Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

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Insights into the composition of lotus rhizome

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Abstract
Nelumbo nucifera Gaertn. (Nymphaeaceae) known as sacred lotus, is a very unique ornamental plant having multiple medicinal properties. The proximate analysis of rhizome were analysed and the results include moisture (72.14%), starch (10.05), carbohydrates (16.03g), protein (2.6g), fat (0.1g), fibre (3.2g), vitamin C (38mg), calcium (40mg), iron (1.07mg), phosphorus (58mg), potassium (450mg). Major chemical constituents that are present in methanol extract of rhizome were analysed through cold maceration method by High Resolution Mass Spectrometry (HR-LCMS) techniques. The compounds include: Betulinic acid, Rutin, Isoquercetin, 2R - aminohexadecanoic acid, Phytosphingosine, Sphinganine, Phorbol, Ginkgolide B, Tetrahydroxy- 2,6- dimethyl anthroquinone, Pseudouridine, p – Hydroxyphenobarbital, Fluoroacetate, Isoamyl nitrite, Metronidazole, Napthaldehyde, Acetoin. The results showed that bioactive compounds identified by HR-LCMS from rhizome of *Nelumbo nucifera* have revealed a very good potential to be explored as food supplements or even pharmaceutical products to improve human health.

Keywords: *Nelumbo nucifera*, HR-LCMS, rhizome, nutrient composition, therapeutic property, bioactive compounds

Introduction
Identification and isolation of active phytocompounds is the preliminary step in designing plant based drugs. Plant extracts and bioactive compounds isolated from medicinal plants are used for antibacterial, antifungal and antiviral therapy (Pawar and Nazreen, 2018). Moreover, a quarter of the allopathic medications are based on compounds isolated from natural products. With increase in drug recalls resulting from severe side effects, the pharmaceutical industry also is interested in finding new drugs from natural sources with fewer or no side-effects. Recently, these traditional medicines are receiving more scientific support which helps in not only authenticating the use of these medicines for treatment but also understanding the mechanism of action of these drugs (Fernandes and Banu, 2012).

Lotus (*Nelumbo nucifera* Gaertn.) produces highly valued flowers and rhizome which is an underutilised vegetable. In addition, leaves, seeds, stems and other parts are edible and have many medicinal properties (Mukherjee *et al*., 2009) [21]. This plant is naturally seen as well as grown throughout the tropics. It has economic value where rhizomes are popular because of its crispness, attractive white colour and abundant nutrients. They can be eaten either as cooked or raw form and are rich in health promoting compounds such as alkaloids, lipids, flavonols, carotenes, aporphine, nuciferine, phospholipids, flavonoids, xanthophylls, and minerals (Li *et al.,* 2017) [15].

Lotus belongs to the *Nelumbolaceae* family and the genus *Nelumbo*. There are only 2 species in this genus: *Nelumbo nucifera* with pink, red or white flowers, distributed in Asia and Oceania, and *Nelumbo lutea* with yellow flowers, distributed in North and South America (Man *et al*., 2012) [17].

Contents of fresh rhizome is described to have 81.42g water, 66kcal energy, 0.07g fat, 0.52g sugars, 1.58g protein, 3.1g fibre, 26mg calcium, 78mg phosphorus, 363mg potassium, 0.9mg iron The vitamins thiamine (0.12mg/100 g), riboflavin (0.01 mg/100 g), niacin (0.30mg/100 g) and ascorbic acid (27.4 mg/100 g) are also present in the rhizomes (Sheikh 2014) [31].

The lotus rhizomes are rich in minerals and are consumed as health foods. It has profuse starch grains throughout the tissue. Fresh rhizome contains 31.2% starch, which shows no characteristic taste or odour (Fatima *et al*., 2018) [6]. Mukherjee *et al*. (1997) found that the methanol extract of the rhizome contained a steroidal triterpenoid – betulinic acid.

Zhao *et al*. (2014) identified the compounds astragaline, rutin, isoquercetin, nuciferine, dauricine, isoliensinine, and neferine. Lotus rhizome contains high levels of polysaccharides. Jiang *et al*. (2011) [12] isolated two antioxidant micro-molecular components (gallocatechin, catechin) and an antioxidant macromolecular component LB2 from lotus rhizome.

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The LB2 was identified as a polysaccharide sulfate containing ß-pyranose and α-furanose, with a molecular mass of 18.8 kDa.

Studies on different parts of *N. nucifera* have shown a variety of pharmacological activities. Extracts of different parts have shown anti-ischaemia, antioxidant, anticancer, antiviral, anti-obesity, lipolytic, hypocholesterolaemic, antipyretic, hepatoprotective, hypoglycaemic, antidiarrhoeal, anti- fungal, antibacterial, anti-inflammatory and diuretic activities (Mukherjee *et al.*, 1996) [22].

Mukherjee *et al.* (2009) [21] suggests that the extract of rhizome of *N. nucifera* stimulate defense system by modulating several immunological parameters.

Tsuruta *et al.* (2012) [33] reported that polyphenolic extract of lotus rhizome can alleviate hepatic steatosis in obese diabetic db/db mice. The condensed tannins present in lotus rhizome can relieves hepatic steatosis by suppressing the lipogenic enzyme activity in the liver of diabetes mice.

The rhizome from lotus is nutritious and could be used for the treatment of diarrhoea, dysentery and dyspepsia for children (Jain, 1993). The rhizome node is traditionally used for the treatment of nasal bleeding, hematuria and functional bleeding of the uterus (Yang *et al.*, 2007) [35].

Two antioxidant micro-molecular components (gallocatechin, catechin) and an antioxidant macromolecular component LB2 were isolated from lotus rhizome, and these compounds have good potential for their antiviral and immunoregulatory activities. The antioxidant components gallocatechin, catechin and LB2 strongly inhibited HIV-1 reverse transcriptase and integrase (Jiang *et al.*, 2011) [12].

Materials and Methods
Plant material
The fresh and healthy lotus rhizomes were collected from farmers' field of Palakkad and Malappuram districts, Kerala, India.

Physico-chemical qualities
Moisture
Moisture content of fresh lotus rhizome was estimated by the method of A.O.A.C (1980) [1]. Moisture content of the sample was determined by taking the known weight of the sample and then drying in a hot air oven at 60^0C to 70^0C, which was then cooled in a desicator and weighed. Until a constant weight was achieved the process of heating and cooling was repeated. Loss in weight during drying gave the moisture content of the sample. The moisture content of the sample was calculated from the loss in weight during drying.

Starch
Starch was estimated colourimetrically using anthrone reagent as suggested by Sadasivam and Manickam (1992) [29]. Rhizome was weighed to 0.5 g and extracted with 80 per cent ethanol to eliminate the sugars. The residue was dried on a water bath and 6.5 ml of 52 per cent perchloric acid and 5 ml water was added and extracted at 0^0C for 20 minutes. The supernatant was agglomerated and made up to 100 ml. The supernatant was pipetted out to 0.2ml and made up to one ml with water and 4 ml of anthrone reagent, heated for 8 minutes, cooled and absorbance was read at the OD at 630 nm.

A standard graph was drawn using serial dilutions of standard glucose solution. From the graph, glucose content of the sample was determined and the value was multiplied by a factor of 0.9 to arrive at the starch content and indicated in g per 100g of sample.

Carbohydrate
The carbohydrate content of rhizome was estimated by the method suggested by Sadasivam and Manickam (1997) [29]. A dried sample was weighed to 100mg and hydrolysed with 5ml of 2.5N HCl for 3 hours by boiling in a water bath and cooled to room temperature. The residue was counteracted with sodium carbonate until effervescence ceases. The volume was made up to 100ml and centrifuged. Pipetted 0.2ml of the supernatant was made up to 1ml and then 4ml of anthrone reagent was added. Heated for 8 minutes in a boiling water bath, cooled rapidly and the intensity of green to dark colour was read at 630nm (OD). A standard graph was constructed using standard glucose by adding the serial dilutions. From the standard graph, the amount of total carbohydrate present in the sample was calculated and expressed in gram per 100g of sample.

Protein
The protein content of lotus rhizome was estimated using Lowry's method given by Sadasivam and Manickam (1997) [29]. A sample of 500mg was extricated using 5 to 10 ml of buffer (Tris buffer GR – tris hydroxymethyl amino methane) and centrifuged. An aliquot 0.1 ml from the supernatant was taken in a test tube, 5 ml alkaline copper solution was mixed well and allowed to stand for 10 minutes. Folin-Ciocalteau reagent of 0.5 ml was added and kept at room temperature in the dark for 30 minutes and the intensity of developed blue colour was read at 660nm (OD). A standard graph was drawn using alkaline copper solution and Folin-Ciocalteau reagent by applying serial dilutions of standard solutions. From the standard graph, the amount of total protein present in sample was estimated and indicated in gram per 100g of sample.

Fibre
The crude fibre content of lotus rhizome was estimated using the method given by Sadasivam and Manickam (1997) [29]. Sample of two grams was boiled with 200 ml of 1.25 per cent sulphuric acid for 30 minutes. It was then filtered and washed with boiling water. The residue was again boiled with 200 ml of 1.25 per cent of sodium hydroxide for 30 minutes. The filtration was repeated through muslin cloth and residue was washed with 25 ml of boiling 1.25 per cent of sulphuric acid, three 50 ml portion of water and 25 ml of alcohol. The obtained residue was taken in an ashing dish (W_1) and dried at 130^0C for 2 hours. The dish was cooled in a desiccator which was reweighed and noted as W_2. The residue was again ignited in muffle furnace at 600^0C for 30 minutes, cooled in a desiccator and reweighed (W_3).

Fat
The fat content of the fresh rhizome was estimated using the method given by Sadasivam and Manickam (1997) [29]. Five gram of sample was taken in a thimble and stoppered with cotton. The material was extricated with petroleum ether for six hours without interruption by gentle heating in a soxhlet apparatus. Extraction flask was then cooled and ether was separated by heating and the weight was noted. The fat content was expressed in gram per 100g of the sample.

Vitamin C
The vitamin C content was estimated by method suggested by Sadasivam and Manickam (1992). Three grams of lotus rhizome sample was extracted with 4 per cent oxalic acid, made up to 100ml with oxalic acid and supernatant was titrated against the 2,6 – dichlorophenol indophenol dye until

the appearance of a pink colour which remained for a few seconds. Vitamin C content was expressed in mg per 100g of the sample.

Total ash
The ash content of the lotus rhizome was analysed using the method given by ISI (1980) [10]. Five grams of sample was weighed in a crucible and then was ignited at 550- 600⁰C in a muffle furnace for 5 to 6 hours. Cooled in a desiccator at room temperature and weighed. The ash content of sample was indicated in percentage.

Calcium
Calcium content present in lotus rhizome was estimated using method suggested by Perkin – Elmer (1982) [26]. One gram of the lotus rhizome was pre-digested using 10 ml of 9:4 ratio of nitric and percholoric acid. The prepared diacid extract of the rhizome sample was used for estimation of calcium in Atomic Absorption Spectrophotometer. The amount of calcium content present in sample was expressed as mg per 100g.

Iron
Iron content present in selected lotus rhizome was analysed using method suggested by Perkin – Elmer (1982) [26]. One gram of the lotus rhizome was pre-digested using 10 ml of 9:4 ratio of nitric and percholoric acid. The prepared diacid extract of the rhizome sample was used for analysing of iron in Atomic Absorption Spectrophotometer. Iron content present in the sample was expressed as mg per 100g.

Phosphorus
The phosphorus content was analysed colorimetrically as suggested by Jackson (1973) [11], which gives yellow colour with nitric acid vandate molybdate reagent. To 5 ml pre-digested aliquot, 5 ml of nitric acid vandate molybdate reagent was added and made up to 50 ml with distilled water. After 10 minutes, the OD was read at 420 nm. A standard graph was plotted by serial dilution of standard phosphorus solution. The phosphorous content was expressed in mg per 100g.

Potassium
Potassium present in lotus rhizome was estimated using method suggested by Jackson (1973) [11] with the help of Flame Photometer. One gram of the rhizome sample was digested using diacid solution. The pre-digested sample was used to measure potassium content in flame photometer and it was expressed as mg per 100g of the sample.

Preparation of plant extract
Cold maceration extraction method using hexane and methanol was done. One hundred gram of lotus rhizome powder was soaked in 250ml of hexane for 24 hours and then filtered. The extraction was repeated thrice in hexane. The residue obtained was then extracted thrice with methanol. Methanol extract was concentrated using rotary evaporator and prepared a 10 ppm solution with methanol. It was then filtered using 0.22 μM PVDF syringe filter and transferred to vial.

High Resolution Liquid Chromatography and Mass Spectrometry (HR-LCMS) analysis
The extract was prepared in methanol and then subjected to HR-LCMS analysis. Chemical fingerprints of lotus rhizome extract was prepared with 0.01% mass resolution (Agilent

high resolution liquid chromatography and mass spectrometry model- G6550A). The acquisition method was set to be MS-minimum range 50 (M/Z) and maximum 1000 dalton (M/Z) with scanning rate each spectrum per second. Gas chromatography had maintained at 250˚C with gas flow 13 psi/minute. Hip sampler with model- G4226A was used with auxiliary speed 100 μl/minute, ejection speed 100 μl/minute, flush out factor 5 μl and 8 μl injection volume used for HR-LCMS. Within 30 min acquisition time, initial 2 min the flow of solvent composition A: B was 95: 5.
Solvent used for HR-LCMS includes
1. 100% Water
2. 100% Acetonitrile

Result and discussion

Table 1: Nutritional composition of *Nelumbo nucifera* rhizome

Nutrient	Amount
Moisture (%)	72.14
Starch (g)	10.05
Carbohydrates (g)	16.03
Protein (g)	2.60
Fat (g)	0.10
Fibre (g)	4.20
Vitamin C (mg)	38.00
Total ash (g)	1.18
Calcium (mg)	40.00
Iron (mg)	1.07
Phosphorus (mg)	58.00
Potassium (mg)	450.00

The nutritional constituents of the fresh lotus rhizomes were estimated. The constituents like moisture, starch, carbohydrates, protein, fibre, vitamin C, total ash, calcium, iron, phosphorus, sodium, potassium content of the samples were analysed. The results pertaining to nutritional constituents of raw rhizome are presented in Table-1.
The mean moisture content in raw lotus rhizome was found to be 72.14 per cent. Moisture content of 75.40 per cent and 77.58 per cent were reported by Li *et al.* (2017) [15] and Khattak *et al.* (2009) [13] in lotus root. Mukherjee *et al.* (2009) [21] found moisture content of 83.80 per cent of lotus rhizome and it is high when compared to moisture content of lotus rhizome in this study.
The starch content of lotus rhizome was found to be 10.05 per cent. According to Mukherjee *et al.* (2009) [21] the starch content of the fresh rhizome was 9.25 per cent which is observed to be slightly lower than the starch value obtained in this study. Faruk *et al.* (2012) [5] reported that the starch content of water chestnut was 8.7 per cent. Syed *et al.* (2012) [32] reported that the fresh lotus root contained 15 per cent of starch and it can be used in the manufacturing of food products such as imparting texture and consistency and as functional ingredients like thickeners, stabilizers and gelling agent. Geng *et al.* (2007) [8] reported that lotus starches had highest percentage of amylose 21.16 per cent and possessed good clarity and gel strength.
The carbohydrate content of lotus was found to be 16.03 g and according to Khattak *et al.* (2009) [13] the carbohydrate content of lotus rhizome were 16.60 g/ 100g and it is related to the value obtained in this study. Sheikh (2014) [31] reported that content of carbohydrate in lotus rhizome was 16.02g.
In the present study, protein content of fresh lotus rhizome was found to be 2.60. This is in line with the observations of Khattak *et al.* (2009) [13] and Mukherjee *et al.* (2009) [21] who

reported the content of protein in lotus rhizome of 2.41and 2.70 per cent. Paudel and Panth (2015) [25] reported protein content of 1.70 per cent in lotus rhizome and it is less when compared to the protein content of lotus rhizome in this study. In the present study, the fat content was found to be 0.10 g. Mukherjee *et al*. (2009) [21] reported that the fat content of the fresh rhizome was 0.11 per cent and a similar findings of Sheikh (2014) [31] revealed that the lotus root contained 0.07g of fat.

In the present study the fibre content were 4.20 per cent. Sheikh (2014) [31] reported that lotus rhizome contain crude fibre of 3.10 g per 100g. Khattak *et al*. (2009) [13] observed the fibre content to be 1.63g. Paudel and Panth (2015) [25] reported fibre content of 0.80 per cent in lotus rhizome and it is less when compared to the fibre content of lotus rhizome in this study.

In the present study the content of vitamin C were 38mg per 100g and Sheikh (2014) [31] reported that the ascorbic acid content of lotus rhizome as 27.4 mg. A similar findings of NIN (2002) [23] revealed that vitamin C content of lotus rhizome was found to be 28mg per 100g.

The total ash content in this study was observed to be 1.18 g. Mukherjee *et al*. (2009) [21] stated that the ash content of the fresh lotus rhizome was 1.10 per cent and it is also in the line with Khattak *et al*. (2009) [13] who reported the ash content of lotus rhizome to be 1.22 per cent. In the study of Faruk *et al*. (2012) [5] the ash content of water chestnut was found to be 1.04% per 100g.

Calcium content of lotus rhizome was found to be 40 mg per 100g. Sheikh (2014) [31] reported calcium content of 27.4mg in lotus rhizome. Faruk *et al*. (2012) [5] stated that calcium content in water chestnut was 0.26% which was found to be low than the calcium content in lotus rhizome.

In the present study the iron content was observed to be 1.07 mg. A similar findings of Sheikh (2014) [31] revealed that iron content in lotus root was 0.9mg. Mukherjee *et al*. (2009) [21] reported the iron content of 0.05 per cent which was found to be low than the iron content in this study.

In the present study the content of phosphorus was 58 mg. Sheikh (2014) [31] stated that the phosphorus content of lotus rhizome was 78mg. Faruk *et al*. (2012) [5] reported that the iron content of water chestnut was 6.77% per 100g.

In this study the potassium was found to be 450mg in lotus rhizome and it is in the line with the observations of Sheikh (2014) [31] who reported potassium of 363 mg per 100g.

Table 2: Bioactive compounds in methanol extract of *Nelumbo nucifera* rhizome

| Compounds	Retention time	Mass	Formula
Betulinic acid	21.28	456.3529	C30 H48 O3
Rutin	16.98	610.1399	C27 H30 O16
Isoquercetin	16.12	464.0916	C21 H20 O12
2R - aminohexadecanoic acid	15.17	271.2451	C16 H33 N O2
Phytosphingosine	14.91	317.2858	C18 H39 N O3
Sphinganine	14.80	273.2650	C16 H35 N O2
Phorbol	14.48	364.1825	C20 H28 O6
Ginkgolide B	14.33	424.1431	C20 H24 O10
Tetrahydroxy- 2,6- dimethyl anthroquinone	13.90	300.0619	C16 H12 O6
Pseudouridine	13.08	244.0665	C9 H12 N2 O6
p - Hydroxyphenobarbital	10.09	248.0782	C12 H12 N2 O4
Fluoroacetate	1.20	78.0112	C2 H3 F O2
Isoamyl nitrite	1.14	117.0762	C5 H11 N O2
Metronidazole	1.03	171.0647	C6 H9 N3 O3
Napthaldehyde	0.89	156.0596	C11 H8 O
Acetoin	0.38	88.0523	C4 H8 O2
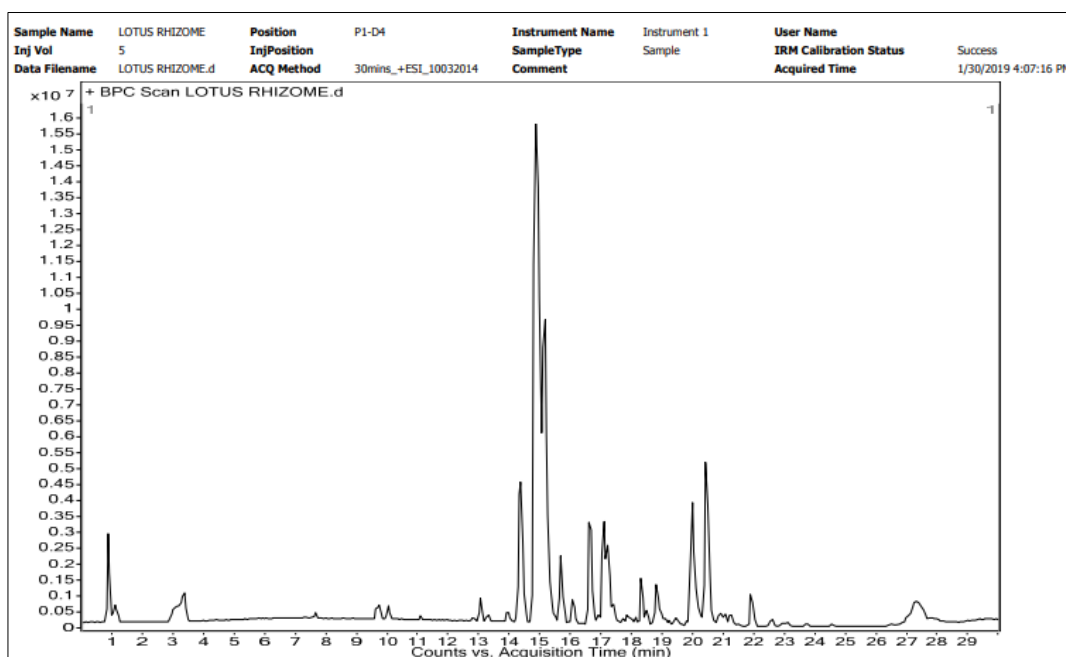
Fig 1: Chromatogram of methanolic Extract of *Nelumbo nucifera* rhizome

HR-LCMS analysis of methanol extract of *Nelumbo nucifera* rhizome showed 6 major peaks indicating the presence of various phytochemical constituents. On comparison of the high resolution liquid chromatography and mass spectra of constituents with the main library all these compounds were characterised and probably identified. Identified compounds were Betulinic acid, Rutin, Isoquercetin, 2R - aminohexadecanoic acid, Phytosphingosine, Sphinganine, Phorbol, Ginkgolide B, Tetrahydroxy- 2,6- dimethyl anthroquinone, Pseudouridine, p – Hydroxyphenobarbital, Fluoroacetate, Isoamyl nitrite, Metronidazole, Napthaldehyde, Acetoin.

Betulinic acid is a triterpenoid isolated from birch trees and even found in other botanical sources (Czuk, 2014). Rios and Manez (2018) [28] stated that it had wide range of pharmacological activities in the fields of immunity and metabolism, namely antidiabetic, antihyperlipidemic, and anti-inflammatory antiviral and antitumor activities.

Isoquercetin is a dietary flavonoid present in a variety of medicinal and dietary plants, including vegetables, herbs and flowers (Morand *et al.*, 2000) [18]. Zhou *et al.* (2014) [36] reported that isoquercetin had numerous therapeutic properties, including anti-inflammatory, antioxidant and anti-allergic activities. Morand *et al.* (2000) [19] also demonstrated that isoquercetin was better absorbed than quercetin and had higher bioavailability.

Harborne (1986) [9] described that rutin is a flavonol, abundantly found in plants, and is a vital nutritional component of food stuff. Ganeshpurkar and Saluja (2017) [7] revealed that it contained a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities.

Phytosphingosine is an endogenous, bioactive sphingolipid present in fungi, plants, and the corneous layer of human skin in low concentrations. It possessed antimicrobial and antitumor property (De Jesus Cortes-Sanchez *et al.*, 2013) [4].

Lloyd-Evans *et al.* (2008) suggested the possibility that endogenous sphinganine may inhibit cholesterol transport in Niemann-Pick Type C (NPC) disease.

Bond *et al.* (2007) reported that administration of phorbol myristate acetate can act effectively against pancreatic cancer.

Anthraquinones constitute an important class of natural and synthetic compounds used as colorants. Anthraquinone derivatives have been used as laxatives, antimicrobial and anti-inflammatory agents. Current therapeutic indications included constipation, arthritis, multiple sclerosis, and cancer (Malik and Muller, 2016).

Van den Broek *et al.* (2012) [34] reported that administration of phenobarbital under hypothermia reduced the transition rate from a continuous normal voltage (CNV) to discontinuous normal voltage in hypothermic asphyxiated newborns, which may be attributed to the additional neuroprotection of phenobarbital in infants with a CNV pattern.

Metronidazole played an important role in anaerobic- related infections and proved to be an antibiotic (Lamp, 1997) [14].

Amyl nitrite are used in the treatments of hypertension or ischemic heart disease, and was discovered to possess novel pharmacologic actions such as modulating hypoxic vasodilation and providing cytoprotection in ischemia-reperfusion injury (Nossaman *et al.*, 2010) [24].

Ramdas *et al.*, (2006) [27] revealed that the phytochemical plays an important role in the treatment of diseases without any side effects, there is a need to search new drugs from natural sources. India is a home to a variety of traditional medicine system that relay to a very large extent on native plant species for new drug materials. Therefore now there is a need to look back towards traditional medicine which can serve a novel therapeutic agent (Chitravadivu *et al.*, 2009) [2]. The pharmacognostical evaluations also give valuable information which is essential to standardize the drug.

Conclusion
Lotus rhizome was nutritionally evaluated and was found to be rich in fibre, vitamin C and minerals. Its negligible fat content contributes it to be a very interesting constituent for fat free diets. The result of HR-LCMS analysis specifies that the methanol extract of *Nelumbo nucifera* rhizome contains various valuable secondary compounds which have various medicinal properties that can be useful for the treatment of various diseases. Since, lotus rhizome has good nutritional profile in addition to medicinal properties, it can be used as a functional ingredient in food industry.

References
1. AOAC. Official and Tentative Methods of Analysis (13th Ed.). Association of Official Analytical Chemists. Washington. D. C, 1980, 1018p.
2. Chitravadivu C, Manian S, Kalaichelvi K. Qualitative Analysis of selected Medicinal Plants, Tamil Nadu, India. Middle-East J Sci. Res. 2009; 4(3):144-146.
3. Csuk R. Betulinic acid and its derivatives: a patent review (2008–2013). Expert Opin Ther Pat. 2014; 24(8):913-923.
4. De Jesus Cortes-Sanchez A, Hernandez-Sanchez H, Jaramillo-Flores ME. Biological activity of glycolipids produced by microorganisms: new trends and possible therapeutic alternatives. Microbiol res. 2013; 168(1):22-32.
5. Faruk MO, Amin MZ, Sana NK, Shaha RK, Biswas KK. Biochemical analysis of two varieties of water chestnuts (*Trapa* sp.). Pak J Biol Sci. 2012; 15(21):1019-26.
6. Fatima T, Iftikhar F, Hussain SZ. Ethno-medicinal and pharmacological activities of lotus rhizome. J Pharm. Innov. 2018; 7(4):238-241.
7. Ganeshpurkar A, Saluja AK. The pharmacological potential of rutin. Saudi Pharm J. 2017; 25(2):149-164.
8. Geng Z, Zongdao C, Yimin W. Physicochemical properties of lotus (*Nelumbo nucifera* Gaertn.) and kudzu (*Pueraria hirsute* Matsum.) starches. Int J Food Sci Tech. 2007; 42(12):1449-1455.
9. Harborne JB. Nature, distribution and function of plant flavonoids. Prog Clin Biol Res. 1986; 213:15-24.
10. ISO. [International Organization for Standardization]. Starch-Determination of moisture content-Oven-drying methods. International Organization for Standardization, 1980. Available:
https://www.iso.org/obp/ui/#iso:std:iso:3593:ed-2:v1:en
11. Jackson ML. Soil Chemical Analysis. Prentice Hall of India Private Ltd, New Delhi, 1973, 299p.
12. Jiang Y, Ng TB, Liu Z, Wang C, Li N, Qiao W, Liua F. Immunoregulatory and anti-HIV-1 enzyme activities of antioxidant components from lotus (*Nelumbo nucifera* Gaertn.) rhizome. Bioscience reports. 2011; 31(5):381-390.
13. Khattak KF, Simpson TJ. Effect of gamma irradiation on the microbial load, nutrient composition and free radical scavenging activity of *Nelumbo nucifera* rhizome. Radiat. Phys. Chem. 2009; 78(3):206-212.

14. Lamp K. Metronidazole–A therapeutic Rewie and Update. Drugs. 1997; 54(5):679-708.
15. Li S, Li X, Lamikanra O, Luo Q, Liu Z, Yang J. Effect of cooking on physicochemical properties and volatile compounds in lotus root (*Nelumbo nucifera* Gaertn). Food chem. 2017; 216:316-323.
16. Lloyd-Evans E, Morgan AJ, He X, Smith DA, Elliot-Smith E, Sillence DJ *et al*. Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. Nat med. 2008; 14(11):1247.
17. Man J, Cai J, Cai C, Xu B, Huai H, Wei C. Comparison of physicochemical properties of starches from seed and rhizome of lotus. Carbohydr Polym. 2012; 88(2):676-683.
18. Morand C, Manach C, Crespy V, Remesy C. Quercetin 3-O-β-glucoside is better absorbed than other quercetin forms and is not present in rat plasma. Free Radic Res. 2000; 33(5):667-676.
19. Morand C, Manach C, Crespy V, Remesy C. Quercetin 3-O-β-glucoside is better absorbed than other quercetin forms and is not present in rat plasma. Free Radic Res. 2000; 33(5):667-676.
20. Mukherjee PK, Das J, Saha K, Giri SN, Pal M, Saha BP. Antipyretic activity of *Nelumbo nucifera* rhizome extract. Ind J Exp Biol. 1996; 34:275-276.
21. Mukherjee PK, Mukherjee D, Maji AK, Rai S, Heinrich M. The sacred lotus (*Nelumbo nucifera*)–phytochemical and therapeutic profile. J Pharm. Pharmacol. 2009; 61(4):407-422.
22. Mukherjee PK, Saha K, Balasubramanian R, Pal M, Saha BP. Studies on psychopharmacological effects of *Nelumbo nucifera* Gaertn. rhizome extract. J Ethnopharmacol. 1996; 54:63-67.
23. NIN. [National Institute of Nutrition]. Nutritive Value of Indian Foods, Indian Council of Medical Research, Hyderabad, 2007, 43p.
24. Nossaman VE, Nossaman BD, Kadowitz PJ. Nitrates and nitrites in the treatment of ischemic cardiac disease. Cardiol rev. 2010; 18(4):190.
25. Paudel KR, Panth N. Phytochemical profile and biological activity of *Nelumbo nucifera.* Evid. Based Complement. Alternat. Med. 2015, 1-16.
26. Perkin-Elmer. Analytical Methods for Atomic Absorption Spectrophotometry. Perkin- Elmer Corporation, USA, 1982, 114p.
27. Ramadas K, Ramachandra YL, Rai PS. Antibacterial activity of the leaf extracts of *Asparagus racemosus*. Geobios. 2006; 33:279-280.
28. Rios JL, Manez S. New pharmacological opportunities for betulinic acid. Planta Med. 2018; 84(01):8-19.
29. Sadasivam S, Manickam A. Biochemical Methods (2nd Ed.). New Age International private limited, New Delhi and Tamil Nadu Agricultural University, Coimbatore, 1997, 254p.
30. Sefa-Dedeh S, Agyir-Sackey EK. Chemical composition and the effect of processing on oxalate content of cocoyam *Xanthosoma sagittifolium* and *Colocasia esculenta* cormels. Food Chem. 2004; 85(4):479-487.
31. Sheikh SA. Ethno-medicinal uses and pharmacological activities of lotus (*Nelumbo nucifera*). J Med. Plants Stud. 2014, 42-46.
32. Syed A, Singh S, Longowal P. Physicochemical, Thermal, Rheological and Morphological Characteristics of Starch from Three Indian Lotus Root (*Nelumbo nucifera* Gaertn) Cultivars. Food Process. Technol, 2012.
33. Tsuruta Y, Nagao K, Shirouchi B, Nomura S, Tsuge K, Koganemaru K *et al*. Effects of lotus root (the edible rhizome of *Nelumbo nucifera*) on the deveoolpment of non-alcoholic fatty liver disease in obese diabetic db/db mice. Biosci Biotechnol Biochem. 2012; 76(3):462-466.
34. Van den Broek MPH, Groenendaal F, Toet MC, Van Straaten HLM, Van Hasselt JGC, Huitema ADR *et al*. Pharmacokinetics and clinical efficacy of phenobarbital in asphyxiated newborns treated with hypothermia. Clin pharmacokinet. 2012; 51(10):671-679.
35. Yang DM, Wang QS, Ke LQ, Jiang JM, Ying TJ. Antioxidant activities of various extracts of lotus (*Nelumbo nuficera* Gaertn) rhizome. Asia Pacific J Clin Nutr. 2007; 16:158-163.
36. Zhou J, Yoshitomi H, Liu T, Zhou B, Sun W, Qin L *et al*. Isoquercitrin activates the AMP–activated protein kinase (AMPK) signal pathway in rat H4IIE cells. BMC Complement Altern Med. 2014; 14(1):42.