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Investigating The Phytochemical, Proximate, And Biochemical Composition Of Walnuts: Implications For Nutrition And Health Benefits.

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ABSTARCT

This study investigates the phytochemical composition and proximate analysis of walnut seed to encourage its consumption as a functional food. The presence of bioactive and nutritive compounds in the seed has made it a genuine source for good health conditions. Walnuts are a good source of dietary minerals. Mineral composition was determined in three walnut cultivars. Microelements such as Na, K, Ca, Fe, Cu and Zn were determined by using different techniques, Mineral element content presented significant differences from one cultivar to the other. Comparing the obtained data with the existing literature indicated that walnut cultivars proved to be important sources of nutritive elements, and walnut kernel consumption can contribute to a well-balanced diet.

Keywords: Phytochemical, proximate, walnut seeds, cultivar, Analysis

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INTRODUCTION

Walnuts are the oldest fruit cultivated in the world. The walnut tree yields high-quality wood and has a high nutritional value. Furthermore, due to their unique biochemical components, walnuts have great economic value as well as medical significance for human health.

For both humans and animals, plants are an excellent source of nutrients. For most of their dietary and therapeutic requirements, humans have relied on plants. Certain plant seeds are required to be included in diets due to their high calorific and nutritional qualities [1]. People have traditionally utilized plants as a means of treating illnesses and disorders [2]. In the 1990s, research on nutrition revealed that eating nuts frequently significantly reduced the risk of heart disease [3].

Walnut plant is a fine hardwood species in the family of *Juglandaceae* occurring in North America and South America, West India, Asia, Japan and Nigeria [4]. The walnut tree is a massive deciduous tree that grows to a height of 25 to 35 meters. Its broad crown and trunk can reach a diameter of up to 2 meters. It is a draught-demanding species that needs full sun to flourish [5]. It is economic plant widely cultivated to produce nuts and is used as delicacies [6].

The walnut tree produces a meaty green drupe that contains nuts. The nut's corrugated wood shell shields the kernel from harm. The huge seed has a comparatively thin shell and a rich flavor when eaten.[7]. Due to their low saturated fat level, walnut seeds are well known for their high omega-3 fatty acid content, which helps to maintain a healthy heart. It is possible that the seeds will improve memory, cognitive function, and overall brain health. Decreases in cognitive function and depression have been related to low consumption of omega-3 fatty acids [8]. A hormone that helps the body control sleep, melatonin, is found in walnut seeds. Strongly combating free radicals, which are the cause of cancer cell formation, is melatonin, an antioxidant [9]. Walnut seeds also contain allergic acids that neutralize cancer causing substances [10]. Due to its ability to lower bad cholesterol, consuming walnut seeds may therefore assist to prevent the growth of cancer cells, heart disease, and high blood pressure [11].

Preparation of sample

The collected seeds were cleaned, air dried and carefully ground into a coarse form using a mechanical blender of sample. The dehulled samples were dried and ground to a fine powder. 2 g of the powdered sample was defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 hours.

MATERIALS AND METHODS

Various Techniques were used to analyse walnut samples. Colorimeter was used to determine the amount of iron, flame emission spectrophotometer was used to determine the composition of sodium & potassium, other metals copper and zinc were determined by atomic absorption spectrophotometer and finally the collected samples were analysed for major physicochemical parameters and phytochemical components. Total carbohydrates and total proteins of walnut samples were determined by anthrone method and biuret method respectively using a UV-spectrophotometer.

Qualitative Phytochemical Analysis

Test for phenol: 2 mL of the sample solution was mixed with few drops of 10 % ferric chloride solution. The formation of green-blue or violet or blue-black coloration was an indication of a positive result.

Test for flavonoids: 2 ml of 10 % sodium hydroxide was added to 2 ml of the sample solution in a test tube. A yellow colour was formed which turned colourless upon addition of 2 ml of diluted hydrochloric acid indicating positive result.

Test for Tannins: 5 drops of 0.1 % ferric chloride were added to 2 ml of the sample solution. Formation of a brownish green or blue-black coloration indicated a positive result.

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Test for Saponins: 2 ml of sample solution was diluted with 2 ml distilled water. It was then agitated in a test tube for 5 minutes. 0.1 cm layer of foam indicated a positive result.

Test for Phlobotanins: 2 ml of the sample was boiled with 1 % aqueous hydrochloride. Deposition of a red precipitate indicated a positive result.

Test for Alkaloids: 2 ml of the sample solution, 2 ml of 10 % hydrochloric acid was added. To the resulting solution containing the acid, 1 ml Hager's reagent (Saturated picric acid solution) was added. Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

Test for Steroids: 2 ml of the sample solution was dissolved in 10 ml of chloroform and then 10 ml of concentrated sulphuric acid was added from the walls of the test tube. The upper layer turned red whereas, the sulphuric acid layer turned yellow with green fluorescence.

Test for Terpenoids: 2 ml of the sample solution was mixed with 2 ml of chloroform and 1 ml of concentrated sulphuric acid was carefully added to form a layer. Clear upper and lower layers with a reddish-brown interphase indicated a positive result.

Test for Glycosides: 2 ml of acetic acid was added to 2 ml of the sample solution. The mixture was cooled in a cold-water bath. 2 ml of concentrated sulphuric acid was added. Colour advancement from yellow to yellowish green shows the nearness of glycosides.

10.Test for Anthraquinones: 2 ml of the sample solution was boiled with 5 ml of 10 % hydrochloric acid for 3 minutes. 5 ml of chloroform was added. 5 drops of 10 % ammonia were further added. A rose-pink coloration indicated a positive result.

Alkaloids Determination

5 grams of the sample was weighed into 250 ml beaker and 200 ml of 20 % acetic acid in ethanol was added and covered to stand for 4 hours. This was filtered and the extract was concentrated to one quarter of the original volume. Then concentrated ammonium hydroxide was added drop wise to the extract to precipitate the alkaloid. This was done until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected by filtration, dried and re-weighed. The percentage alkaloid was calculated as the difference in weight [12-13].

$$\frac{W2 - W1}{W} \times 100$$

W= weight of the sample
W1= weight of empty filter paper
W2= weight of sample along with empty filter paper

Flavonoids Determination

Ten grams of the test sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to constant weight [14].

$$\% \text{ Flavonoid} = \frac{\text{Weight of dried sample}}{\text{Weight of sample}} \times 100$$

Total Phenols

The extraction of the phenolic content: The fat free sample was boiled with 50 ml of ether in 15 min. 5 ml of the extract was pipetted into a 50 ml volumetric flask. Then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of amyl alcohol were also added. The mixture was made up to mark and

left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at a wavelength of 505 nm [12].

Proximate Analysis of Walnut

This was carried out according to the method of A.O.A.C. [16].

Determination of Moisture Content

Two grams of the sample was weighed into a dried weighted crucible. The sample taken in a crucible was put into a moisture extraction oven at 105°C and heated for 3 hours. The dried sample was put into a desiccator and allowed to cool and reweighed. The process was repeated until constant weight was obtained. The difference in weight was calculated as the percentage moisture content.

Ash Content Determination

Two grams of the sample was weighed into a crucible heated in a moisture extraction oven for 3 hours at 100 ^{o}C before being transferred into a muffle furnace at 550^{o}C until it turned white and free of carbon. The sample was then removed from the furnace and cooled in a desiccator to room temperature and reweighed immediately. The weight of the residual ash was then calculated as Ash content.

$$\% \text{ Ash content} = \frac{\text{Weight of Ash}}{\text{Weight of original sample}} \times 100$$

Fat Content Determination

Two grams of the sample was loosely wrapped with a filter paper and put into a thimble which was fitted to a dried weighed clean round bottom flask. The flask contained 120 ml of petroleum ether. The sample was heated with heating mantle and allowed to reflux for 5 hours. The heating was then stopped and later weighed. The difference in weight was taken as the mass of fat and expressed as percentage fat content of the sample.

$$\% \text{ Fat or Oil content} = \frac{W2 - W1}{W3} \times 100$$

W1 = Weight of the empty extraction flask
W2 = Weight of the flask and oil extracted
W3 = Weight of the sample

Minerals

Metal composition was measured by making ash of the samples. Ash was then dissolved in acid and digested for some time and then filtered and diluted to the mark in volumetric flask. This sample solution was used for the measurement of trace elements [17-20].

Determination of total Carbohydrates

Carbohydrates are dehydrated by conc.H2SO4 to form furfural; furfural condenses with Anthrone to form a blue colored complex, which is measured calorimetrically at 620 nm.

Determination of total Proteins

Bovine Serum Albumin (BSA)is treated with specific biuret reagent and the colour developed is measured at 540nm in a photoelectric colorimeter. Biuret is given by those substances whose molecules contain at least two carbonyl groups joined directly/through a single atom of nitrogen/carbon. i.e amino acids and dipeptides do not show positive biuret reaction but tripeptides which contain at least two carbonyl groups and other peptides give

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a positive biuret reaction. Protein responds positively due to the formation of Cu-Co-ordination complexes with their peptide bonds.

RESULTS

The collected samples were analysed for phytochemical characteristics and quantitative estimation of metals.

Table 1: Metal composition of walnut samples

Metal	Type of sample		
	Sample-1	Sample-2	Sample-3
Sodium (ppm)	17	13	12
Potassium (ppm)	2.16	1.8	1.2
Calcium (ppm)	6.04	4.8	3.88
Iron (ppm)	0.61	0.43	0.46
Zinc (ppm)	0.025	0.55	0.83
Copper (ppm)	0.00	0.062	0.062

Table 2: Qualitative Phytochemical components result of walnut

Phytochemical component	Type of sample		
	Sample-1	Sample-2	Sample-3
Phenol	+	+	+
Flavonoids	+	+	+
Tannins	-	-	-
Saponins	-	-	-
Phlobotanins	-	-	-
Alkaloids	+	+	+
Steroids	+	+	+
Terpenoids	-	-	-
Glycosides	-	-	-
Anthraquinones	-	-	-

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

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Table 3: Quantitative Phytochemical components result of walnut

Phytochemical component	Type of sample		
	Sample-1	Sample-2	Sample-3
Phenols	1.42	1.87	1.72
Flavonoids	1.45	1.56	1.64
Saponins	0.89	1.09	1.19
Steroids	1.43	1.34	1.04
Alkaloids	1.67	1.27	1.04

Table :4. Proximate Composition of walnut

Proximate Composition	Type of sample		
	Sample-1	Sample-2	Sample-3
Moisture	22.63	32.54	48.57
Ash	2.68	2.06	2.54
Total Carbohydrates (ppm)	20.65	21.14	28.20
Total Proteins (ppm)	10.42	12.07	10.58
Fiber	4.62	4.98	3.58
Fat	3.88	4.59	4.05

DISCUSSION

Plants have become a source of important bioactive compounds that are beneficial to human and animal, through their ability to affect metabolic and physiological activity. According to a research article published by Hayes et al, it was observed that Due to their purported cytotoxic qualities, walnuts' polyphenol and other phytochemical content makes them a desirable study subject for preventing free radical-induced nucleic acid damage [21]. In the current research it was identified that in addition to polyphenols, a very sizable number of phytochemicals were identified, which have potential medicinal properties. In an article published in international journal of environmental research and public health, mineral composition of walnuts and oils were published by Juranović et al. as per their studies, explained the beneficial effects of macro and micronutrients in walnuts [22]. In the current study many trace elements and their composition were identified and duly reported, specific methodologies, including the use of atomic absorption spectrophotometry was suggested in the evaluation of the mineral composition and their potential benefits.

CONCLUSION

According to this study, there are a significant number of phytochemicals and phytonutrients in walnut seeds. The proximate analysis reveals a high protein and carbohydrate content. These strongly back the plant's reported anti-cancer and cardio-protective properties. Consumption of the walnut seeds will help to relieve the trauma and pains of people suffering from these debilitating ailments.

According to this study, walnuts are a significant source of phytonutrients and bioactive substances. They include tannins and flavonoids that are thought to have analgesic effects. Flavonoids and tannins are

among those thought to have analgesic effects. 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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