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Phytochemical Contents and Proximate Analysis of Walnut Kernel (*Tetracarpidium conophorum*)

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Abstract Plants are a source of medicine in Africa and almost all developing countries of world, due to availability, accessibility and belief to have least side effect than orthodox counterpart. This research investigates the phytochemical constituents and proximate composition of walnut kernel to accomplish, standard protocols were adopted. The result of phytochemical screening reveals the presence of cardiac glycosides, terpenoids, cardinolytes, tannins, phalobatannins and carbohydrates. The proximate composition of walnut kernel was found to be moisture 5.006%, ash 2.4106%, crude protein 8.0746%, fat 0.8623%, carbohydrate 81.5315%, and crude fiber 2.1150% respectively. The presence of metabolites and nutritive constituents in the walnut kernel shows that it can be used as source of food as well as drug formulation.

Keywords Kernel, Metabolites, Nutritive, Phytochemical, Proximate

Introduction

Medicinal plants are those plants that are used (parts, extract etc) in treating and preventing ailments and diseases that affect human beings. Hence the important role of medicinal plants in health care delivery (services) cannot be over emphasized [1].

In the last decade, people have become aware of the use of medicinal plants in the treatment of diseases. There has been reports to the effect that over 80% of the rural populace relies on herbal medicine [2-3]. Natural substances of botanical origin have been used throughout the world for human and animal health care [4-5] especially in Africa. Thus, plants are a source of medicine in Africa and almost all developing countries of the world. Herbs and herbal usage has become a global issue in recent times, especially due to the high poverty level and the cheapness of herbal medicines and the belief that it has less side effects [6].

Walnut is a climbing shrub grown in southern Nigeria and western Cameroon for its leaf, root and nut[7] reported that the presence of tannins, carbohydrate in the raw walnuts. Walnut has been used globally in human nutrition since ancient times. The high protein and oil contents of the kernels of walnut make this fruit, indispensable for human nutrition. Therefore, the walnut is classified as a strategic species for human nutrition and is included in the FAO list of priority plants [8]. The seed part of the fruit (kernel) is consumed fresh, toasted, or mixed with other confectionaries. Walnuts are nutrient-rich food due to high content of fats, proteins, vitamins and minerals. They are also good source of flavonoids sterols, pectics substances, phenolic acids and related polyphenols. The nutritional contents differ from the cultivar to another which can be influenced by genotype, cultivator, different ecology and

different soil [9-10] examined the effect of walnut diet on motor and cognitive ability in aged rats for 8 weeks. The three treated groups (2%, 6% and 9%) revealed that the 2% walnut diet improved performance on rod walking, while the 6% walnut diet improved performance on the medium plank; the higher dose of the 9% walnut diet impaired reference memory, however, the researcher attributed this to the number of polyphenolic compounds that could be negatively effecting reference memory at a higher dose [11].

Walnuts are edible even when raw and give a bitter taste and a stimulating effect like kola.

They can be cooked, roasted or sun dried and the roasted seeds could be ground like melon seeds and used as a thickener in soup preparation. The plant is known in Africa especially in the Eastern and Western parts of Nigeria for its antibacterial efficacy [12]. Decoction of leaves and seeds serve as beverage which relieves abdominal pains and fever [13]. Dried walnuts can be ground and turned into flour which can be used as composite flour during baking or in-place of milk in tea preparation.

This work is designed to evaluate phytochemical contents and proximate analysis of extract of walnut kernel. This will help in ascertaining the bioactive and nutritional ingredients of this plant for drugs formulation as well as malnutrition alleviation.

Materials and Methods

Sample Collection and Identification

The walnut kernel was purchased from Monday market in Maiduguri, Borno State, Nigeria and identified by a plant Taxonomist at the Department of Biological Sciences, University of Maiduguri. It was pulverized into fine powder and kept in the research laboratory, Chemistry Department, University of Maiduguri.

Extraction and Preliminary Phytochemical Screening

The powdered air dried material (200g) was extracted in methanol using cold infusion (maceration) technique; it was filtered and the filtrate was concentrated *in vacuo* using rotatry evaporator at reduced pressure. The extract was subjected to Phytochemicals evaluation using standard procedures.

Test for Alkaloids

Preliminary Test for Alkaloids

The extract (0.5 g) was stirred with 5 ml of 1% aqueous HCl on water bath then filtered. Three milliliters (5ml) of the filtrate was taken and divided equally into 3 portions in a test tube. To the first portion, few drops of Dragendoff's reagent were added. The occurrence of orange red precipitate was taken as the indication for the presence of alkaloids. To the second portion; 1 ml of Mayer's reagent was added and the appearance of buff-coloured precipitate was taken as indication for the presence of alkaloids; to the third portion, 1 ml of Wagner's reagent was added and a dark-brown precipitate was taken as an indication of the presence of alkaloids [14].

Test for Flavonoids

Ferric Chloride Test

The extract (0.5 g) was boiled with distilled water and then filtered. To a 2 ml of the filtrate, few drops of 10% ferric chloride solution were added. A green-blue colouration was taken as an indication of the presence of phenolic hydroxyl group [15].

Shinoda's Test

The extract (0.5 g) was dissolved in (3 ml) of ethanol, warm and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by few drops of concentrated hydrochloric acid. A pink to purple colouration was taken as an indication of the presence of flavonoids [16].

Lead ethanoate Test

The extract (0.5 g) was boiled with 5 ml of distilled water and then filtered. To 5 ml of the filtrate, 3 ml of lead ethanoate solution was added. The appearance of a buff coloured precipitate was taken as an indication of the presence of flavonoids [14].

Sodium hydroxide Test

The extract (0.5 g) was dissolved in 5 ml of water and filtered. To the filtrate, 2 ml of 10% aqueous sodium hydroxide was added to produce a yellow colouration. A change in the colour from yellow to colourless on addition of dilute hydrochloric acid was taken as an indication for the presence of flavonoids [15].

Test of glycosides

Lieberman Burchard's test

To (0.5 g) of the extract, 2 ml of acetic anhydride was added. The mixture was cooled in ice and then 1ml of concentrated tetraoxosulphate (VI) acid was added carefully. Colour development from violet to bluish-green was recorded as an indication of the presence of a steroidal ring [17].

Salkowski's test (test for steroidal nucleus)

To (0.5 g) of the extract, 2 ml of chloroform was added. Then, 2ml of tetraoxosulphate (VI) acid was carefully added by the side of the test tube to form a lower layer. Appearance of a reddish-brown colour at the interphase was taken as an indication of the presence of steroidal ring [17].

Test for free anthraquinones (Borntrager's Test)

To the extract (0.5 g), 10 ml of benzene was added and shaken. The mixture was filtered. 5 ml of 10% ammonia solution was added to the filtrate. The mixture was then shaken. The appearance of violet colour in the lower interphase was taken as indication of the presence of anthraquinones [15].

Test for combined anthraquinones (Borntrager's Test)

To the extract (0.5 g), 10 ml of aqueous tetraoxosulphte (VI) acid was added and shaken and then was filtered while it's still hot. The filtrate was shaken with 5 ml of benzene. The benzene layer was separated and was added half its own volume of 10% ammonia solution. The presence of a pink colouration in the ammonical (lower) phase was taken as an indication for the presence of combined anthraquinones [15].

Test for Terpenoids

The extract (0.5 g) was dissolved in 3ml of ethanol. One milliliter (1 ml) of acetic anhydride was added, followed by the addition 1ml of concentrated tetraoxosulphate (VI) acid. colour change from pink to violet was taken as an indication of the presence of terpenoids [17].

Test for Saponins Glycosides

The extract (0.5 g) was boiled with 5 ml of distilled water and filtered. The filtrate was divided into 2 portions to the first portion about 3 ml of distilled water was added and shaken for about 5 minutes. Frothing which persist on warming was an evidence for the presence of saponins [18]. To the second portion, 2.5 ml of a mixture of equal volume of Fehling's solution A and B was added. The appearance of brick-red precipitate was taken an indication for the presence saponin glycosides [19].

Test for Tannins

To extract (0.5 g), 10 ml of distilled water was added and stirred. The mixture was filtered. The filterate was used for the following test; to 2 ml of the filtrate, few drops of 1% ferric chloride solution were added. The occurrence of a blue-black precipitate was an indication for the presence of tannins. A mixture of equal volume of 10% lead ethanoate was added to 2 ml of the filtrate. The formation of white precipitate was an indication of the presence of tannins. The filtrate of the extract was boiled with 3 drops of 10% HCl and a drop of methanol. A red precipitate was taken as indication for the presence of tannins [18,15].

Test for Phlobatannins

The extract (0.5 g) was boiled with 5 ml of distilled water and then filtered. The filtrate was further boiled with 1% aqueous HCL. The appearance of red precipitate indicates the presence of phlobatannins [15].

Test for Carbohydrate

Molish's Test

The extract (0.5 g) was dissolved in 5ml of distilled water in a test tube. To the mixture, few drops of the Molish's reagent was added, and then followed by 1ml of concentrated tetraoxosulphate (VI) acid by the side of the test tube. The mixture was allowed to stand for two minutes and then was diluted with 5 ml of distilled water. Formation of a dull violet colour at the inter phase of the two layers was taken as a positive test [20,15].

Test for Monosaccharide (Barfoed's Test)

The extract (0.5 g) was dissolved in 5ml of distilled water and filtered. One (1 ml) of the filtrate was mixed with 1ml of Barfoed's reagent in a test tube. This was heated on a water bath for two minutes. A red precipitates of cuprous oxide was considered as positive test [14].

Test for Combined Reducing Sugar

The extract (0.5 g) was mixed with 5 ml dilute hydrochloric acid and boiled. The mixture was neutralized with sodium hydroxide solution. Few drops of Fehling's solution was added to it and then heated on a water bath for two minutes. Reddish brown precipitate of cuprous oxide was an indication of the presence of combined reducing sugars [15].

Test for Free Reducing Sugar (Fehling's Test)

The extract (0.5 g) was dissolved in 5 ml of distilled water and filtered. To the filtrate, 5 ml of equal volumes of Fehling's solution A and B was added. Formation of red precipitates of cuprous oxide was an indication for the presence of reducing sugar [15].

Test for Soluble Starch

The extract (0.5 g) was boiled with 1m of 5% potassium hydroxide and cooled. To the mixture tetraoxosulphate (VI) acid added. A yellow colouration was indication for the presence of soluble starch [19].

Test for Pentoses

To the (0.5 g) of the extract 1 ml of hydrochloric acid was added and a little quantity of phoroglucinol. The mixture was heated with low flame. The appearance of red colour was indication the presence of pentose [19].

Salivanoff's Test (Standard Test for Ketones)

To (0.5 g) of the extract, few crystals of resorcinol and 2 ml of hydrochloric acid were added and the mixture boiled for 5 minutes. The appearance of red colouration was indication of the presence of ketones [19].

Proximate Content Analysis

The sample was analyzed for dry matter, crude protein, crude fibre, ether extract or fat, ash, carbohydrate and nitrogen free extract (N.F.E) according to [21].

Dry Matter

The dry matter content of the sample was determined by weighing 10g of sample into petri dish while placed in hot oven at 105^{o}C for 24hours. Then removed and placed it in dissicator to cool, after cooling it was reweighed. The dry matter content was calculated using the formula:-

$$\text{Dry Matter Content} = \frac{w1-w2}{w2-w1} \times 100\ \%$$

Where

W_1 : Weight of petri dish with sample in grammes before oven dried

W_2 : Weight of petri dish with sample in grammes after oven dried

W : Weight in grammes of empty petri dish

Crude Protein

Crude protein content was analysed using Keljedal tablets and 1g or 2g of sample was weighted into a digestion tube and 1 or 2 Keldedahl tablets was added, 10 or 20ml of concentrated sulphuric acid (Conc. H_2SO_4) was added onto the tube and digested at 420^{o}C for 3 to 5 hours. After cooling, 80ml or 90ml of distilled water was added into digested solution. About 50ml of 40% caustic soda (NaOH) was added onto 50ml of digested and diluted solution and then placed on heating section of the distillation chamber, 30mls of 4% boric acid, bromocresol green and methyl red as an indicator were put onto conical flask and placed underneath the distillation chamber for collection of ammonia, the solution changed from orange to green colour. About 0.1 normal solution of hydrochloric acid (HCl) was weighed into burette. It was titrated until the colour changes from green to pink. The burette reading was taken. The crude protein was calculated using the formula:-

$$\%CP = \frac{(\mathrm{A}-\mathrm{B}) \times \mathrm{N} \times \mathrm{F} \times 6.25}{\text{mg of Samples}} \mathrm{X}\ 100$$

where A: mls of acid used for titrating the sample
B: ml of acid used for titrating blank sample (0)
N: Normallity of acid used for titration
F: Factor =14.007
6.25: is constant

Crude Fibre

Crude fibre was determined by weighing 2g of samples, it was placed in a round or flat bottom flask and 50ml of tri-chloroacetic acid reagent (T.C.A) was added the mixture was boiled and refluxed for 40minutes. The flask was removed and cooled to room temperature. Filter paper was used to filter the residue. The residue obtained was washed 4 times with hot water and once with petroleum ether then the filter paper and the sample were folded together and dried at $30^oC - 60^oC$ in an oven for 24hours. It was reweighed and then ashed at 650^oC and then cooled and reweighed again. Crude fibre was determined using the formula:

$$\%\text{CF} = \frac{\text{Difference in weighing}}{\text{Weight of sample on Dm basis}} \times 100$$

Ether Extract (Fat)

The ether extract was determined by using soxhlet apparatus, 1or 2g of the feed sample was weighed into a thimble and 200ml of petroleum ether was measured with measuring cylinder, the solution was put into round or flat bottom flask and was heated at 45^oC for 1-2hours. The collecting flask was removed, and cooled into dessicator for 15minutes and percentage fat sample was determined using the formula:-

$$\%Fat = \frac{Weight\ of\ fat}{Weight\ of\ sample} \times 100$$

Ash

Ash was determined 1 or 2g of sample was weighed into crucible and dried at 105^oC for 24hours, then cooled in the dessicator for 15minutes and re-weighed, it was then charred at 600^oC or 650^oC in muffle furnace for 2-3hours. Then cooled in desicator for 15minutes and re-weighed. It was determined using the formula:

$$\%ASH = \frac{Loss\ in\ weight}{Initial\ weight} \times 100$$

Nitrogen Free Extract

Nitrogen free extract was determined by computing indirectly by difference using the formula:

% N. F. E. =100 – (%CP + %CF + %EE + %ASH)

Carbohydrate

Percentage carbohydrate was determined by computing indirectly by difference using the formula:

%Carbohydrate = 100 – (%MC + %ASH + %CP + %CF)

Result

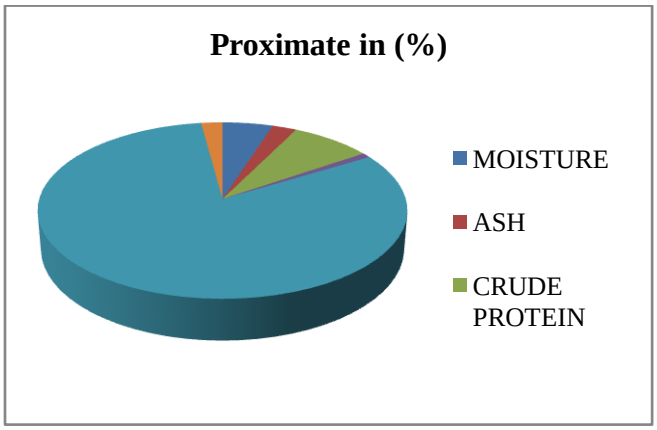
Table: 1. Result of phytochemical screening of *Tetracarpidium conophorum*

Constituent	Result
Alkaloids	-
Flavonoids	+
Cardia glycosides	+
Anthraquinones	-
Terpenoids	+
Tannins	+
Phlobatannins	+
Saoponins	-
Carbohydrates	+

Key: (+)= Present, (-) = Absent

Table 2: Result of Proximate Analysis of *Tetracarpidium conophorum*

Proximate	Contents (%)
Moisture	5.006
Ash	2.4106
Crude Protein	8.0746
Fat	0.8623
Carbohydrate	81.5315
Crude Fiber	2.1150



Discussion

Phytochemical evalution of this plant revealed the presence of some secondary metabolites such as tannins, phlobatannins, cardiac glycosides, flavonoids and Phenolic compounds, these compounds have been reported to serve as an antioxidant, and exhibit a wide range spectrum of medicinal properties such as anti-cancer, anti-inflammatory and anti-diabetes [22]. Many of the therapeutic actions of phytochemicals are ascribed to their biologically active polyphenol components, such as phenolic acids and flavonoids, which have potent antioxidant activities [23]. Earlier literature data have shown that many of these antioxidant compounds possess anti-inflammatory, cardioprotective antitumor, anticarcinogenic, hepatoprotective, and antibacterial activities to a greater or lesser extent [24-26]. However, alkaloids, saponins and anthraquinones were not detected in the plant.

The result of proximate analysis of walnut kernel revealed the presence of following; moisture 5.006%, Ash 2.4106%, protein 8.0746%, fat 0.8623%, crude fiber 2.1150% and carbohydrates 81.5315% respectively. The proximate composition of walnut seed is shown in Table 2. Protein content was found to be 8.0746%. The nuts are a good source of protein. Walnuts are high in protein food and are excellent addition to vegetarian diet. Plant proteins still remain a veritable source of food nutrient for the less privilege population in developing countries including Nigeria where cost of animal protein is beyond their per capital income [27]. Walnut seeds contain an appreciable amount of fat 0.8623%. Fats of walnut not only taste great but are rich source of healthy mono-saturated fats and an excellent source of omega-3-fatty acid [28]. Among the proximate analyzed the carbohydrate was found to be higher in percentage than all the compositions analyzed. However, protein and moisture were also having reasonable percentage. Nevertheless, the ash content was found to be 2.4106%. While the fat (0.8623%) content was very minute compared to the other contents analyzed.

Conclusion

This research shows that walnut kernel contain significant amount of bioactive compounds and phytonutrient as well. Amongst which flavonoids and tannins believed to have analgesic properties. Due to the reasonable amount of

carbohydrates and protein in this plant it may be used as a good source of energy given food to alleviate malnutrition in the world, especially in developing countries.

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References made published literatures have been duly acknowledged.

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