

Ethnomedicinal, phytochemical and nutritional analysis of *nelumbium nucifera* gaertn rhizome

Abstract

The present study aims to evaluate the phytochemical and nutritional analysis of *Nelumbium nucifera* rhizome from district Charsadda KPK (Pakistan). Descriptive research design was adopted in this study. Study and analyzes were done in Quaid-i-Azam University in Pakistan, in a 3month time frame. The proximate parameters like ash, crude fat, crude fiber, crude protein, carbohydrates, moisture contents and energy values were analyzed and obtained using Association of Official Analytical Chemists methods. Total phenolic contents was found to be (25.49±1.15)mg GAE/g and (17.6±3.80)mg GAE/g dry extract whereas the total flavonoid contents were found to be (10.84±0.16)mg RUE/g and (6.86±0.76)mg RUE/g in methanol and ethanolic extract respectively. Eventually concentration of moisture (55.3 ±0.80) and carbohydrates (23±0.26%) were detected higher than concentration of fat (3.1±1.67%) and fiber (2.5±0.86%) contents. Results showed that the *N. nucifera* rhizome has various phytochemicals such as cardiac glycosides, flavonoids, saponin, tannin, phlobatanins, phenolic compounds and alkaloids. Methanolic extract of *N. nucifera* rhizome showed the high contents of flavonoids and phenols. Also indicates that rhizome is a good source of carbohydrates and proteins. It possesses significant nutrient properties which give it an advantage of being used in bakery, food production and good experimental system for further researches on renewable resources.

Keywords: ethnomedicine, *nelumbium nucifera*, nutritional analysis, phytochemical

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Abbreviations: AOAC, association of official analytical chemists; TPC, total phenolic contents; TFC, total flavonoid contents; CE, chloroform extract

Introduction

Nelumbium Adans is a genus belongs to the monogeneric family Nelumbonaceae. Genus *Nelumbium* has two species around the world, *Nelumbium nucifera* Gaertn and *Nelumbium lutea* Willd. *Nelumbium nucifera* is a perennial aquatic edible plant commonly known as Sacred Lotus, which has been used as a medicinal herb in China and India.¹ In traditional herbal system of cure, the different parts of *Nelumbium nucifera* is reported for beneficial effects for the treatment of smallpox, pectoralgia, cough, dysentery, epistaxis, haematemesis, haemoptysis, haematuria, metrorrhagia, fever, cholera.¹

N. nucifera has pink, red or white flowers, widely spread in Asia and Oceania, and *N. lutea* has yellow flowers, spread in North and South America.¹ *N. nucifera* is an aquatic perennial herb known by a number of other names including simply lotus, Indian lotus, bean of India, Chinese water lily and sacred lotus.² *N. nucifera* is described to have used in traditional medicine by people for its enormous health benefit in different parts of the World. It has been used to treat sunstroke, diarrhea, dysentery, hemorrhoids, dizziness, vomiting of blood, uterine bleeding ailments, promoting conception, improving the skin condition, regulatory burning sensation, against infections, cough, hypertension, fever, urinary problems, hematemeses, epistaxis, hemoptysis, hematuria and metrorrhagia.³ In Asian culture, whole parts of *N. nucifera* are eaten as food or used for medicinal purposes, containing seeds, rhizomes, nodes, leaf, roots, young shoots, stamens, petals, stalks, and pericarps.¹ Many pharmacological studies on lotus have proven its anti-diarrheal, anti-inflammatory, antipyretic,

hypoglycemic, immunomodulatory, antioxidant, lipolytic, antiviral, anticancer and hepatoprotective activities.⁴ Medicinal and aromatic plants contain some phytochemicals which are responsible for certain physiological act on the human body and these bioactive phytochemicals consist of terpenoids, steroids, tannins, alkaloids and carbohydrates.⁴ These phytochemicals are present universally in all the medicinal plants and are generally divided into two main groups' primary metabolites and secondary metabolites. Phytochemical screening has been reported that *N. nucifera* yields a number of important medicinal secondary metabolites.⁵ Nutrition plays an important role in health, by not only providing vital nutrients, however also promoting health and inhibiting ailment.⁶ Different researchers have been reported the significance of these biochemicals.⁷ Moreover the nutritional value such as fiber, moisture, ash contents and the energy values of individual vegetable and plant species have also been reported that they have importance to the human health. The pharmacological investigations carried on *N. nucifera* have important activities and *N. nucifera* is a well known plant in ancient medical sciences, some research have been done on the whole, different parts and rhizomes of the plant.⁸

The purpose of the study is to evaluate the phytochemical and nutritional analysis of *N. nucifera* rhizome. Also this research aim is to evaluate various ethnomedicinal, phytochemical, nutritional, potential usefulness, renewable resources and allied approaches rhizome of *N. nucifera*.

Methodology

The present study was carried out to evaluate the phytochemical, ethnomedicinal and nutritional analysis of *N. nucifera* rhizome. Phytochemicals were extracted from *N. nucifera* rhizome using

different solvents like methanol, acetone, and chloroform. The proximate parameters like ash, crude fat, crude fiber, crude protein, carbohydrates, moisture contents and energy values were obtained using the Association of Official Analytical Chemists (AOAC) methods. Descriptive research design was adopted in this study. Different parts of the *N. nucifera* (A) whole plant and (B) rhizome is shown in Figure 1.

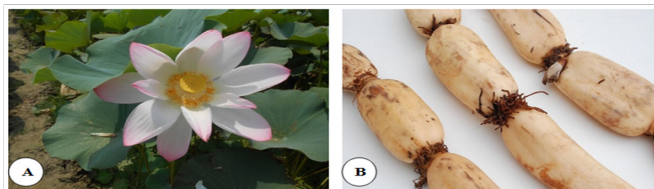


Figure 1 Different parts of *N. nucifera* (A) whole plant (B) rhizome.

Collection and identification of *N. nucifera*

The rhizomes of *N. nucifera* were collected from the fields of irrigated areas of the village Majokey, district Charsadda, KPK, Pakistan by taxonomist Raess Khan. Identification of species was carried out in Herbarium of Quaid-i-Azam University with Flora of Pakistan by taxonomist Raess Khan. The voucher specimens have been deposited in Quaid-i-Azam University (Islamabad-Pakistan) herbarium.

Extraction of *N. nucifera* rhizome

Fresh rhizomes were washed with distilled water to eliminate mud. The rhizomes divided into very small pieces with a sharp knife. Then the rhizome was dried under shadow, separated, crushed by a mechanical grinder and passed through a mesh sieve. A total of 20g of the crushed plant material was extracted for 4 days in 2% chloroform ethanol, acetone and methanol solvents. The detached extracts were then filtered by Whatman filter paper product number Z274852. Dried solvent extract was kept at 4°C for further analysis. Fresh rhizome was used for the determination of the moisture contents.

Qualitative phytochemical analysis

The qualitative phytochemical analysis of *N. nucifera* rhizome was carried out using the Association of Official Analytical Chemists (AOAC) method to detect the presence of different classes of phytochemical such as alkaloids, flavonoids, terpenoids, tannin, saponins, glycoside, phenol, phlobatanins, coumarin and cardiac glycoside.⁹

Test for alkaloids: To 1mL of extract, 2.5mL of concentrated HCL was mixed in a test tube. Then few drops of Mayer's reagents were added dropwise. The formation of a white precipitate indicated the occurrence of alkaloids.

Test for tannins

2mL of extract and 2.5mL of 5% ferric chloride were taken in a test tube. Formations of dark blue or greenish black was indicated the presence of tannins.

Test for saponins

1mL of extract and 1.5mL of distilled water were mixed in a test tube and shaken in graduated cylinder for 10 minutes lengthwise. The presence of foam shows the presence of saponins.

Test for flavonoids

To 2mL of plant extract and 2mL of 2N sodium hydroxide were added in a test tube. A yellow color formation shows the presence of flavonoids.

Test for quinones

1mL of plant extract and 1mL of concentrated sulphuric acid were taken in a test tube. The presence of red color shows the presence of quinones.

Test for cardiac glycosides

To 1mL of extract and 2.5mL of glacial acetic acid and few drops of 5% ferric chloride were added in a test tube. This was under layered with 1mL of concentrated sulphuric acid. The appearance of a brown ring at the interface shows the presence of glycosides.

Test for terpenoids

To 2mL of extract and 1.5mL of chloroform were mixed in a test tube and followed by carefully adding 3mL concentrated sulphuric acid. The appearance of red brown color at the interface indicates the presence of terpenoids.

Test for coumarone

To 1mL of extract and 2mL of 10% NaOH were added in a test tube. The appearance of yellow color shows the presence of coumarone.

Test for phenols

To 2mL of the plant extract and 2mL of distilled water followed by few drops of 10% ferric chloride were added in a test tube. A blue or green coloration indicates the presence of phenols.

Test for phlobatanins

To 1.5mL of plant extract and a few drops of 2% HCL were mixed in a test tube. The presence of a red color precipitate shows the presence of phlobatanins.

Quantitative phytochemical screening

Determination of total phenol content: Total phenolic content was determined according to the method of¹⁰ with certain modifications 200mg/ml methanolic solutions for both methanolic and ethanolic extracts were prepared for the analysis. The reaction mixture was made by mixing 100µl of the plant extract solution, 900µl of Folin-Ciocalteu's reagent dissolved in water and 860µl of 6% of NaCO₃ aqueous solution. The samples were incubated in the dark condition for 40min. The absorbance was estimated by spectrophotometer at wave length=630nm. The samples were prepared in replicate for all analysis and the mean value of absorbance was attained.

Determination of total flavonoid content: Total flavonoid content

was estimated according to the method of¹¹ with some modification using rutin as a standard. Rutin (100 to 300mg) was dissolved in methanol. Then, the stock solution was diluted to provide a series of concentrations. To this 150µl of 10% aluminum chloride and distilled water was added. Then 100µl 1M potassium acetate, 200µl extract was added. They were incubated for 40minutes in dark at room temperature. The absorbance was measured at 430nm.

Nutritional analysis

Proximate parameters such as moisture content, carbohydrates, fats, ash, crude protein, fiber and energy value in *N. nucifera* rhizome were evaluated using standard procedures and below formulas.¹² When we place the related data in the places of the values in the formulas, we get the results which were showed in the main text.

Determination of moisture content: The moisture content of the sample was determined as loss in weight of the original sample and the moisture percentage content was determined by formula as:¹²

$$\text{Moisture}(\%) = \frac{\text{Wt. of fresh sample}(W1) - \text{Wt. of dried sample}(W2)}{\text{Wt. of fresh sample}(W1)} \times 100$$

Crude protein determination: Crude protein was determined by the Kjeldahl method. The Crude protein percentage was obtained by formula.¹²

Determination of crude fats: Fat was determined by the soxhlet extraction method using 200ml n-hexane as the extracting solvent in soxhlet apparatus. Crude fats were expressed in percentage as:¹²

$$\text{Crude Fats}(\%) = \frac{\text{Wt. of extracted fats} + \text{Flask}(W3) - \text{Wt. of empty flask}(W4)}{\text{Wt. of sample used}(W5)} \times 100$$

Estimation of crude fiber: The crude fiber determination was performed through the process of AOAC method.¹⁰

$$\text{Crude fibre}(\%) = \frac{\text{Weight of loss in ignition}}{\text{Weight of fat free sample}} \times 100$$

Carbohydrates determination: The percentage of carbohydrate was calculated by using below formula.¹³

$$\text{Total carbohydrates content} = 100 - (\% \text{moisture} + \% \text{crude protein} + \% \text{crude fat} + \% \text{crude fiber} + \% \text{ash})$$

Energy value: Energy value was calculated for each sample as follows:¹⁴

$$\text{Energy value} = 4x\% \text{protein} + 9x\% \text{fats} + 4x\% \text{carbohydrates}$$

Results and discussion

The material collected has been analyzed and the total phenolic contents were found to be (25.49±1.15)mg GAE/g and (17.6±3.80)mg GAE/g dry extract whereas the total flavonoid contents were found to be (10.84±0.16)mg RUE/g and (6.86±0.76)RUE/g in methanol and ethanolic extract respectively. Further results revealed high moisture content (55.3±0.80%) and carbohydrates (23±0.26%) content and low concentration of fat (3.1±1.67%) and fibre (2.5±0.86%). Moreover the present study results also have shown that the *N. nucifera* rhizome has rich in terms of glycosides, flavonoids, saponin, tannin,

phlobatanins, phenolic compounds and alkaloids (Table 1); also it is understood through this study, that the *N. nucifera* rhizome is a good source of carbohydrates, proteins and it possesses significant functional properties which give it an advantage of being used in bakery and food production. In addition *N. nucifera* has important ethnomedicinal uses in the district of Charsadda KPK of Pakistan. Preliminary phytochemical screening of *N. nucifera* rhizome show positive results for the presence of phytochemical constituents such as; flavonoids, tannins, alkaloids, phenols, coumarin, cardiac glycosides, phlobatanins and saponins. The total phenolic content of ethanolic and methanolic extracts were determined by the Folin-Ciocalteu's reagent whereas the total flavonoid content were determined using the aluminium chloride method.

Ethnomedicinal uses of *N. nucifera*

Medicinal and aromatic plants have been used as a medicinal source by mankind since ancient times. The indigenous knowledge of many tradition communities has been formulated, been documented and eventually become organized systems of medicine used as a diuretic and anthelmintic and in the treatment of strangury, vomiting, leprosy, skin diseases and nervous exhaustion.¹⁵ In popular medicine rhizomes of this plant were prescribed as demulcents for haemorrhoids and are beneficial in dysentery, chronic dyspepsia, and have nutritive, diuretic and cholagogue activities.¹⁶ The stem of this plant is used in medicine as diuretic, anthelmintic, to treat strangury, vomiting, leprosy, skin disease and nervous exhaustion. The leaves are used for the treatment of haematemesis, epistaxis, haemoptysis, haematuria, metrorrhagia and hyperlipidaemia.¹⁷ The flowers of this plant are useful in the treatment of diarrhoea, cholera, fever and gastric ulcers; the seeds and fruits of *N. nucifera* are used as a health food in Asia and to treat many ailments and the seed powder mixed with honey is useful in treating cough.¹⁸

The present study documented the ethnomedicinal uses of *N. nucifera* in district of Charsadda KPK, Pakistan. The rhizomes (locally known as barsanday) are often consumed as a vegetable sold in the market. The market rate was Rs 60-80 per kilogram. These are commonly sliced and cooked with or without meat. The powdered seed is used against cough. The rhizome is also used as an antidiabetic and antiobesity. The flower was used to cure liver ailments and a range of other ailments like fever, hypertension, diarrhoea, weakness etc. The rhizomes of *N. nucifera* were locally used as an antifertility and inflammation. The flower is used for vomiting, cholera, diarrhoea and intermittent fevers and the plant was also utilized for intra-uterine growth hindrance, dysentery, diarrhoea, and skin darkening. Roots aid in the elimination of toxic waste from the body, and are also useful in decreasing body heat. The roots and rhizomes are beneficial in caring for throat complications, small pox, pigmentation problems in skin and diarrhoea. The cooked root was good for the stomach and the reproductive organs. Rhizome paste is useful in ringworm and coetaneous affections. Stem aids in the healthy growth of the fetus. Leaves were used against diarrhoea and high fever. The flowers and pedicels were used as a cardiac and hepatic tonic. The seeds were used for cutaneous ailments and also consumed as a raw.

Qualitative phytochemical screening

The current study carried out on the *N. nucifera* showed the presence of medicinal active components. The phytochemical active components of *N. nucifera* rhizome were qualitatively analysed and the results are presented in Table 1.

Total phenolic content (TPC): The absorbance of standard compound (GAE) was shown in this Table 2 and total phenolic content of *N. nucifera* for different extracts are presented in Table 3 Standard curve of gallic acid equivalent indicated the equation $Y=0.0002X+0.0731$, $R^2=0.9857$ clarified in Figure 2.

The results of total phenols contents that were measured by Folin Ciocalteu's reagent in term of gallic acid equivalent presented in Table 3 and Figure 3.

Table 1 Phytochemical constituents of the rhizome extracts of *N. nucifera*

Phytochemical compounds	ME	AE	CE
Tannin	+	+	+
Saponin	-	-	+
Phlobatanins	-	-	-
Coumarin	+	+	-
Terpenoids	+	+	+
Alkaloids	+	-	-
Flavonoids	-	+	-
Phenols	+	+	+
Glycosides	+	-	-
Cardiac glucosides	+	+	+

Present (+), Absent (-) (ME methanol extract AE acetone extract CE (chloroform extract))

Table 2 Absorbance of standard compound (GAE) at 630nm

Gallic acid concentration mg/ml	Absorbance at 630nm
100	0.099
300	0.135
500	0.19

Table 3 Total phenolic contents in different *N. nucifera* rhizome extracts

<i>N. nucifera</i> extracts	Total phenolic contents GAE mg/g dry extract \pm SD
Methanol	25.49 \pm 1.15
Ethanol	17.4 \pm 3.80

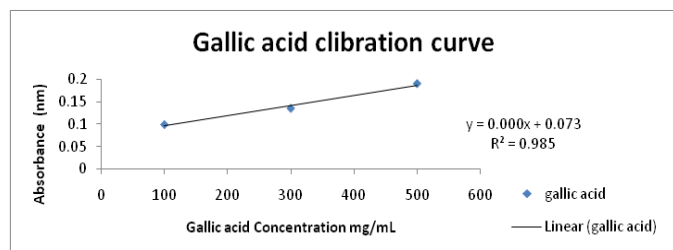


Figure 2 Standard curve of gallic acid.

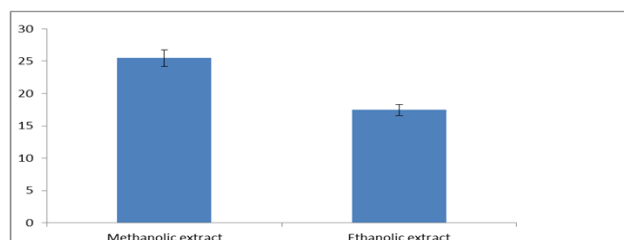


Figure 3 Total phenolic contents of methanolic and ethanolic extract of *N. nucifera* rhizome.

Total flavonoid contents

Absorbance of standard compound (Rutin) at 430nm in *N. nucifera* was shown in Table 4 and Figure 4.

The present study showed the results of total flavonoid contents was calculated from the calibration curve of Rutin and expressed as milligram of Rutin Equivalent per gram of dry extract (mg RUE/g dry extract) and shown in Table 5 and Figure 5.

Table 4 Absorbance of standard compound (RUE mg/mL) at 430nm

Rutin Concentration mg/ml	Absorbance at 430nm
100	0.033
200	0.068
300	0.099

Table 5 Total flavonoid contents in different *N. nucifera* extracts

<i>N. nucifera</i> extracts	Total flavonoid contents RUE mg/g Dry Extract \pm SD
Methanol	10.84 \pm 0.16
Ethanol	6.86 \pm 0.76

Nutritional analysis

Proximate composition: The proximate composition of rhizome powder of *N. nucifera* is presented in Table 6. The table shows the Mean \pm SD (g/100g) of moisture, crude protein, crude fat, crude fiber carbohydrates and ash content.

Phytochemical screening

The present study was carried out on *N. nucifera* which showed the presence of medicinally active constituents. The phytochemical of *N. nucifera* rhizome were qualitatively analysed and the results are presented in Table 1. In these screening process alkaloids, glycosides, saponins, phenol, tannins, phlobatanins, coumarin, flavonoids, cardiac glycoside and terpenoids showed different types of results in various solvent extracts. The current study reports that tannins were present in acetone extract whereas phlobatanins and alkaloids were absent. According to a research, tannins and alkaloids were absent in acetone extracts while phlobatanins were present in acetone extract.¹⁹ The study also reported that glycoside and flavonoids were absent while saponin was present in chloroform extracts. Glycosides, flavonoids and saponins all were presents in chloroform extract. Saponins have been reported to have an inhibitory effect on inflammation.²⁰ In current

study coumarin, phenol and terpenoids were presents in methanol extracts. In another study phenols were present while coumarin and terpenoids were absents in methanolic extract.²¹

Total phenolic contents (TPC)

The total phenolic contents in extracts were measured by Folin-Ciocalteu's reagent with reference to Gallic acid. Figure 2 show total phenols expressed as mg/g gallic acid. The total phenolic contents of *N. nucifera* rhizome methanolic and ethanolic extracts were estimated from gallic acid standard curve and expressed as GAE mg/g dry extract (Figure 3). The attained values of the total phenol for methanolic and ethanolic rhizome extract of *N. nucifera* were found to be 25.49 and 17.45mg GAE per gm correspondingly. Similar study has been reported that total phenolic content for methanol extract of *N. nucifera* was found to be 85.01 mg GAE per g.²²

Total flavonoid contents (TFC)

The estimation of total flavonoid content was determined from the calibration curve of Rutin and expressed as (mg) of Rutin Equivalent per gram of extract. In the current study the total flavonoid contents were found to be higher 10.84±0.16 RUE mg/g in methanolic extract, whereas in ethanolic extract the flavonoid content was low 6.86±0.76mg RUE/g respectively (Figure 5). According to another

study total amount of flavonoids in methanolic extract was found to be 41.86mg QE g of dry extract. In similar studies the total flavonoid content of *N. nucifera* stem extract was found to be 77.8mg Rutin equivalent/100ml for ethanolic extract.¹⁹

Proximate composition

The proximate analysis of *N. nucifera* rhizome was presented in Table 6. The present study shows that the moisture content was higher (55.3±0.80) than those reported by Shukla et al.,²³ which was low i.e. 6.86±0.17g/100g.²³ The current study result of ash content (g/100g) was 6.2±0.57 which was higher when compared to the study carried out the ash content was 3.88g/100g.¹⁹ The crude protein content of *N. nucifera* rhizome was (9.9±1.45) which were close to the results of Shad et al.²⁴ reporting that the protein content of lotus rhizome was (8.48±0.25).²⁴ The high content of protein in lotus rhizome highlights their value as an important source of nutrients. In our result the fat content of *N. nucifera* rhizome was (3.1±1.67) likewise the fat content of *N. nucifera* seed was found to be (2.5±0.28).²⁵ The fiber content (g/100g) of lotus rhizome recorded was (2.5±0.86) while comparing to the study of Mohammed et al.²⁵ That the fiber content of *Nymphaea lotus* seeds powder was (1.60±0.20). According to the present study the carbohydrates content of *N. nucifera* rhizome was (23±1.68) which was higher than those reported by Gana et al.¹⁹

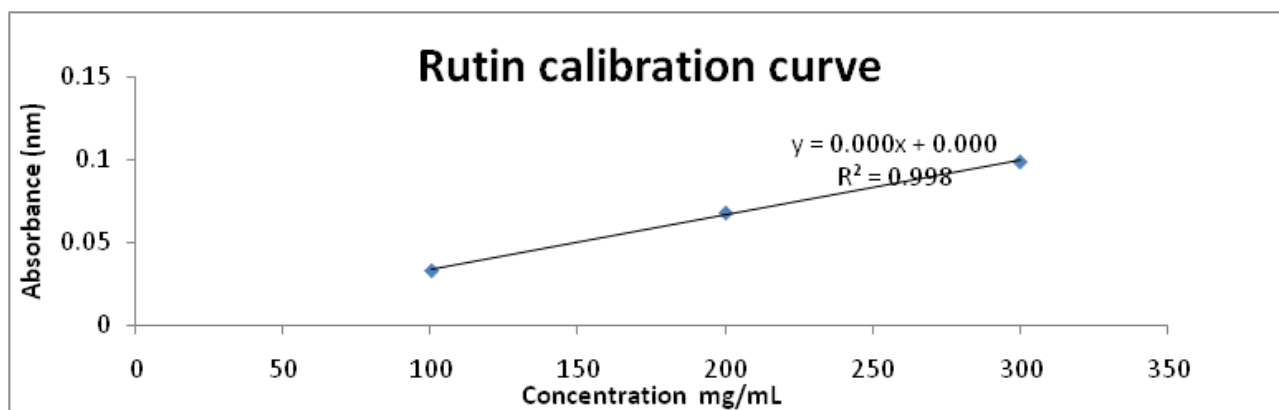


Figure 4 Standard calibration curve of rutin.

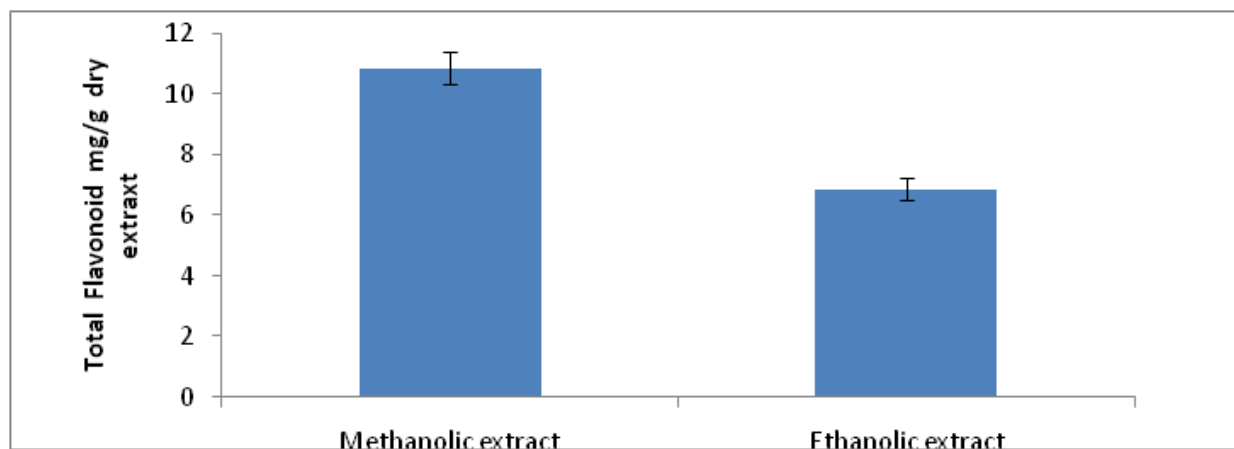


Figure 5 Total flavonoid contents of different *N. nucifera* rhizome extracts.

Table 6 Proximate composition of *N. nucifera* rhizome

Contents g /100 g	<i>N. nucifera</i> ±SD
Moisture content	55.3±0.80
Crude Fat	3.1±1.67
Ash	6.2±0.57
Crude Fiber	2.5±0.86
Crude Protein	9.9±1.45
Carbohydrates	23±0.26
Energy Value (K cal)	154.4

Conclusion

The ethnomedicinal, phytochemical and nutritional investigations carried on *N. nucifera* showed that it has important properties in traditional medicine, phytochemical composition and pharmacological activities. *N. nucifera* is also reported to contain a wide range of chemical constituents. These important chemical compounds could serve as leads in the search for novel medicinal agents. With the availability of primary investigations, further studies on *N. nucifera* should be designed to investigate the molecular mechanism(s) of action of isolated important chemicals using specific biological screening models and clinical trials, and also to discover novel leads from them. Also studies should be extended to standardize the various extracts of *N. nucifera* for the purpose of their use in specific herbal formulations and herbal medicine. The present study results showed that the *N. nucifera* rhizome has various phytochemicals such as cardiac glycosides, flavonoids, saponin, tannin, phlobatanins, phenolic compounds and alkaloids. The quantitative screening indicated that the methanolic extract of *N. nucifera* rhizome showed the highest contents of flavonoids and phenols. The results of this study also indicated that the lotus rhizome is a good source of carbohydrates and proteins. It possesses significant functional properties which give it an advantage of being used in bakery and food production. In addition *N. nucifera* has important ethnomedicinal uses in the district of Charsadda KPK of Pakistan.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

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