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Biochemical Characterisation of Selected Edible Nut Seeds

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ABSTRACT

The present study was focused to evaluate the phytochemical screening and biochemical estimation of ethanolic and aqueous extracts of edible nuts of *Arachis hypogea, Anacardium occidentale, Prunus dulcis and Juglans regia*. The phytochemical screening showed the presence of carbohydrate, protein, phenol and tannin, flavonoid, glycoside, steroid, alkaloid in all the studied nuts. The quantification of *A. hypogeae, A. occidentale, P. dulcis and J. regia* were performed to determine the total amount of carbohydrate, protein and phenol. The highest amount of Carbohydrate was present in *A. occidentale* (244.7 ± 6.3 mg GLC/g), The Protein content was highly present in *J. regia* (455.14 ± 3.7 mg BSA/g) and the highest amount of Phenol was present in *P. dulcis* (355.78 ± 7.2 mg GAE/g).

KEYWORDS: Human dietary, nutritional rich food, anti-oxidant, phytochemical constituents.

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INTRODUCTION

Nuts are described as a valuable commercial product among the food commodity, consisting of high-water content, sweet and sour. Nuts along with vegetables called "Protective food". They are considered as little factories of good health which is called as Powerhouse of energy or a complete package type of food[1]. Nuts are fruits, consists of a single seed, a husk or a pod, covered with a indehiscent outer layer which protects the edible part kernel, provides nutrients for humans and wildlife. Nuts are a rich source of protein, lipids, dietary fiber, vitamin E, vitamin B2, folate, monosaturated fatty acids and unsaturated fatty acids compared to saturated fatty acids[2]. Consumption of nuts is also due to the presence of organoleptic chemical property[1]. They supply various nutrition which plays an essential role in human nutrition thus maintaining in our human health. Nuts has a beneficial effect on human health such as proper regulation of blood cholesterol, supports heart health, high in fat, prevents problems in digestion, helps in preventing cancer, anemia, chronic inflammation and dehydration, helps in weight loss, effective in treating mental disorders. Phytochemical compounds enable the biomolecular function and analysis of phytochemicals access the presence of chemical compounds in a determined proportion.

Arachis hypogea Linn. (Ground nut) belongs to the family Fabaceae, Peanuts, unlike other legumes have symbiotic nitrogen – fixing bacteria in their root nodules, called "The king of Oil Seeds". Daily consumption of ground nut helps in reducing bad cholesterol, improves heart health and also a rich source in omega 3 fatty acids, vitamin E. *Anacardium occidentale* Linn. (Cashew nut) belongs to the family Anacardiaceae, cashews consist of 30% carbohydrates, 44% fat and 18% protein. They are rich sources of copper, manganese, phosphorus, and magnesium vitamin K. Cashews helps in treating diabetes, gastro intestinal ailments and skin problems. *Prunus dulcis* (Mill.). D(Almond) belongs to the family Rosaceae, Almonds have 22% carbohydrate, 21% protein, and 50% fat. It contains vitamin B and E, calcium, copper, iron, magnesium, MUFA and PUFA and also increases the brain power[3]. Almonds increases the anti-oxidant, reduces the risk of heart disease and reduces blood pressure. *Juglans regia* Linn. (Walnut) belongs to the family Juglandaceae,15% Protein, 65% fat, and 14% carbohydrates, walnuts are rich in anti- oxidant and a high quantity of PUFA[4] and alpha- linolenic acid.

MATERIALS AND METHODS

Plant Material

The selected nuts of*Arachis hypogea* (Groundnut), *Anacardium occidentale* (Cashew nut), *Prunus dulcis* (Almond) and *Juglans regia* (Walnut) were collected in and around local market from Coimbatore district.

Preparation of nuts sample

Kernels of *A. hypogea, A. occidentale, P. dulcis and J. regia* were removed from their pods and roasted for a few minutes before grind into fine powder and stored in an airtight container for further studies.

Phytochemical Screening

Methods of Extraction

The powdered samples of *A. hypogea, A. occidentale, P. dulcis and J. regia* were measured and weighed for 15 grams then introduced with 100mL of ethanol and hot water extraction were carried out by shaker for 72 hours. The extract was stored in refrigerator at 4˚C for further studies. The extracts of selected samples were filtered and then tested for protein, phenol, flavonoid, alkaloid, tannin, saponin, terpenoid, carbohydrate, steroid, glycoside. The phytochemical screening of the extracts was carried out by following the method[9]

Test for Carbohydrate

Molisch's Test

Crude extract was mixed with 2mL of Molisch's reagent and the mixture was shaken vigorously. After that, 2mL of Concentrated H_2SO_4 was poured carefully along the sides of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

Test for Protein

Ninhydrin Test

Crude extract was boiled with 2mL of 0.2% Solution of Ninhydrin, Violet colour appeared suggesting the presence of amino acids and proteins.

Test for Phenols and tannin

Crude extract was mixed with 2mL of 2% solution of $FeCl_3$. A blue - green or black coloration indicated the presence of phenols and tannin.

Test for Flavonoid

Alkaline reagent Test

Crude extract was mixed with 2mL of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoid.

Test for Saponin

Crude extract was mixed with 5mL of distilled water in a test tube and it was shaken vigorously. The formation of stable foam indicated the presence of saponin.

Test for Glycoside

Liebermann's Test

Crude extract was mixed with each of 2mL of Chloroform and acetic acid. The mixture was cooled in ice. Concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Test for Steroid

Crude extract was mixed with 2mL of Chloroform and concentrated H_2SO_4 was added to the side of the test tube. A red colour produced in the lower chloroform layer indicated the presence of steroid. Another test was performed by mixing crude extract with 2mL of chloroform. Then 2mL of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish colour indicated the presence of steroid.

Test for Terpenoid

Crude extract was dissolved in 2mL of Chloroform and evaporated to dryness. To this 2mL concentrated H_2SO_4 was added and heated for about 2 minutes. A greyish colour indicated the presence of terpenoid.

Test for alkaloid

Crude extract was mixed with 2mL of 1%HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate confirmed the presence of alkaloid.

BIOCHEMICAL ESTIMATION

Estimation of Carbohydrate by the Anthrone Method

Anthrone reagent

200 mg of Anthrone was weighed and dissolved in 100 mL of ice-cold 95% of H_2SO_4.

Stock Solution

100 mg of Glucose dissolved in 100 mL of distilled water.

Working solution

10mL of stock solution was dissolved in 100mL of distilled water.

Procedure

100 mg of sample was weighed, hydrolyzed with 10mL of 2.5 N of HCL by using a motor and pestle. The mixture was transferred into boiling test tube at room temperature of 100˚ C for 3 hours and a pinch of Sodium carbonate was added until the effervescence ceases. The Sample mixture was made up to 50 mL and centrifuged for 15 minutes. The standards were prepared by 50, 100, 150, 200, 250 µL of working standard solution and '0' serves as blank. 1 mL of aliquots serves as unknown. The volume was made to 1mL in all the tubes including the sample tube by adding distilled water followed by 4 mL of Anthrone reagent in all tubes heated and for 10-15 minutes in boiling water bath. Then it was cooled rapidly and read absorbance and observed the colour change from green to dark green colour at 630nm by using spectrophotometer A standard graph was drawn by plotting the concentration of the standard on the X-axis on the Y-axis. From the graph the amount of carbohydrate present in the sample tube was calculated[7].

Estimation of Protein by Lowry's Method

Reagent A

2% of sodium carbonate in 0.1N NaOH.

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Reagent B

0.1% of copper sulphate solution in 1 % of Sodium potassium tartrate solution.

Reagent C

50mL of Reagent A was mixed with 1mL of Reagent B were mixed.

Reagent D

Equal volume of Folin – Ciocalteu reagent and distilled water was taken in equal volume.

Buffer (Phosphate buffer)

Solution A – 0.2 M solution of Monobasic sodium phosphate.

Solution B – 0.2 M Solution of dibasic sodium phosphate.

Stock solution

100 mg of BSA (Bovine serum albumin) was dissolved in 100 mL of distilled water.

Working standard solution

10 mL of stock solution was diluted in 100 mL of distilled water in a standard flask.

Procedure

100 mg of sample was weighed and grinded well in 5-10 mL of Phosphate buffer. The mixture was centrifuged for 15 minutes. The working standard of 50, 100, 150, 200, 250 µL were pipetted out into a series of test tubes and 1 mL of aliquots serves as unknown. The volume is made up to 1mL in all the tubes and a test tube with the 1mL serves as blank. 4 mL of reagent C was added to each test tube including the blank and mixed well and allowed to stand for 10 minutes. 0.5mL of reagent D was added to all the test tube mixed well and incubated at room temperature in the dark for 30 minutes. Blue colour change was observed and the readings were taken at 660 nm. A standard graph was plotted and the amount of protein present in the sample is calculated7.

Estimation of Phenol

Stock solution

100 mg of Gallic acid was dissolved in 100 mL of distilled water.

Working standard solution

10 mL of stock solution was taken and dissolved in 100 mL of distilled water.

Sodium carbonate solution

3.5 % of sodium carbonate was dissolved in 100 mL of distilled water.

Folin- Ciocalteu reagent

Folin Ciocalteu Reagent and distilled water was taken in equal volume.

Procedure

1g of sample was dissolved in 10 mL of Methanol and centrifuged for 15 minutes 1 mL of aliquots was used as unknown of the working standard of 50, 100, 150, 200, 250 µL was pipetted out in all the test tubes with '0' serves as blank. The volume was made to 1mL in all the test tubes including the sample tube by adding distilled water. 5 mL of FC reagent was added in all test tubes followed by 5 ml of Sodium carbonate solution was added in all the tubes and incubated in dark room for 40 minutes. Blue colour was indicated the presence of phenol and the values were taken in spectrophotometer at 725 nm A standard graph was plotted and the amount of protein in the sample was calculated[7].

RESULTS AND DISCUSSION

Phytochemical screening of nut samples *Arachis hypogea, Anacardium occidentale, Prunus dulcis and Juglans regia.*

The qualitative preiliminary phytochemical analysis of ethanolic and hot water extraction in dried samples of *Arachis hypogea, Anacardium occidentale, Prunus dulcis and Juglans regia* were performed to confirm the presence of secondary metabolites. The results revealed that the carbohydrate, phenol, tannin, flavonoid and alkaloids were highly in all nuts studied, whereas saponin and terpenoids were absent in all the nut samples. Similarly, the phytochemical contents such as saponins, tannins, steroid, alkaloids & flavonoids of ground nut varieties are shown in proximate composition mg/100g in bar graph. Thus, the results showed that all nut varieties are rich in carbohydrate, protein[4]

Table 1. Showing the Phytochemical screening of ethanolic and hot water extracts of *Arachis hypogea, Anacardium occidentale, Prunus dulcis and Juglans regia.*

Sl.No.	Phytochemicals constituents	*Arachis hypogea*		*Anacardium occidentale*		*Prunus dulcis*		*Juglans regia*	
		Ethanol extract	Water extract	Ethanol extract	Water extract	Ethanol extract	Water extract	Ethanol extract	Water extract
1.	Carbohydrate	+++	+++	_	_	++	+	+++	+++
2.	Protein	_	_	+	+++	+	+	+++	+++
3.	Phenol and tannin	+++	+++	+	+++	++	+	+	+++
4.	Flavonoid	++	+++	++	+	+	+++	++	+++
5.	Saponin	_	_	_	_	_	_	_	_

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6.	**Glycoside**	+	_	_	_	_	+++	_	+
7.	**Steroid**	_	++	+	_	_	+++	+	+++
8.	**Terpenoid**	_	_	_	_	_	_	_	_
9.	**Alkaloid**	++	+++	++	+++	+++	+++	+	_

(+++) – highly present ; (++) – moderately present ; (+) – low ; (-) – Absent

Biochemical Estimation of nut samples *Arachis hypogea, Anacardium occidentale, Prunus dulcis* and *Juglans regia.*

Biochemical estimation of carbohydrate, protein and phenol of *Arachis hypogea, Anacardium occidentale, Prunus dulcis* and *Juglans regia* were determined. The highest amount of Carbohydrate was present in *Anacardium occidentale* **(244.7 ± 6.3 mg GLC/g)** when compared to *Arachis hypogeae* (228.57 ± 3.7 mg GLC/g), *Prunus dulcis* (22.09 ± 1.3 mg GLC/g) and *Juglans regia* (36.7 ± 2.3 mg GLC/g). The highest amount of protein was present in *Juglans regia***(455.14 ± 3.7 mg BSA/g)** when compared to *Arachis hypogea* (14.2 ± 0.9 mg BSA/g), *Anacardium occidentale* (311.07 ± 5.7 mg BSA/g), *Prunus dulcis* (17.78 ± 0.6 mg BSA/g). The highest amount of phenol was present in *Prunus dulcis* **(355.78 ± 7.2 mg GAE/g)** when compared to *Arachis hypogea* (28.04 ± 1.0 mg GAE/g) *Anacardium occidentale* (14.75 ± 0.6 mg GAE/g) and *Juglans regia* (104.30 ± 2.1 mg GAE/g). Similarly, the phytochemical characterization of *A. hypogea* extract and they suggested that peanut consumption is safe for healthy uses, such as nutrition and phytomedicine[2].The carbohydrate and protein in nut sample as Ground nut, Cashew nut, Pista & Almond by using DNSA method. Regular nut consumption is an indispensable component of any healthy, plant-based dietary pattern[6].

Table 2 : Showing the total amount of carbohydrate, protein and phenol present in the dried sample of *Arachis hypogea, Anacardium occidentale, Prunus dulcis* and *Juglans regia.*

Biochemical constituents	***Arachis hypogea***	***Anacardium occidentale***	***Prunus dulcis***	***Juglans regia***
Carbohydrate(mg GLC/g)	228.57 ± 3.7	**244.7 ± 6.3**	22.09 ± 1.3	36.7 ± 2.3
Protein (mg BSA/g)	14.2 ± 0.9	311.07 ± 5.7	17.78 ± 0.6	**455.14 ± 3.7**
Phenol (mg GAE/g)	28.04 ± 1.0	14.75 ± 0.6	**355.78 ± 7.2**	104.30 ± 2.1

GLC- Glucose, BSA- Bovine serum albumin, GAE- Gallic acid equivalents

CONCLUSION

Nuts play a role in wide range of supplying the source of nutrients and energy which are responsible for our dietary health also nourishes the metabolism in human body. Hence the present study was focused to determine the phytochemical constituent and to compare the biochemical composition of *Arachis hypogea, Anacardium occidentale, Prunus dulcis* and *Juglans regia.* All the tested edible nuts have remarkable presence of secondary metabolites involved in several biological activities. Highest amount of carbohydrate, protein and phenol was observed in all the studied nuts. Hence, the present study is useful for human nutrition and consuming nuts on the regular basis is strongly recommended for well-balanced diet for obtaining key nutrients for maintaining our balanced diet.

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