions of the seed and their phytochemical constituents.

Bioactive compounds	Methanolic fraction	n -hexane fraction	Chloroform fraction	Aqueous Extracts	Powdered drug
Alkaloids	Weak +	++	++	++	++
Saponins	++	++	++	+++	+++
anthraquinones	–	–	–	–	–
phlobatannins	NT	NT	NT	–	–
Flavonoids	–	–	–	–	Weak +
Tannins	+++	–	–	–	+++
Cardiac glycosides	++	+	+	+	+
Resins	NT	NT	NT	NT	–
Protein	++	++	++	++	+
Steroids	++	++	–	++	+
Carbohydrate	+	–	–	+++	+++
Terpenoids	NT	NT	NT	NT	+

NT= not tested

Proximate analysis: The result of the proximate analysis are shown in table 2. The crude protein is very significant as compared to other known protein sources like cowpea [20]. With regards to the lipid content and the carbohydrate; it could be recommended for diabetic patients. The caloric value is 384.33 ± 0.52 Kcal/100g; it can contribute to the caloric requirement of the body.

Table 2. Proximate Composition of *Bucchozia coricea* seeds.

Parameters	Percentage (%) composition	Percentage dry matter
Moisture content	13%	1.34±0.02
Crude fat	0.45%	2.50±0.06
Ash content	3.918%	4.53±0.07
% tage nitrogen	1.96%	-
Crude protein	12.8%	13.28±0.38
Crude fibre	-	1.70±0.09
Carbohydrate	69.832%	77.18±0.27
Caloric value (Kcal)	-	384.33±0.52

(Values are means of triplicate determinations ±SD.)

The level of crude fibre is appropriate; for digestion and absorption of glucose and fibre.

(Values are means of triplicate determinations ±SD.)

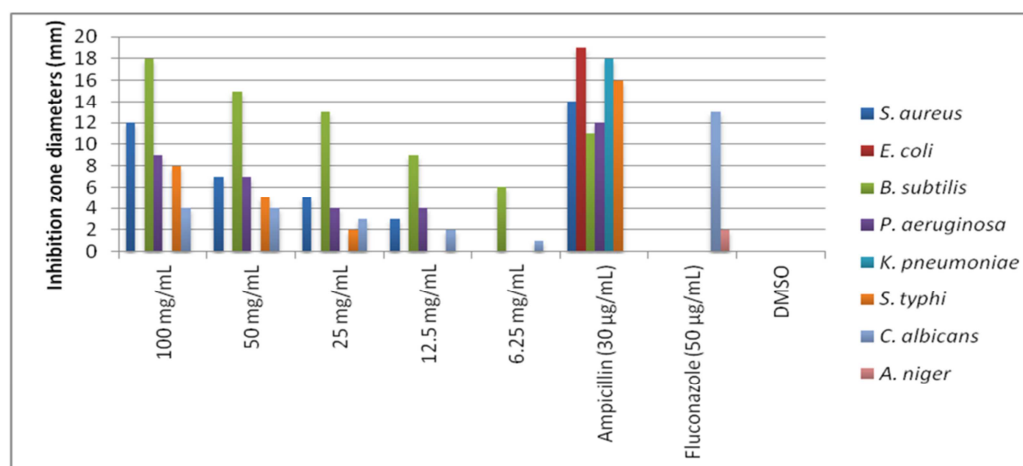


Figure 1. Result of antimicrobial screening of aqueous seed extract.

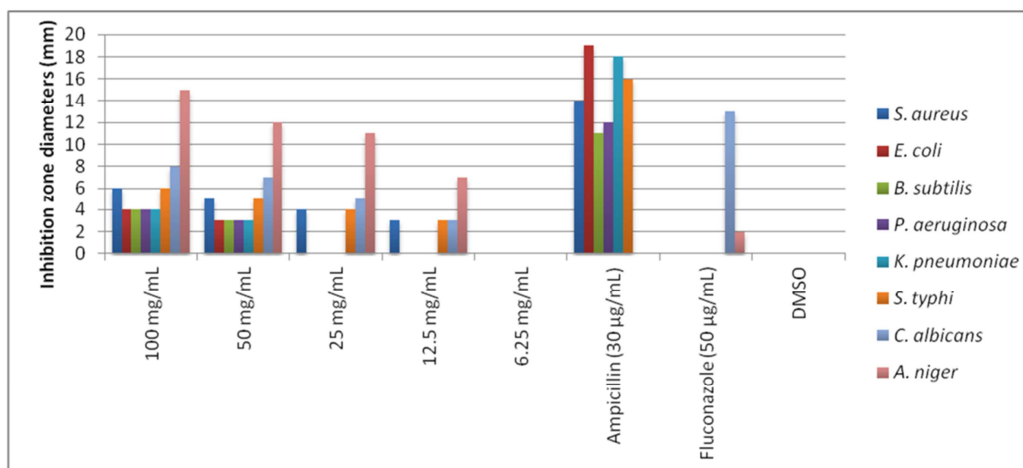


Figure 2. Result of antimicrobial screening of methanolic seed extract.

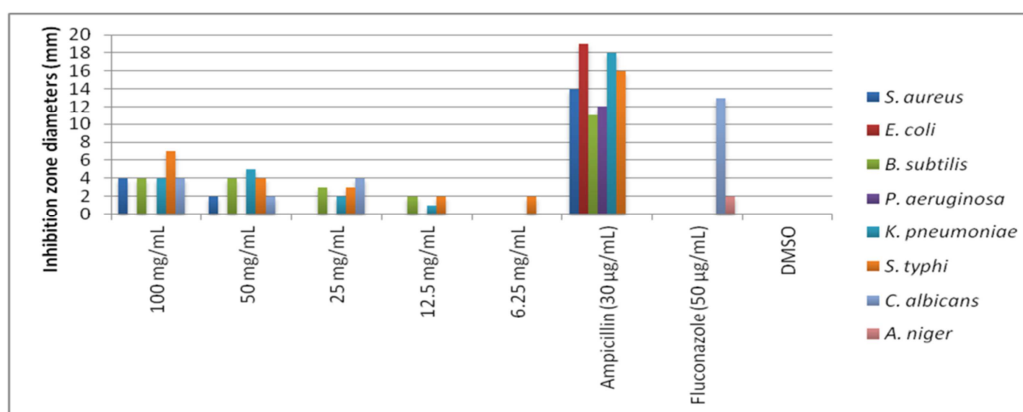


Figure 3. Result of antimicrobial screening of n-hexane seed extract.

From the results of the anti microbial screening presented in Figure 1 above, it can be observed that the aqueous seed extract of *B. coriacea* recorded antibacterial activity against the bacterial test isolates (except *E. coli* and *K. pneumoniae*), with the best activity recorded against *B. subtilis*. Antifungal activity was recorded only against *C. albicans*.

Also looking at figure 2, it can be observed that the methanol seed extract of *B. coriacea* recorded antibacterial activity against all the bacterial test isolates. Also, antifungal activity was recorded both against *C. albicans* and *A. niger*. The methanol extract recorded better antifungal activity than antibacterial with best activity against the mold, *A. niger*.

In the n-hexane fraction (Figure 3), *B. coriacea* recorded antibacterial activity only against *S. aureus*, *B. subtilis*, *K. pneumoniae*, and *S. typhi*. Antifungal activity was recorded only against *C. albicans*.

Wherefore, from the antimicrobial evaluation of the aqueous, methanol, and n-hexane extracts of *B. coriacea*, it can be seen that at the concentrations analysed (6.25-100 mg/mL), the inhibition zone diameters (IZDs) produced by the aqueous extracts against the test isolates ranged from 0-18 mm (figure 1); the methanol extract recorded IZDs that ranged from 0-15 (figure 2); while the n-hexane extract recorded IZDs that ranged from 0-7 mm (figure 3).

Several research works have confirmed the antimicrobial

activity of *B. coriacea* [21-23]. From the result of the antimicrobial screening, *B. coriacea* as shown in figure 1-3, it can be observed that the aqueous extracts recorded the best antibacterial activity. The methanolic extract showed best antifungal activity against both *C. albicans* and *A. niger*. Further work will therefore include; isolation and characterization of the major secondary metabolites of this wonder seed; that could be responsible for the recorded activities. A recent review on this wonder plant [24] as well as the available information from the literature, to the best of our knowledge suggests that we have slim if not no available report of anti-inflammatory activity of wonderful kola seed.

Acute toxicity

No mortality was observed among the mice and rat groups administered 100-5000mg/kg body weights of ethanolic and aqueous extracts of *Buchholzia coriacea* after two weeks of post treatment observation.

Egg albumen-induced edema in Rats

Changes in paw diameter of the induced oedema in rats treated with 200-400mg/kg of the methanol and aqueous extracts of *B. coriacea* seeds followed a dose and time dependent pattern up to 4hrs. *B. coriacea* did not produce significant activity; however, a dose dependent inhibition of oedema was observed to maximum of 40% inhibition with aqueous extracts and 20% inhibition with methanolic extract

at 400mg/kg (Table 3).

Table 3. Percentage inhibition of egg albumin induced paw oedema.

Treatment	Dose (mg/kg)	Percentage inhibition (%)			
		1h	2h	3h	4h
Aqueous extract	200	12	16	24	36
	400	8	24	36	40
Methanol fraction	200	4	8	8	20
	400	8	8	8	20
aspirin	100	12	20	24	28
Tween 80	10%	8	8	12	24

Dose of extract in mg/kg i.p, n/gp = 5

The results of the GC-MS analysis are given in Table 4. *B. coriacea* seed oil contains a mixture of long chain saturated and unsaturated fatty acids, alcohols and their esters and high concentration of Estra-1, 3, 5 [10]-trien-17 β -ol (Figure 4). The major constituent is a steroid which differs from estradiol, a sex hormone, with the absence of an OH group at carbon atom number 3. Steroids though similar in basic structure, have extreme specificity [25] as the steroid in the oil cannot be said to function like estradiol.

Oleic acid, have been confirmed present in several plants, being unsaturated is considered as a healthy source of fat in human diet. Many fatty acids are also known to have anti-bacterial and anti-fungal activity [26]. As acclaimed by herbal medical practitioners; these constituents with other identified metabolites play possible role in curative properties attributed to this wonder plant.

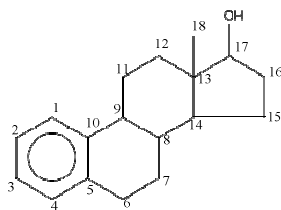


Figure 4. Structure of the major constituent identified in the seed oil.

Table 4. Chemical Composition of *B. coriacea* seed oil.

S/N	Compounds	Percentage (%)
1	Estra-1, 3, 5 [10]-trien-17 β -ol	35.26
2	Oleic Acid	6.49
3	Hexadecanoic acid	6.49
4	1-(+)-Ascorbic acid-2,6-dihexadecanoate	5.98
5	2-methyl Hexadecanoic acid	3.98
6	Tert-Hexadecanoic acid	3.68
7	Docosanoic [Behenic] acid	2.85
8	Hexadecanoic acid Methyl ester	2.74
9	Arachidic [Eicosanoic] acid	2.12

4. Conclusion

Our investigation has demonstrated that the methanolic extract and to a larger extent the aqueous extract contain a comparable anti-inflammatory agent. The aqueous extract has consistently shown better activity in both antimicrobial and inflammatory studies which supports hot water decoction as the best mode of intake. Indeed this study has undoubtedly

provided scientific evidence and confirmation for the safety and use of the seeds as food and in the treatment of several infectious diseases and inflammatory related diseases in Nigeria. The isolation and characterization of the major secondary metabolites from the non-lipophilic extracts of this wonder seed, is therefore eminent.

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