



ORIGINAL RESEARCH ARTICLE

Nutritional analysis of nuts extract of *Juglans regia* L.Raja G.^{1*}, Ivvala Anand Shaker¹, Inampudi Sailaja², Swaminathan R.³, Kondaveeti Suresh Babu¹ and S. Saleem Basha¹¹Department of Biochemistry, Melmaruvathur Adhiparasakthi Institute of Medical Science, Melmaruvathur-603319, Tamil Nadu, India.²Department of Botany, PVP College of Arts, Science and Commerce, Pravaranagar at / P-Loni (kd) 413731, Tal-Rahata, Dist Ahmednagar, Maharashtra, India.³Parthys Reverse Informatics Analytic Solution (P.) LTD., Taramani, Chennai -13, Tamilnadu, India.

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Abstract: *Juglans regia* L. (Walnut) is the most widespread tree nut in the world. Nuts are used by mankind for food, edible oils, spices, condiments or beverages. They have been an important food source from prehistoric times and are among the most nutritionally concentrated of human foods, high in protein, oil, energy, minerals and vitamins. Nuts that are only rarely used as famine food have been excluded from this present study partly because of the paucity information available but mainly because they are not normally considered edible. Nuts used solely for spices or condiments have also been largely excluded since they are used sparingly, to flavor food and not as a food; traditionally they are considered separately from edible nuts. Nuts that are largely used as commercial sources of edible oil are not discussed in any great detail since they are already adequately dealt with in the literature. Present study is an attempt to determine the amount of biomolecules, mineral and vitamins present in nuts extract of *Juglans regia* L. The study was set up to determine the quantity of biomolecules, mineral and vitamins present in nuts extract of *Juglans regia* L. using various analytical models. Total carbohydrates, total protein, alkaloids, phosphorus, Iron, vitamin C, E and catalase activity of nuts extract of *Juglans regia* L. were determined using various biochemical experimental models. The nutritional analyses of nuts extract of *Juglans regia* L. were determined in different experimental models and it was observed that presence of the test compounds in all the models.

Keywords: *Juglans regia* L.; Tocopherol; Ascorbic Acid; Catalase.

Introduction

In recent years, there is gradual increase of the importance of a healthy diet, nutritional composition and fruit quality because of increasingly aware of health promoting components of foods by consumers [1]. The 'hidden hunger' caused by nutrient imbalance is an invisible threat to human health. Currently, over three billion people are micronutrient malnourished [2]. Since 20th century, it has reached broad consensus that rich nutrition and balanced food by cultivating and modifying crops will do good to human health [3]. The phenomenal growth that occurs in adolescence, second only to that in the first year of life, creates increased demands for energy and nutrients. Total nutrient needs are higher during adolescence than any other time in the lifecycle. Nutrition and physical growth are integrally related; optimal nutrition is a requisite for achieving full growth potential. Failure to consume an adequate diet at this time can result in delayed sexual maturation and can arrest or slow linear growth [4]. Nutrition is also important during this time to help prevent adult diet-related chronic diseases, such as cardiovascular disease, cancer and osteoporosis.

Juglans regia L. (walnut) is a very popular nut fruit in India from the genus Juglandaceae. It is traditionally cultivated both for fruit and high quality timber production. It is native in a region stretching from the Balkans eastward to the Himalayas and southwest China. Among dietary plants used worldwide, *Juglans regia* L. were ranked second only to *Rosa canina* in their antioxidant activity [5]. Nuts are a main source of unsaturated fatty acids, vitamin E, fiber, magnesium and potassium [6]. The health benefits of these nuts are usually attributed to their chemical composition. Walnuts are good sources of essential fatty acids (linoleic acid is its major fatty acid), tocopherols and tocotrienols, proteins, fibers, melatonin, sterols, folate, tannins and other polyphenols [7]. Among the nuts, which contain mostly monounsaturated fatty acids, walnuts are considerably rich in omega-6 and omega-3 polyunsaturated fatty acids [8]. The objective of this study was the composition of total carbohydrate, total protein, alkaloids, and minerals of phosphorus, iron content and vitamin E and C and catalase activity of nuts extract of *Juglans regia* L. were determined using various test models.

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Materials and Methods

Collection and Preparation of sample extracts:

J.regia L. Nuts with shell were obtained from the local market. The Plant nuts were identified by the Professor of Department of Botany and Sample specimen was voucher. The shells were peeled-off and broken, kernels were shade dried for 4-6 weeks, and minced and powdered in mixer grinder in a dry manner and sieved twice to obtain fine powdered. 100 gm of dried powder of nut of *Juglans regia L.* were extracted with Soxhlet extractor using aqueous solution. The extract was dried till constant weight was obtained. This residue was boiled in water bath for 5 minutes, cooled and centrifuged at 4000 rpm for 10 minutes. The clear supernatant was used for evaluating in various quantitative assays.

Estimation of Total starch: The content of starch was determined using anthrone method by E.W. Yemm *et al.*, 1954[9]. Anthrone reagent was prepared by adding 200mg of anthrone dissolved in 100ml of concentrated sulphuric acid and stored in 4°C. 0.01ml aliquot of various concentrations of (50 -500µg) nuts extract, made up to 1ml with distilled water and 3ml of anthrone reagent. Boiled for 10 minutes and blue color developed was read at 620nm. The same procedure was carried out for glucose standard and blank. All determinations were carried out in triplicate. The content of starch was calculated in terms of mg %.

Estimation of Total protein: The content of protein was determined by Lowry *et al.*, 1951[10]. The method containing alkaline copper solution which was prepared by adding 2% of sodium carbonate in 0.1N sodium hydroxide (Solution A), 0.5% of copper sulphate in water (Solution B) 1% of sodium potassium tartarate in water (Solution C). 50ml of solution A is mixed with 0.5 mL of solution B and 1 mL of solution C just before use. Another reagent the commercially available Folin-Ciocalteu reagent was diluted to 1:2 ratios with distilled water prior to use. BSA serve as a standard solution, 100 mg of crystalline BSA was dissolved in 100 mL of distilled water. 0.5ml aliquot of various concentrations of (100 -500µg) nuts extract, made up to 1ml with distilled water and 4.5 mL of alkaline copper reagent. The content was left to stand for 10 minutes at room temperature 0.5mL of diluted Folin-Ciocalteu reagent was added. After the 20 minutes of incubation the absorbance of blue color development was read at 640 nm. The same procedure was carried out for BSA standard and blank. All determinations were carried out in triplicate. The content of protein was calculated in terms of mg %.

Estimation of alkaloids: The alkaloids content was determined by the method Harborne J.B 1992

[11]. To 5 g of the sample in 250 mL beaker, 200 mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed. The content of alkaloids was calculated in terms of g %.

Estimation of iron: The iron content was determined by using the W.N.M. RAMSAY 1952 method [9]. The method containing 2, 2'-Dipyridyl reagent, which were prepared by 100 mg of 2, 2'-dipyridyl dissolved in 3% glacial acetic acid and made up 100ml with water. Ferrous sulphate serve as a standard solution, 50mg of ferrous sulphate, 1 drop of concentrated sulphuric acid and the solution made up to 100 mL using distilled water. 0.05ml aliquot of various concentrations of (50-500µg) nuts extract, made up to 2 ml with distilled water. The contents were heated on the boiling water for 5 minutes. The absorbance of pink color development was read at 520 nm. The same procedure was carried out for standard and blank. All determinations were carried out in triplicate. The values are expressed as mg %.

Estimation of phosphorus: The phosphorus content was determined by using Fiske-Subbarow method 1925 [12]. The method contains molybdate I and II and ANSA reagent. Molybdate I was prepared by 5 g of ammonium molybdate is dissolved in 40mL of distilled water. To this 100mL of 10 N sulphuric acid added and made up to 200mL using deionized water. Molybdate II was prepared by 5mg of ammonium molybdate is dissolved in 4mL of distilled water and the content is transferred to 60 mL of 10 N sulphuric acid. 1, 2, 4 amino naphthol sulphonic acid [ANSA] reagent prepared by 0.25 g of ANSA is added to 98 mL of 15% sodium bisulphite and 2.5mL of 20% sodium sulphite. The content is stored in brown bottle at 4°C. Standard phosphorus solution was prepared by 35 mg of potassium dihydrogen orthophosphate in deionized distilled water in 100mL add 1mL of concentrated sulphuric acid. 0.05ml aliquot of various concentrations of (80-800µg) nuts extract, made up to 7ml with distilled water. 1mL of molybdic acid-I in all the standard tubes including blank expect test tube and 0.4 mL of ANSA in all the tubes added. The test tubes add 1mL of molybdic acid-II was added. A blank containing 7.0mL of deionized water and standard containing aliquot of potassium dihydrogen orthophosphate and the contents were left to stand for 10 minutes at room temperature. The absorbance of blue color

development was read at 670nm. All determinations were carried out in triplicate. The phosphorus content values calculated in terms of mg %.

Estimation of Vitamin C: The ascorbic acid content was determined by ROE, J. H. *et al.*, 1943[14]. To this method 2, 4-dinitrophenyl hydrazine [DNPH] reagent was prepared by 2 g of DNPH is dissolved in 100mL of 9N sulphuric acid. 6% (W/V) trichloro acetic acid used for protein precipitation. 0.05ml aliquot of various concentrations of (20 - 200 μ g) nuts extract, made up to 2.5 ml with distilled water. 0.5mL of 10% thiourea and 2, 4-DNPH reagent. The content was left to incubate for 3 hours at 37°C. Then 2.5 mL of 85% Sulphuric acid was added and cooled and the color development was read at 540 nm. The same procedure was carried out for standard and blank. The ascorbic acid content calculated in terms of mg⁰%.

Determination of Vitamin-E: The Vitamin-E content was determined by Barker H. *et al.*, 1968 [15]. To test tubes marked S1 – S5, 0.5 – 2.5 mL of standard tocopherol solution of concentration 0.5 – 2.5 mg is added. 1.5mL of the extract taken in tube T along with a duplicate. 3.5mL of distilled water is taken as the blank (B) and the total volume in all the test tubes is made up to 3.5mL with the same. 1.5mL of xylene is added to all the tubes followed by 1mL of 2, 2' - dipyridyl reagent, mixed well and the absorbance of the test and standard are read against the blank at 460 nm. 0.3 mL of ferric chloride solution is added all the tubes and again the absorbance is read at 520 nm. All determinations were carried out in triplicate and the values expressed as mg %.

Determination of activity of catalase: The catalase activity was determined by Sinha A.K. 1972 [16]. The method consists of 0.02 M phosphate buffer [pH 7] which was prepared by 3.12 mg of sodium dihydrogen orthophosphate in 100 mL of distilled water (Solution A), 282 mg of disodium hydrogen orthophosphate in 100 mL of distilled water (Solution B). 39 mL of solution A and 61mL of solution B are mixed and pH is adjusted to 7. Dichromate acetic acid reagent was prepared by 350mg of potassium dichromate in 7mL of distilled water to this solution and 21 mL of acetic acid added. This stock solution was diluted to 1:5 ratio using distilled water. Hypotonic phosphate buffer was prepared by 0.4g of sodium chloride in 100 mL of phosphate buffer. Hydrogen peroxide standard solution (6.8 mg/mL) prepared by 2.2 mL of 30% hydrogen peroxide is dissolved in 100 mL of distilled water. An aliquot of the sample was made up to 5.0 mL with distilled water and 1.0 mL of phosphate buffer and 2.0 mL of potassium dichromate acetic acid solution in all the tube. A blank containing 5.0mL of water and

standard containing aliquots of hydrogen peroxide solution were also treated similarly. The contents were left to stand for 10 minutes in boiling water bath. The absorbance of blue color developed was read at 620 nm. The catalase activity was determined by different tube marked as blank and test tubes. All the tubes are made up to 5.0mL with water, and 1.0mL of phosphate buffer were added to blank tube, and 2mL of dichromate in all the tubes were added to blank The 0.5mL of extract was added only in test tubes and added 0.5mL hydrogen peroxide was added. 0, 15, 30 and 60 seconds interval were added dichromate solution and all the tubes are boiled for 5 minutes and cooled. The absorbance of blue color developed was read at 620nm. The values are expressed as mL of hydrogen peroxide utilized/minute.

Results and Discussion

The nutritional compositions of nut extract of *Juglans regia L.* were shown in table.1. *Juglans regia L.* nuts appear to be a fair source of carbohydrates and proteins. They serve as good sources of iron, phosphorus, vitamin C and E and minor amount of alkaloid has also been quantified. The total carbohydrates were 13.7 % of nut extract. The low value carbohydrate is useful for the dietary source of diabetic patients. It has been proved walnut have good source of fiber, reported total dietary fiber contents ranging from 31 to 52 g kg⁻¹ kernel (dry matter) in walnut cultivars from New Zealand [17]. The total protein content was 15.2 % and Walnut flour may be obtained from kernel press-cake. It provides appreciable amounts of proteins (450 g kg⁻¹ on average). They are mainly composed of glutelins (about 70% of the total seed proteins) together with lesser amounts of globulins (18%), albumins (7%) and prolamins (5%). The majority of total walnut polypeptides have estimated molecular weights in the range 12–67kDa [18]. The amino acid (AA) composition of walnut flour is dominated by the acidic AA residues of aspartate and glutamate together with relatively high levels of arginine [19, 20]. Walnut proteins contain all essential AAs required for the needs of a human adult. However, compared with the AA requirements of a 2–5-year-old child, lysine is the first limiting AA [21]. The lysine/arginine ratio in walnut proteins is lower than those observed in other common vegetable proteins, and this fact has been identified as a positive feature in the reduction of atherosclerosis development [22].

Table 1: Quantitative analysis of *Juglans regia* L. nuts

Nut extracts of <i>Juglans regia</i> L.	
Substances	Values
Carbohydrates	13.7 ^a
Protein	15.2 ^a
Alkaloids	2.2 ^a
Iron	40 ^b
Phosphorous	340 ^b
Vitamin – C	40 ^b
Vitamin – E	24.5 ^b
Catalase activity	102 ^c

[^a denotes % values, ^b denotes mg%, ^c denotes mg H₂O₂ utilized/min]

Value = Mean ±SD of 3 determinations, *significant values and p < 0.001

The quantitative value of alkaloids was 2.2mg%. The general meaning of alkaloid was taken to be an “alkali like” compound of plant origin, or as Bentley (1954) interpreted, a “vegetable alkali”. Alkaloids means Alkali likes. The Pharmacist W. Meissner proposed the term Alkaloids in 1819. According to him "Alkaloids (alkali = base, oid=like sub) are basic nitrogenous compd. of plant origin which have complex molecular structure & many pharmacological activities. The characteristic features Alkaloids are basic nitrogenous plant origin, mostly optically active & possessing nitrogen hetero cycles as their structural units with physiological action. Presence of alkaloids in walnut is harmful to disease causing pathogens.

The iron content was 40 mg%. Iron is vital for transporting oxygen in the bloodstream and for preventing anemia. For both male and female adolescents, the need for iron increases with rapid growth and the expansion of blood volume and muscle mass. The onset of menstruation imposes additional iron needs for girls. Iron needs are highest during the adolescent growth spurt in males and after menarche in females. The RDA for iron is 8 mg/day for 9-13 year olds, 11 mg/day for males' ages 14-18 and 15 mg/day for females ages 14-18. Estimates of iron deficiency among adolescents are 3-4% for males and females ages 11-14, 6-7% for females ages 15-19, and 0.6% for males ages 15-19 [23]. More than 80% of the iron consumed is in the form of non-heme iron. Bioavailability of non-heme iron can be enhanced by consuming it with heme sources of iron or vitamin C. Because the absorption of iron from plant foods is low, vegetarians need to consume twice as much iron to meet their daily requirement [24]. So that vegetarians can consume walnut for their iron requirements.

The content of phosphorus present in nut extract was 340 mg%. Calcium and Phosphorus are essential to human life. In vivo, the ionic forms of calcium and phosphorus combine to form calcium phosphate. During the process of bone hardening or aging, the Ca:P ratio gradually increases from

1:1 to 1.67. Research has shown that intake of high daily levels (1000 to 1500 mg) of calcium to prevent or treat osteoporosis can bind up to 500 mg of phosphorus, making it unavailable to the body [25]. Osteoporosis is a common form of bone disease, and is characterized by low bone mass and deterioration of the bone structure. While many osteoporosis prevention studies have focused on calcium, the emphasis for some of these studies has now shifted toward the importance of the balance of calcium and phosphorus in treatment for this disease. Walnut is good source of phosphorus and it helpful in the mineral requirements.

The vitamin-C content of nut extract of *Juglans regia* L. was 40mg %. Vitamin C is an important water soluble vitamin and the classic disease of vitamin C deficiency is scurvy. This condition is produced by inadequate ascorbic acid, which plays an important role in the hydroxylation of lysine and proline residues in the collagen protein of connective tissue and joints [26]. The well-publicized role of vitamin C as a powerful antioxidant overshadows the many other functions it has in the body. In the forms of ascorbate (ascorbic acid) and dehydroascorbic acid, vitamin C acts as a cofactor or co-substrate for eight different enzymes in the body [27]. Vitamin C was shown to protect neutrophils from reactive oxygen species generated during phagocytosis [28]. Evidence further suggests that vitamin C provides indirect antioxidant protection by regenerating other biologically important antioxidants such as glutathione and vitamin E to their active state [29]. *Juglans regia* L. nut serves as a good source of vitamin C as a natural form.

The vitamin-E content of nut extract of *Juglans regia* L. was 24.5mg %. Vitamin E may not be directly linked to growth, but its roles in immune response, nerve and muscle function, and its powerful antioxidant properties make it vital to the health of the young growing horse. Together with selenium, vitamin E acts to maintain normal muscle function, aid in the prevention of muscular disease, and provide antioxidant protection to body tissue, particularly cell membranes, enzymes and other intracellular substances, from oxidation induced damage [30]. A deficiency of vitamin E may cause a variety of different symptoms and pathological changes, which may include nutritional muscular dystrophy (weak and poorly oxygenated muscles) and poor immunity to diseases [31]. *Juglans regia* L. nut serves as a good source of vitamin E and helpful for nutritional requirements.

The catalase activity also determined in nut extracts, the catalase activity expressed in 102 mg of H₂O₂ utilized per minute, suggests that the role of these nuts in scavenging free radicals when used

in dietary sources without subjecting to much temperature and pH conditions.

Conclusion

In conclusion, the result of the present study apparently indicates that *Juglans regia* L. nuts (walnut) constitute a suitable source of vitamin C which possibly can be used as alternative natural antioxidants in food industries. Walnuts better source of protein and unsaturated fatty acids, these compounds are important in treatment of cardiovascular disease and various protein deficiency disorders. Presence of fair amount of phosphorus, walnut can be used in the treatment of bone disorder. Walnut possess good source of iron, so that it may be useful to the treatment of iron deficiency anemia. Vitamin E also one of component in *Juglans regia* L. nut, for this it can be used to infertility treatments. In general nuts serves has a good dietary source of protein, iron, phosphorus, vitamin C and E, which suggest their potential therapeutic and preventive uses in pathological conditions like, protein malnutrition, iron deficiency anemia, scurvy, sterility, impotency, atherogenic and inflammations. Further trials in humans are required to determine the efficacy of walnut extract or one or more of its constituents and to establish what, if any, adverse effects are observed.

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