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Proximate, nutraceutical composition and antimicrobial activities of *Uvaria chamea* (Udagu) seeds and oil

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ABSTRACT

The study investigated proximate, nutraceutical composition and Antimicrobial activities of *Uvaria chamea* (Udagu) seeds. Standard methods were used to analyze the plant seeds for the present of nutraceutical and proximate component of the plant *Uvaria chamea* (Udagu) seeds. While disk diffusion method was employed to assay the the potent of the seed oil as an antimicrobial agent. Result of the proximate revealed that *Uvaria chamea* (Udagu) seeds contains ash (10.19 %), moisture (5.2 %), crude protein (0.09 %), crude fiber (51.81 %), total fat (26.19 %) and carbohydrate (6.52 %). While nutraceutical study showed that *Uvaria chamea* (Udagu) seeds contains cardiac glycosides (0.046 %), tannins (0.2 %), saponins (0.01 %) and alkaloids (0.45 %).The proximate result implies that the plant contains essential component such as crude fiber, total fat and ash in significant quantities, and can be utilized as food and in treating several ailments. The presences of alkaloids, tannins, Cardiac glycoside, and saponins in the plant seed indicate it can be used as a medicinal source.The oil presented no antimicrobial and antibiotic activities against *Staphylococcus aureus* and *Salmonella typhi.* The results suggest that *Uvaria chamae* seed have a potential role as a new source of health-promoting diets (can be used as a dietary fiber supplement in obesity management) with high oxidative stability. And the oil is a fixed oil that could be an acceptable substitute for cooking vegetable oil that do not have antimicrobial/antibiotic activities.

Keywords: Proximate; nutraceutical; fiber; alkaloids; tannins

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INTRODUCTION

Plants offer a large range of Phytochemical natural chemical compoundsbelonging to different molecular families which havevarious properties to humans as therapeutic agents that reduce the risk of cancer due to dietary fibers, polyphenol, antioxidants and anti-inflammatory effects [1]; [2]. The well-recognized father of modern medicine, Hippocrates (460-377 BC), stated "Let food be thy medicine and medicine be thy food" to predict the relationship between appropriate foods for health and their therapeutic benefits. Today phytochemicals (non-nutritive plant chemicals that have protective or disease preventative properties) in foods have been identified and new discoveries are being made surrounding the complex benefits of phytochemicals. A combination of the words "nutrition" and "pharmaceutical," the term nutraceutical was coined by combining the terms "Nutrition" and "Pharmaceutical" in 1989 by Dr Stephen DeFelice, Chairman of the Foundation for Innovation in Medicine, encompasses foods or food products that claim to prevent/ treat various ailments ranging from heart diseases to cancer, improve health, delay the aging process, and/or increase life expectancy [3].

Edible seed foods can be classified based on the amount of nutrients and the type of nutrients they provide for subsistence and survival and are composed of macronutrients or nutrients that are needed in large quantities (carbohydrates, proteins, fats, fiber, and water) and micronutrients or nutrients that are needed in small amount (include vitamins and minerals) in varying proportions which is essential for and nutritionally important [4]; [5]; https://doi.org/10.4060/ca9692en; [6]; [7].Plants capture and utilize solar energy by means of photosynthesis, thereby storing carbohydrates which serve as source of energy [8] with fiber and water, while fatty acids and *proteins*, *can* also *serve* as *energy sources* when they are funneled through appropriate enzymatic pathways [9]. Thus, these products serve as a source of two types of energy which can be expressed as calories. The first energy being heat which serve to maintain the body temperature. the second is free energy, which is available for work [10]. Complex macromolecule polymers of amino acids (protein) joined in peptide linkage that is very important for growth, development and maintenance that compose of 50 % of dry weight of living cells called protein that bind to other selected molecules and how their activity depends on such binding [9]; [11].

Plant fats and oils is an energy storing molecules and most important kind of lipid. They function as a Structural component of membranes, aid in transportation of fat-soluble nutrients that are important for good health and as a storage form of metabolic fuel [12]; [13].Carbohydrate nutrient are mainly derived from plants and they are polyhydroxy aldehyde or ketone or substances that yield such compounds on hydrolysis. Our body breaks down carbohydrates into glucose or blood sugar that is the main source of energy for your body's cells, tissues, and organs [14]. The primary function of the carbohydrates in living organisms is as an energy source. Many of them are easily digested by animals where they are converted back into carbon dioxide and water, with a concurrent release of energy [4]; [15].

Fiber is mainly a carbohydrate that the body can't digest but keep the digestive system healthy. A diet high in especially fiber, can help solve constipation, high cholesterol, type 2 diabetes and cardiovascular diseases, and more [16]; [17]; [18]; [19]; [20]; [21]. Fiber can help burn down trouble and stubborn fat in the body. As such fiber can help combat weight gain and keep the weight off essential for a long healthy life [22].As a universal solvent, the moisture content (water) from food material plays an important role in living systems. It is an indispensable nutrient and involved in the metabolism of a food digestion, elimination of body wastes, transportation of nutrients, regulation of body temperature, secretion of enzymes, hormones, lubricates the joints and tissues, maintains healthy skin, and for proper digestion and other biochemical activities [23]; [24]; [25]; [26]; [27];http://css.umich.edu/factsheets/us-food-systemfactsheet;https://www.nap.edu/read/10925/chapter/6#102).

Edible seeds are important sources of nutrients and energy especially among the resource-poor populations where protein- energy malnutrition (PEM) has continued to hamper optimal growth and development [28]; [29] and can sustain livestock production by ensuring the availability of various sources of nutrients that are required for the formulation of animal feed [30].

Seed oils which are mainly triacylglycerols have range of physical and chemical compositional parameters that determine its applications [31]; [32]. The little or no information on the composition

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and utilization of the many and varied lesser seed oils indigenous to the tropics are more of problem than the real shortage of oils [33].

Materials and methods

Sample collection and preparation

The sample was ripped fruit of *Uvariachama*and collected from Oghe Town in Ezeagu Local Government of Enugu State on 22 July 2022. The plant was identified and authenticated by Prof. J.C. Okafor of Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology. The ripped fruits were sun dried for two months and the seeds dehulled from the dried fruits and separated by the help of gravity. The whole seed was ground into powder with the aid of manual grinder and kept in a sterile plastic bag at room temperature for further studies.

Collection of Micro-organism

The test organisms include *Staphylococcus aureus* and *Salmonella typhi* and were obtained from the Microbiology Department of Emex Medical Laboratory, Abakpa Nike Enugu. They were sub-culture on nutrient agar and kept at 37℃.

Moisture content determination (AOAC, 1999)

The percent difference in weightof exactly 2g of the ground sample after 8 hoursin an electric oven at 105 ℃ and allowed to cool down to room temperature in a desiccator containing calcium chloride for about 40 minutes [34].

Calculation

$$\% \, Moisture == \frac{loss \ in \ weight}{weight \ of \ sample} \text{X } 100$$

Ash determination (AOAC, 1999)

Two grams of the ground sample was weighed into a fire-polishedempty crucibleand transferred into muffle furnace and heated to 550°C for 30 minutes and allowed to cool down to room temperature in a desiccator and weighed.

Calculation

$$\% \, Ash = \frac{Weight \ of \ ash}{Weight \ of \ sample} \times 100$$

Crude protein determination (AOAC, 1999)

The samples (1 g) was digested with 25 ml concentrated sulphuric acid, using 3 g of copper sulphate and sodium sulphate (10:1) as catalyst to convert organic nitrogen to ammonium ions. Employing macrokjeldahl method of AOAC (1999).Thekjeldahl flask was inclined and heated gently at first until frothing ceased, then heated strongly with shakings, at intervals, to wash down charred particles from sides of the kjeldahlflask. Heating was continued until the mixture become clear and free from brown or black colour. The digest was allowed to cool down to room temperature and the volume made up to 100 ml with distilled water. Forty milliliters (40 ml) of 40% Sodium hydroxide was added to 20 ml of the diluted digest and the liberated ammonia is distilled with few pieces of granulated zinc and anti-bumping granules into 50 ml of 2 % boric acid receiving conical flask. The distillate was titrated with 0.01 N hydrochloric acid to a purple coloured end point (Vml).

Calculation

$$\% \, Nitrogen \ = \frac{1.4 \ \times Titer \ volume \ \times total \ volume \ of \ digest}{1000 \ \times weight \ of \ sample \ \times \ aliquot \ distilled} \times 100$$

Crude Protein (%) = %Nitrogen x 6.25

Crude fat determination (AOAC, 1999)

Five grams (5 g) of the ground sample was weighed into a thimble bag (W) and inserted into the extraction column with the condenser. The total fat content was extracted with 300 ml of the extracting solvent (n-hexane) at 60°C for six hours. After extraction, the thimble was removed and the solvent distilled out. The total fat content was placed on a pre-weighed beaker (W_1) and the solvent evaporated off in a water bath and after transferred to an oven and heated for some minutes to

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evaporate the remaining solvent and moisture to complete dryness. It was cooled in a desiccator and weighed (W_2).

The percent oil yield was calculated using

Percent $Total\ fat$ yield(%) $= \frac{W2 - W1}{W} \times 100$

$= \frac{\text{weight of oil}}{\text{weight of Sample}} \times 100$

Crude fiber determination

Two grams of the defatted sample (above) obtained during fat determination was air dried and heated with 200ml of 1.25% sulphuric acid for 30 minutes and filtered. The insoluble matter was washed with boiling distilled water until the filtrates is free from acid. The insoluble matter was washed back into the flask with 200 ml of 1.25% NaOH and boiled for 30 minutes and filtered immediately. The insoluble matter was wash with boiling water until no base is detected in the filtrate. The whole insoluble matter was washed with 1% HCl and finally with boiling water until free from acid. Then it was washed twice with 95 % ethanol and three times with ether and oven-dried at 100°C to constant weight. The crucible and its content were ash in muffle furnace at 550°C and re-weighed. The difference between the weight of ash and the weight of insoluble matter gave the weight of the crude fiber.

Calculation

$$\%\ Crude\ fiber\ = \frac{Weight\ of\ dried\ insoluble\ matter - weight\ of\ ash}{Weight\ of\ sample\ before\ deffating} \times 100$$

Determination of total carbohydrate

The total percentage carbohydrate content was determined by difference of 100.
%Carbohydrate: = 100 – (%Protein + %Moisture + %Ash + % Fibre)

Quantitative phytochemical screening

Determination of total alkaloids

Twenty grams of ground*Uvariachamea* (Udagu) seedswere extracted with 500 ml of methanol: (1:1v/v) mixture and filtered with Wathmann filter paper. The filtrate was evaporated. The resultant residue was mixed with 200ml of 1 % H_2SO_4 and partitioned with ether to remove unwanted materials. The aqueous left was then extracted five times with chloroform to obtain the alkaloid fraction. The chloroform extract was combined and concentrated to dryness. The alkaloid was weighed and the percentage was calculated with reference to the initial weight of the sample powder,[35].

$$\%\ Alkaloids = \frac{weight\ of\ alkaloid\ residue\ \times 100}{weight\ of\ sample}$$

Determination of total saponins

Twenty grams of groundsample was boiled under reflux with 300ml of 50% ethanol for 30 minutes and filtered while hot through a coarse filter paper. Activated charcoal (2 g) was added to the content to adsorb impurities (pigments), boiled and filtered while hot. The filtrate was cooled and an equal volume of acetone added to precipitate saponins. The separated saponins precipitate was dissolved in the least amount of boiling 95% alcohol and filtered while hot to remove any insoluble matter [35]. The filtrate was allowed to cool to room temperature and the precipitated saponins collected and suspended in 20ml of ethanol and filtered through a pre-weighed filter paper. The pre-weighed filter paper and the residue was transferred to a desiccator containing anhydrous calcium chloride and left to dry for 24 hrs. They were weighed with reference to the weight of sample used.

Calculation

$$\%\ Saponins = \frac{weight\ of\ saponins\ residue\ \times 100}{weight\ of\ sample}$$

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Determination of tannins

Ground (0.1g) sample was gently heated to boiling with 50 ml water for 1 hour in 100 ml conical flask and filtered. The residue was washed several times and the wash water combined with the filtrate and the solution made up to the 50 ml volume with distilled water. To 10ml of sample solution in a 50ml volumetric flask, 2.5ml of Folin - Denis reagent and 10ml of $NaCO_3$ solution were added and made to volume with distilled water and allowed to stand for 20 minutes. The optical density was measured at 760 nm using spectrophotometer [35].

Calculation

$$Tannic\ acid\ (mg/ml) = \frac{absorbance\ of\ sample}{absorbance\ of\ standard\ \times conc.of\ standard}$$

Determination of cardiac glycosides

One gram of the ground sample was extracted with 10ml 70% alcohol and filtered. Eight milliliters of the filtrate werediluted to a 100ml in a volumetric flask with distilled water. Eight millilitersof the diluted solution were added to 8ml of 12.5% lead acetate (to precipitate resins, tannins and pigments). The mixture was shaken well and the volume made up to 100mlwith distilled water and filtered. The filtrate (50ml) was pipette into another 100ml volumetric flask and 8ml of 4.7% disodium hydrogen phosphate (Na_2HPO_4) solution was added to precipitate excess lead. The mixture was made up to the volume with distilled water and stirred. This was filtered twice with filter paper. Baljets reagent (10ml) were added to 10ml of the purified filtrate and to the blank sample of 20ml of distilled water. The two solutions were incubated at room temperature for one hour (time necessary for maximum colour development). The intensity of the colour was read at 495nm using spectrophotometer. The colour wasstable for several hours [35].

Calculation

$$\%\ glycoside = \frac{A \times 100\ g\ \%}{17}$$

Antimicrobial assay

The antimicrobial assay of *Uvariachamea*seed oil was performed by agar well diffusion method. Varying quantities of the oil was dissolved in Dimethyl sulfoxide (DMSO) to obtain 6.25, 12.5, 25, 50, 100 and 200 mg/ml respectively [36]. A fixed volume of Mueller Hinton agar, a standard concentration of bacteria was smeared evenly onto the surface of an agar plate. Next, disks of filter paper, each impregnated with varying concentration of the oil, were applied to the portions smeared with the organisms respectively (*Staphylococcus aureus* and *Salmonella typhi*) respectively. The plates were incubator at 37°C for 24 hours. The antimicrobial activities were observed by measuring the zone of clearance on the bacterial gene near the impregnated disc. The antibiotic activities were also performed with disc impregnated with synthetic antibiotics and zone of inhibition measured.

RESULTS AND DISCUSSION

Table1revealed that *Uvaria chamea* (Udagu) seeds contain some Nutraceutical composition which also plays their own vital role. The result showed that the pant seed contains0.046 % cardiac glycoside, 0.2 % tannins, 0.01 % saponin and 0.45 % alkaloids. This propertyis also due to the plant genetic makeup.

Table 1:Nutraceutical composition of *Uvaria chamea* (Udagu) seeds

S/N	Parameter	Composition (%)
1	Cardiac glycoside	0.046
2	Tannins	0.2
3	Saponins	0.01
4	Alkaloids	0.45

The findings of the study showed that the seeds of *Uvaria chamea* (Udagu)
plant contain phytochemicals such as alkaloids, tannins, Cardiac glycoside, and saponins. Evidence from the study indicated that the concentration of the parameters varies. The presences of alkaloids, tannins, Cardiac glycoside, and saponins in the plant seed indicate that the plant can be used as medicinal source. The findings also corroborate with the findings of [37] who in their study said that

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tannin content of the thirty browse plants was 0.13 %-1.07 %and the small quantity in tannin content of the *Uvaria chamea* seeds may likely suggests that the seed is not rich in tannins. The percent ash, moisture, crude protein, crude fiber, total fat and carbohydrate contents of the *Uvaria chamea* (Udagu) seeds were analyzed using simple chemical methods respectively. Result in Table2 shows that the *Uvaria chamea* (Udagu) seeds contain 10.19 % ash, 5.2 % moisture, 51.81 % crude fiber, 0.09 % crude protein, 26.19 % total fat and 6,48 % carbohydrate. This result implies that the plant contains essential component such as crude fiber (51.81 %), total fat (26.19 %) and ash (10.19 % ash), and can be utilized as food and in treating several ailments. This result may also be attributed to genetic composition of the plant The *Uvaria chamea* (Annonaceae) which has been reported to have potent activity against lymphocytic leukemia in mice [38], anti-malarial activity [39], it also has the anticarcinogenic [40]; [41], exhibited significant in vitro cytotoxicity against the KB cancer cell line [42], has antidiabetic activity [43] has antihemolytic properties [44], has antimicrobial activity [45] has high content of phenolic compounds and high antioxidant activity [46-49].

Table 2: Proximate Determination and percent content of *Uvaria chamea* (Udagu) seeds

S/N	(%) Parameter	Composition (%)
1	Ash	10.19
2	Moisture	5.2
3	Crude protein	0.09
4	Crude fiber	51.81
5	Total fat	26.19
6	Carbohydrate	6.52

The proximate content of the plant with their varying concentration such as that of carbohydrate (6.52 %) and crude fiber (51.81 %) indicates that the plant can be a good source of energy and fiber for animals. The findings of the study that the seed is not a good source of crude protein.Evidence from the study also showed that *Uvaria chamea* (Udagu) seedsalso is a good source of oil. The most interesting components.

The antimicrobial activity of the *Uvaria chama* seed oil was performed using disc diffusion method and Mueller Hinton agar. The results are presented in Table 3.

Table 3: Antibacterial activity of *Uvariachama*seed oilagainst pathogenic microbes.

Microbes	***Uvarianchaemie*seed oil**
Staphylococcus aureus	No activity
Salmonella typhi	No activity

There were no antimicrobial activities against the two microbes (*Staphylococcus aureus* and *Salmonella typhi*) used in this study.

Table 4: Antibacterial-zone inhibition (mm) activity of antibiotic disc on the test organism.

Test organism	Zone of inhibition (mm)									
Gram +ve bacteria	**APX**	**Z**	**AM**	**R**	**CPX**	**S**	**SXT**	**E**	**PEF**	**CN**
S. aureus	0.0	19	0.0	0.0	22	18	0.0	15	18	0.0
Gram –ve bacteria	**CH**	**OFX**	**SP**	**SXT**	**PEF**	**S**	**CPX**	**AM**	**AU**	
E. coli	20	17	17	20	16	21	18	0.0	0.0	

Key:

Gram +ve = APX → Ampiclox; Z → Zinnacef; AM → Ampicilin; R → Recephin; CPX → Cyproflacin; S → Streptomacin; SXT → Septrin; E → Erytromycin; PEF → Reflacin; CN → Gentamycin.

Gram –ve = CH → Chlorophenicol; OFX → Tarvid; SXT → Septrin; PEF → Reflacin; S → Streptomacin; CPX → Cyproflacin; AM → Ampicilin; AU → Augmentin

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The results obtained shows that Zinnacef, Cyproflacin, Streptomacin, Erytromycin and Reflacin were potent against the gram positive test organisms. While Chlorophenicol, Tarvid, Septrin, Reflacin, Streptomacin, Cyproflacin and Augmentin have *potent* against gram nagetive test bacteria.

CONCLUSION

Evidence from the study clearly indicated that *Uvariachamea* (Udagu) seedshas some potential medicinal properties (alkaloid, tannins, saponin and cardiac glycoside). This phytochemical and other founds in the plant makes the *Uvariachamea* (Udagu) seedsa potential medicinal plant. The fiber and oil content are significant and hence, it can be accepted that the plant seed can be harnessed for nutrition, obesity management and industrial purposes. The results of the present investigation clearly demonstrate that the oil of *Uvariachamea*seeds have no activity against the two test organisms (*S. aureus* and *E. coli*) respectively. This work justifies the standard antibiotics have potent against the two test organisms (*S. aureus* and *S. typhi*) respectively with exception of ampiclox, ampicillin, septrin and gentamycin that have no potent against gram positive bacteria's, while for gram negative bacteria's only ampicillin and augmentin that have no potent.

Suggestion for further study

Study should be carried out on the effect of *Uvariachamea* (Udagu) seeds oil for human consumption.

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