Research Article

Nutrient Analysis, Phytochemical and Antioxidant Activity of a Food Product Formulated with Fox Nuts (*Euryale ferox*)

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Submission Date: 07-06-2023; Revision Date: 14-08-2023; Accepted Date: 02-09-2023.

ABSTRACT

Aim/Background - Fox nuts (*Euryale ferox*), a South Asian native crop, are found to be high in protein and micronutrient content and are shown to have antioxidant, hypocholesterolemic and anticarcinogenic properties. **Materials and Methods** - The purpose of this research was to formulate an energy and nutrient-dense food product using fox nuts as the primary ingredient along with almonds, dates, flax seeds and clarified butter. An ethanolic extract of the product was used to carry out qualitative phytochemical analysis and antioxidant assays. **Results:** 100 g of the formulated product was found to have 485 Kcal of energy, 10.7 g of protein, 27.7 g of total fat, 48.3 g of carbohydrate, 15.5 mg of iron and 133.3 mg of calcium. The presence of phenols, flavonoids, alkaloids, terpenoids, tannins and glycosides was detected in the formulated product. IC_{50} value of the product obtained via DPPH● assay was 55.43 µg/mL indicating strong antioxidant activity. The reducing power of the product via FRAP and Phosphomolybdenum assays were found to be 2.08 and 2.03 respectively at the highest concentration of 120 µg/mL. **Conclusion:** Data from the present study showed that the formulated product had a good nutrient profile and free radical scavenging potential.

Keywords: Antioxidant, Fox nuts, Nutrient profile, Phytochemicals.

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INTRODUCTION

Fox nut (*Euryale ferox* Salisb.) is a freshwater crop belonging to the Nymphaeaceae family and is commonly referred to as 'Makhana' in the Indian subcontinent. The seeds are called 'black diamonds' and are widely consumed in popped form among populations in the central and northeast regions of the country. Fox nuts also known as gorgon nuts and lotus seeds, are popular in India and other Southeast Asian countries.[1] Much of India's fox nut cultivation and production (80%) comes from Bihar.[2]

The consumption of fox nuts has greatly increased due to its nutritional benefits. Fox nuts are low in calories, contain negligible amounts of fat and have a high mineral content. Substituting nutrient-dense fox nuts frequently in a diet plan can reduce the incidence of micronutrient deficiencies. Fox nuts can be consumed in popped form or as a powder incorporated into various food items because of their nutrient content.[3]

Popping of fox nuts is said to have a beneficial impact on the phytochemical profile and other bioactive compounds.[4] Fox nuts contain a flavonoid called kaempferol, also present in almond skin, which contributes to its phytochemical activity. Studies have shown that due to its high amino acid index, it plays a major role in cell metabolism by aiding muscle recovery after an exercise session. It has been shown to improve the recovery of elasticity in arteries and veins and contribute to the maintenance of healthy tissues, thereby having an anti-ageing potential.[5]

The presence of HBAC, an essential compound in fox nuts, has been shown to release insulin from the pancreatic β-cells thereby improving glycaemic control.[6]

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DOI: 10.5530/ajbls.2023.12.38

In Traditional medical treatment, it has shown to prevent the build-up of toxins and facilitate the elimination of harmful substances from the human body.[5] Laboratory studies have shown the anti-antioxidant property of fox nut extracts to be high based on the IC_{50} values obtained after conducting various assays.[7,8] Hepatoprotective property of fox nut seed coat was studied in Wistar rats. After supplementation, an overexpression of the CYP2E1 gene, responsible for maintaining a healthy liver, was observed in rats induced with hepatic steatosis compared to the control group.[8,9] The anticarcinogenic activity of *Euryale ferox* ethanolic extract was determined by detecting the presence of anti-apoptotic compounds such as resveratrol, allicin, and gallic acid using HPLC and, observing a reduction in tumour size in A549 Human Caucasian Lung Carcinoma Cancer cell line in male immunodeficient mice.[8,10,11]

Since fox nuts is gaining recognition as a 'super food' due to the above-mentioned health-promoting properties, the main purpose of this research was to formulate a food product using fox nuts as the main ingredient. The secondary objective was to analyse the nutrients, phytochemicals and antioxidant activity of the formulated product.

MATERIALS AND METHODS

Product Development

Popped fox nuts were first roasted and then powdered to flour. The flour along with roasted almonds (with skin), roasted flax seeds, dates and clarified butter was made into balls.

Place of Analysis

Nutrient analysis of the formulated product was carried out at Scientific Food Testing Services (SFTS) Pvt. Ltd., Chennai. The extract preparation, qualitative phytochemical analysis and antioxidant assays were carried out at Armats Biotek Training and Research Institute, Chennai. Both the laboratories had NABL accreditation.

Nutrient analysis

Energy, carbohydrate, protein, total fat, iron and calcium for 100g of the formulated product were estimated. Energy was estimated via calorimetry, carbohydrate was estimated using the anthrone method, protein was estimated via the Kjeldahl method, total fat content was estimated via AOAC 920.39, and iron and calcium were estimated via AOAC 984.27.

Preparation of the extract

100 g of the formulated product was soaked in 99% ethanol for 72 hr at standard room temperature. The resulting supernatant was filtered by Whatman filter paper 1 and condensed at room temperature. A pale-yellow ethanolic extract that was yielded was utilized for additional investigation.

Qualitative analysis of phytochemicals

Different phytochemical tests were performed on the ethanolic extract of the formulated product to determine the presence of phenols, flavonoids, alkaloids, terpenoids, tannins and glycosides in the extract.

Antioxidant assays

DPPH● (1, 1- diphenyl 2-picrylhydrazyl) assay

One mL of 0.1 mM DPPH solution in methanol was combined with different quantities of the sample extract (20-120 g/mL). The mixture was then incubated in the dark for 30 min. As a control, 1 mL methanol was combined with 1 mL DPPH solution. Ascorbic acid, which was prepared in the laboratory from sorbitol, was used as the standard. A UV-spectrophotometer was used to detect absorbance at 517 nm, after which the percentage of inhibition was computed as follows:

$$\% \text{ of Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample Extract}}{\text{Absorbance of Control}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) assay

The extract was combined with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% potassium ferricyanide at various doses (20-120 g/mL). For 20 min, the mixture was incubated at 50°C. 1 mL of 10% trichloroacetic acid was added to the mixture, followed by 1 mL of freshly produced ferric chloride at 0.1%. A UV-spectrophotometer was used to detect absorbance at 700 nm, after which the percentage of reduction was computed as follows:

$$\% \text{ of Reducation} = \left[\left(\text{Abs (sample extract)} - \frac{\text{Abs (control)}}{\text{Abs (sample extract)}} \right) \right] \times 100$$

Wherein,

Abs = Absorbance

Phosphomolybdenum assay

Extract concentrations ranging from 20 to 120 g/mL were mixed with 1 mL of Phosphomolybdenum reagent. The tubes were sealed and placed in a 95°C water bath

for 30 min and allowed to cool to room temperature. A UV-spectrophotometer was used to detect absorbance at 695 nm, after which the percentage of reduction was computed as follows:

$$\% \text{ of Reducation} = \left[\left(\frac{\text{Abs (sample extract)} - \text{Abs (control)}}{\text{Abs (sample extract)}} \right) \right] \times 100$$

Wherein,
Abs = Absorbance

RESULTS

Nutrient Content

The macronutrients, iron and calcium content of the formulated product is presented in Table 1.

The formulated product was found to be nutrient-dense. The energy content of the formulated product contributed to 22.9% and 29.2% of the Recommended Dietary Allowance (RDA) for Indian men and women involved in sedentary activities respectively. Carbohydrate content was found to provide 37.1% of RDA for both men and women and, protein content contributed to 19.8% and 23.4% of RDA for men and women respectively. The total fat of the formulated product was found to make up 12% of total calories for men and 15% of total calories for women. The iron content of the product was found to be 81% of RDA for men and 53.4% of RDA for women. With respect to calcium, the product was found to make up 13.3% of RDA for both men and women.[12]

Qualitative Analysis of Phytochemicals

Table 2 indicates the qualitative presence of phytochemicals in the formulated product.

All the phytochemicals listed in the table were found to be present in the formulated product, indicating this to be a food with good antioxidant potential.

Table 1: Nutrient composition per 100 g of the formulated product.

Nutrient	Nutritive value
Energy (Kcal)	485
Protein (g)	10.7
Total Fat (g)	27.7
Carbohydrate (g)	48.3
Iron (mg)	15.5
Calcium (mg)	133.3

Table 2: Phytochemical screening of formulated product.

Phytochemicals	Present / Absent
Phenols	+
Flavonoids	+
Alkaloids	+
Terpenoids	+
Tannins	+
Glycosides	+

+ Present; - Absent

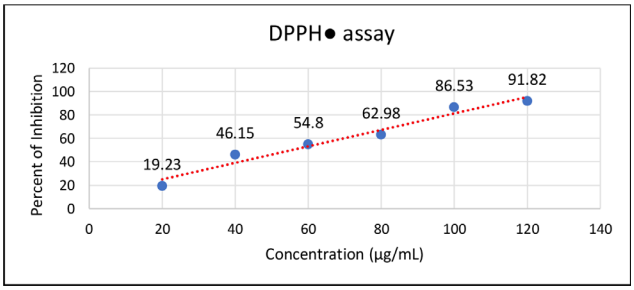

Figure 1: DPPH● free radical scavenging activity of formulated product.

Table 3: IC_{50} value for DPPH● assay.

IC_{50} value	Formulated product	Standard Ascorbic acid
	55.43 µg/mL	21.27 µg/mL

µg/mL – micrograms per millilitre

Antioxidant Activity

DPPH● assay

Results of DPPH● assay of formulated product are presented in Figure 1 and IC_{50} values of the product and standard ascorbic acid are presented in Table 3.

From Figure 1, it was observed that there was a commensurate increase in the percentage of inhibition and the concentration of the extract. Lower the IC_{50} value of a product, the higher the DPPH● radical scavenging activity. An IC_{50} value of a product between 50-100 µg/mL indicates strong antioxidant activity, whereas <50 µg/mL indicates very strong antioxidant activity. From Table 3, the IC_{50} value of the formulated product was higher than the standard ascorbic acid.

FRAP and Phosphomolybdenum assays

The results of the FRAP and Phosphomolybdenum assays of the formulated product are presented in Table 4. Based on the absorbance value, the reducing power of the formulated product at the highest concentration of 120 µg/mL was three times higher than ascorbic acid in the FRAP assay and two times higher than ascorbic acid

Table 4: FRAP and Phosphomolybdenum assays of formulated product.

Concentration (µg/mL)	FRAP assay Absorbance at 700nm		Phosphomolybdenum assay Absorbance at 695nm	
	Sample	Ascorbic acid	Sample	Ascorbic acid
20	0.79	0.32	0.47	0.65
40	1.13	0.44	0.99	0.72
60	1.51	0.52	1.32	0.80
80	1.54	0.59	1.61	0.83
100	1.74	0.63	1.70	0.85
120	2.08	0.69	2.03	0.89

µg/mL – micrograms per millilitre; nm – nanometres

in the Phosphomolybdenum assay, indicating a high antioxidant potential of the product.

DISCUSSION

Nutrient Content

Raw or 'unpopped' fox nuts undergo the process of popping to give it an appearance similar to popcorn. Studies have indicated that popped fox nuts on roasting have better texture and acceptability, and enhanced nutrient bioavailability.[4,13,14] A study[14] has indicated a significant increase in the mineral, protein and fibre content of roasted fox nuts compared to the unroasted equivalent. The nutrient content of popped fox nuts on a variety called 'Swarna Vaidehi' in Bihar has shown a calorific value of 358%, 79.8% carbohydrate, 8.7% protein, 0.5% fat and 0.2% crude fibre. Iron and calcium content was estimated to be 1.4% and 18.5% respectively.[13] Another study conducted on raw *E. ferox* seeds in Bangladesh has shown a protein content of 15.6%, 7.6% fibre, 61.2% carbohydrate and 1.3% fat.[15] Fox nuts have a high 'amino acid index' as they contain 7 out of 9 essential amino acids, except phenylalanine and tryptophan.

The formulated product was found to have a good protein, energy and mineral content. The total fat, iron and calcium content of the formulated product were found to be 27.7%, 15.5% and 133.3% respectively. The addition of almonds may have contributed to the product being nutrient-dense. Almonds contain appreciable amounts of protein, fibre, vitamin E, magnesium and potassium, and have shown improved cardioprotective functions.[16]

Phytochemical Assays

The presence of phytochemicals namely phenols, flavonoids, alkaloids, terpenoids, tannins and glycosides was detected in the formulated product. Phenols and flavonoids are known for their free radical scavenging potential thereby enhancing antioxidant activity.[17] Anti-allergic, antimicrobial, anti-inflammatory, antiviral, hepatoprotective and anti-carcinogenic activities are some of the other functions of phenolic compounds and flavonoids.[18,19] In a study in Maharashtra, phytochemicals such as alkaloids, terpenoids, tannins and glycosides were detected in addition to phenols and flavonoids in an ethanol-water extract of fox nut seed coat.[20] Alkaloids are found abundantly in seeds and roots of plants among which the commonly studied ones are caffeine and atropine. They reportedly have excellent bioactivity and are effective central nervous stimulants, anaesthetics, pain killers, hypoglycaemic and antioxidant agents.[21] In addition, they possess muscle relaxant, analgesic, antiplatelet, anticoagulant functions and, antimicrobial properties to combat pathogens.[22,23] Terpenoids, one of the largest groups of phytochemicals, have also reportedly shown anticarcinogenic, antioxidant, anti-inflammatory, and anti-malarial activities as well as protection against numerous bacterial and viral infections.[24] Tannins, a water-soluble polyphenol, exhibit antioxidant, anticarcinogenic and antimutagenic properties, and are also useful in reducing serum lipid and blood pressure levels.[25] They also possess properties such as antihelmintics, antimicrobial, anti-haemorrhagic and antiseptic.[26,27] Glycosides are said to boost immunity and reduce inflammation thereby preventing cardiovascular and neurodegenerative diseases and cancers.[28]

The adult population is required to consume 500-900 mg of total polyphenols and total flavonoids per day to prevent the occurrence of chronic illnesses.[29] Studies showed that Total Phenol and Flavonoid Content (TPC and TFC) were reduced in popped fox nuts (1.12 ± 0.06 mg GAE/g and 1.26 ± 0.18 mg RE/100g) compared to raw form (2.22 ± 0.06 mg GAE/g and 3.23 ± 0.28 mg RE/100g) due to the breakdown of heat-sensitive compounds during popping.[4] Another study revealed that TPC and TFC of the fox nut seed coat extract were found to be 50.96 ± 0.10 mg GAE/g and 16.27 ± 0.73 mg QE/g respectively.[20] In a study,[14] popped fox nuts were either roasted or left unroasted (control) for analysis of phytochemicals. Results revealed that for roasted variety and control, TPC was 470.62 mg GAE/100g and 346.02 mg GAE/100g respectively. TFC was 4.43 mg CE/g for the roasted variety and 4.15 mg CE/g for the unroasted variety, indicating a higher phytochemical content in the roasted form.[14] Further research is needed to reveal the TPC and TFC of the formulated product in the present study

to discover whether it lies within the desirable range, and in what quantity it needs to be consumed to bring about beneficial health functions.

Antioxidant Activity

Research has shown that when the concentration of selenium-*Euryale ferox* Salisb. Polysaccharide was increased, the radical scavenging rate of DPPH● also correspondingly increased (60.5-89.7%) in a dose-dependent manner.[30] One study has shown that the IC_{50} value of ethanol: water seed coat extract of fox nuts (1.62 µg/mL) was less than ascorbic acid (2.28 µg/mL) indicating very strong antioxidant activity.[20] IC_{50} values between 50-100 µg/mL indicate a strong antioxidant activity and <50 µg/mL indicate a very strong antioxidant activity as per studies conducted previously.[31] In the present study, the IC_{50} value of the formulated product indicated a strong antioxidant capacity as it lay between 50-100 µg/mL. However, the product's ability to scavenge free radicals was not as strong as the standard ascorbic acid, the latter's value being <50 µg/mL.

A study in Pakistan has shown that DPPH● and FRAP activities of roasted fox nuts were substantially greater ($p<0.05$) than the unroasted form. Roasting fox nuts has a positive effect on its ferric-reducing ability thereby enhancing antioxidant activity.[14] Research on Phosphomolybdenum activity on walnuts[32] revealed lesser absorbance in 100 µg/4 mL concentration (0.382±0.59) than in the present study. Other studies have shown that the Phosphomolybdenum activity of 80% ethanolic extract of almonds was found to be 0.57 g/100g, which was lower than walnuts and hazelnuts.[33] There is a lack of studies till date on the Phosphomolybdenum antioxidant activity in fox nuts. In the present study, results for FRAP and Phosphomolybdenum assays showed that the reducing power of the formulated product was greater than the standard ascorbic acid, indicating strong antioxidant activity.

The other ingredients added to the formulated product may have also indicated a good antioxidant activity. A study on the total antioxidant capacity of almonds revealed that almonds with skin had significantly higher antioxidant activity than almonds without skin (27.8 vs 3.5) suggesting that much of the phenolic compounds are found in the skin of these nuts.[34] Hence, the consumption of roasted almonds with the skin intact proves beneficial to the health of an individual. Dates are a good source of antioxidants as well as sugars and micronutrients such as iron, zinc and vitamin C.[16] They are especially beneficial when consumed by pregnant women to shoulder the iron needs of mother and foetus and, to combat iron-deficiency anaemia.[35] Dates also show anti-tumour, anti-diabetic and anti-inflammatory properties.[36] Research among recreational runners has shown that date seed powder holds the potential to alleviate oxidative stress, reduce inflammation, decrease total cholesterol levels and, improve exercise performance by reducing fatigue.[37] Flax seeds contain abundant amounts of omega-3 fatty acids, α-linolenic acid and dietary fibre, and possess anti-inflammatory and anti-carcinogenic properties. The existence of bioactive substances lends credence to its usage as an antioxidant and, it is incorporated into a wide variety of commonly consumed foods.[38] 'Ghee' also known as clarified butter contains fat-soluble vitamins, free fatty acids, carotenoids, tocopherols and traces of iron, phosphorus and calcium. The presence of natural antioxidants in ghee prevents its oxidation thereby prolonging shelf life when added to any food product.[39]

CONCLUSION

In this study, the phytochemical and antioxidant properties of the formulated food were found to be good. Since studies have indicated fox nuts to have a rich nutritional profile, this can be a good alternative for processed snacks and also contribute to nutrient-dense calories. Propagating the consumption of fox nuts in various forms such as popped form or incorporated with other foods would improve the quality of the diet and encourage the use of locally available ingredients among the Indian population. This could contribute towards promoting healthy eating behaviours, and also prevent or postpone the onset of lifestyle-related disorders.

ACKNOWLEDGEMENT

The authors would like to thank the University Grants Commission (UGC) of India for providing financial support.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

ABBREVIATIONS

AOAC: Association of Official Agricultural Chemists; **A549 cells:** Adenocarcinomic human alveolar basal epithelial cells; **CE:** Catechin equivalent; **CYP2E1:** Catalase and cytochrome P450 2E1;

DPPH: (1, 1- diphenyl 2-picrylhydrazyl); **FRAP:** Ferric Reducing Antioxidant Power; **GAE:** Gallic acid equivalent; **HBAC:** 2β-hydroxybetulinic acid 3β-caprylate; **HPLC:** High-performance liquid chromatography; **IC_{50}:** Half-maximal inhibitory concentration; **NABL:** National Accreditation Board for Testing and Calibration Laboratories; **RDA:** Recommended Dietary Allowances; **RE:** Rutin equivalent; **SFTS:** Scientific Food Testing Services; **TFC:** Total flavonoid content; **TPC:** Total phenol content; **UV:** Ultraviolet.

SUMMARY

- 100 g of the formulated product was found to have 485 Kcal of energy, 10.7 g of protein, 27.7 g of total fat, 48.3 g of carbohydrate, 15.5 mg of iron and 133.3 mg of calcium.
- The presence of phenols, flavonoids, alkaloids, terpenoids, tannins and glycosides was detected in the formulated product.
- IC_{50} value of the product obtained via the DPPH● assay was 55.43 μg/mL indicating strong antioxidant activity.
- The reducing power of the product via FRAP and Phosphomolybdenum assays were found to be 2.08 and 2.03 respectively at the highest concentration of 120 μg/mL.
- Propagating the consumption of fox nuts in various forms such as popped form or incorporated with other foods would improve the quality of the diet and encourage the use of locally available ingredients among the Indian population.

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Cite this article: Joseph A and Ramesh G. Nutrient Analysis, Phytochemical and Antioxidant Activity of a Food Product Formulated with Fox Nuts (*Euryale ferox*). Asian J Biol Life Sci. 2023;12(2):279-85.