IJPSR (2022), Volume 13, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 227 March 2022; received in revised form, 15 May 2022; accepted, 06 June 2022; published 01 November 2022

CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF SEED EXTRACT OF *N. NUCIFERA*

Saya Tyagi [1], Anuradha Shukla [1], Pankaj Jain [2] and Rashmi Tripathi [*1]

Department of Bioscience and Biotechnology [1], Department of Pharmacy [2], Banasthali Vidyapith, Banasthali - 304022, Rajasthan, India.

Keywords:

Antioxidant activity, FTIR, GCMS, Hydroethanol, *N. nucifera* and phytochemical analysis

Correspondence to Author:
Dr. Rashmi Tripathi

Assistant Professor,
Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali - 304022, Rajasthan.

E-mail: tripathi.rashmi@gmail.com

ABSTRACT: *N. nucifera*, often recognized as lotus, is an aquatic plant. Extracts of various solvents have shown therapeutic potential against many diseases. This research focused on screening preliminary qualitative phytochemicals and investigating the seed's antioxidant activity. Primary and secondary metabolites were discovered in a hydroethanolic fraction of different extracts of lotus seeds. The extractive value, total ash (3.56±0.36) water-soluble (0.86±0.68), and acid insoluble ash value (2.09±0.88) were determined. The solvents benzene, chloroform, ethyl acetate, n-butanol, and aqueous were used as extracts. TPC and TFC content of ethyl acetate extract of seeds were found to be 366.6±10.77 (mg GAE/ g^{-1} dry weight) and 344.4±3.71 (mg RE/ g^{-1} dry weight), respectively. The ethyl acetate fraction of the seed extract showed the lowest value determining the highest free radical scavenging activity (105.30 ± 12.06µg/ml) in DPPH and FRAP (142.2± 0.0509 mgAA gm^{-1}). The values were found to be close as compared to the standard (56.40±23.63µg/ml).The results support high antioxidant content in the seed extract of *N. nucifera*. FTIR and GCMS did the characterization of compounds. The results conclude that seeds of *N. nucifera* can be included in medicine as it shows pharmaceutical and therapeutic potential.

INTRODUCTION: Plants have been a part of our lives for ages and are an ancient form of medicine known to human. The various products which are obtained from the plant are not only good for health but also play an important role in our existence. Herbal medicine is a type of medicine that uses natural plant compounds to prevent and treat illnesses [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(11).4608-19

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(11).4608-19

Alkaloids, glycosides, terpenes, steroids, flavonoids, tannins and other secondary metabolites are found in herbal plants. Some chemical substances, such as tannins and phenols or their oxygen-substituted derivatives, whereas others may include nitrogen or sulphur and are physiologically active and beneficial in the treatment and prevention of illness in humans and animals [2, 3].

Antioxidant chemicals are found in many medicinal plants and protect cells from the harmful effects of reactive oxygen species (ROS) [4, 5]. When the balance between ROS production and antioxidant defence is disrupted, oxidative stress occurs, triggering a cascade of events that disrupt cellular function and result in diseases such as ageing,

arthritis, asthma, liver disorders, carcinogenesis, diabetes, rheumatism and a variety of neurodegenerative diseases [6]. The plant *N. nucifera* has been used for its medicinal properties for ages. It is cultivated in India and is a national flower. In India, *N. nucifera* is commonly known as lotus, Kamala; the different parts being used in herbal medicines for ages. The seeds of the lotus *N. nucifera* are edible, medicinally significant, and a key component of age-old conventional medical treatments such as indigenous medicine [7, 8]. Seeds are sold as kamal gatta and consumed as a vegetable. The seeds contain hepatoprotective and free radical scavenging properties [9, 10]. This research aimed to look into the pharmacognostical and antioxidant properties of *N. nucifera* seed extract. The presence of secondary metabolites in its extracts was discovered using phytochemical screening. The total flavonoid and phenolic content were calculated using the aluminum chloride colorimetric method and Folin–ciocalteu's, respectively, to determine the extract's quantity of flavonoids and phenols. Various functional groups and chemicals were determined using FTIR and GCMS analysis.

MATERIALS AND METHODS:

Accumulation of Plant Sample: The natural healthy plant seeds of *Nelumbo nucifera* were acquired from the Drona Sagar, Kashipur, Uttarakhand, India in November 2018. Taxonomists from the department recognized and authenticated the plant, which Dr. P. K. Mishra subsequently confirmed, HOD, Dept. of Botany, VBU, Hazaribagh, and a voucher specimen with the accession no. VBU/BOT/00368/2004 has been reserved in the Department of Botany, Vinoba Bhave University, Hazaribagh.

Plant Material: Fruit is a clump of indigestible nutlets. The ripe nutlets are ovoid, roundish, or oblong in shape, measuring up to 1.0 cm in length, and 1.5 cm in width, with a firm, smooth greyish black pericarp and one seed. The lotus seeds were crushed in a mortar pestle, and the outer seed coat was removed. The seeds collected were grounded in a mixer to get fine powder, then kept in an airtight jar for future testing and analysis.

Phytochemical Profiling of *N. nucifera* Seed Extracts Prepared in Different Solvents: The pharmacognostic characteristics were investigated [11, 3].

Assessment of Total Ash Content: The total ash value was determined in crucibles. The crucibles were washed, dried, and weighed. About 2gms of the plant material powder was placed in each crucible, weighed, and ashed in a muffle furnace at 450°C for six hours. The crucibles were cooled and weighed.

$$\text{Total ash value (sample)} = (Z\text{-}X) \times 100 / Y$$

Estimation of Acid Insoluble Ash Content: The ash obtained after the incineration of the plant material was taken with 25ml of dilute hydrochloric acid in a beaker. The content was boiled for 5 min and then passed through an 'ash less' filter paper. The remains on the filter paper were taken in the crucible and kept in the muffle furnace at 450°C for 4-5 h. The acid-insoluble fraction of the ash obtained on the filter paper was then weighed accurately after cooling the crucibles.

$$\text{Acid insoluble ash value (sample)} = a \times 100 / y$$

Determination of Water-Soluble Ash Content: This is determined using the same procedure as acid insoluble ash, with the exception that instead of dil HCl, 25ml of water is used.

Determination of Extractive Value: To determine the extractive value, 5gms of powder was taken and mixed in 100ml of solvents namely benzene, chloroform, n-butanol, ethyl acetate and aqueous. All the beakers were kept on a rocker shaker for 6h and left undisturbed for 18 h at room temperature. The weight of empty glass petriplates was taken and noted. The solutions passing through filter paper was poured into glass petri plates and kept in oven for drying. After drying weight of each petriplates were taken, and moisture was determined using the formula:

$$\% \text{ yield} = \text{weight of residue} \times 100 / \text{Sample weight}$$

Fluorescence Characteristics Analysis: To estimate the fluorescence characteristic, the seed powder was taken, and foreign particles were removed with the help of a sieve. The powder was taken on the watch glass, and the solvents were added. Then watch glasses containing samples were observed with UV fluorescence analysis

cabinet under different visible wavelengths UV short 254nm and UV long 366 nm[12].

Plant Extraction: The fine powder of the seed, about 10 gms was extracted with 100ml of hydro-ethanol solvent, with the proportion of ethanol, and water, respectively, in 70:30 ratios. The extract was then kept on a rotor shaker for 24h at normal temperature and filtered using Whatman filter paper, and further extraction was carried. After completing, the final extract was evaporated and dried at room temperature.

Separation of Extracts in Different Solvents: The separation was performed using a separating funnel, 3 gms of the extract was dissolved properly in 60 ml distilled water. This was introduced into a separating funnel, and 60 ml benzene was added and mixed well. The separation resulted in the formation of two layers, then the upper layer was separated, and the same process was repeated thrice. The same technique was used for all solvents, including benzene, chloroform, ethyl acetate, n-butanol, and aqueous.

Phytochemical Screening: Standard techniques were used to test the phytochemical content of an *N. nucifera* seed extract. To detect significant natural chemical groups, phytochemical screening was carried out in the extracts using various solvents. Tests for tannins, saponins, quinones, flavonoids, terpenoids, phenols, steroids, coumarins, anthocyanins, betacyanin's, carbohydrates, and protein were performed following standard protocols [13, 14, 15].

Estimation of Total Phenolic Content: The Folin–ciocalteu's method was followed to estimate the total phenolic content [16]. Added to the sample (1mg/ml) was 500 µl of the previously diluted Folin- Ciocalteu reagent (1:1). It was then incubated for 5min. 1ml of 20% sodium carbonate and 12.5 ml DW were put in and mixed into the mixture. It was left for 30min to 2h in the dark, and observation of the sample was taken at 720nm. TPC was estimated as mg gallic acid/100g as a standard compound. The gallic acid dissolved in methanolin the range 10-50 mg/ml to prepare the standard curve. To calculate the TPC Equation 1 was used.

T= CV / M

Where: T = Total phenolic concentration C = Concentration of gallic acid from calibration curve (mg mlG1) V = Volume of extract (ml) M = Weight of plant extract.

Total Flavonoid Content: The concentration of total flavonoids was measured using the aluminum chloride colorimetric technique ($AlCl_3$) with minor modifications using rutin as a reference [17]. Each extract 50 µl at a concentration of (1mg/ml) 1 ml sodium nitrite (5%) and 0.15 ml aluminum chloride (10%) were added. 1 ml 1N NaOH was added and vortexed vigorously. The OD was measured at 415 nm. The rutin standard solution in methanol was used to create the standard curve. The extracts' total flavonoid concentration was measured in milligrams of rutin equivalents per gram of dry weight. The total flavonoid content may be determined using the following formula.

T= CV / M

Where: T = Total phenolic concentration C = Concentration of quercetin from calibration curve (mg mlG1) V = Volume of extract (ml) M = Weight of plant extract

DPPH Assay in Antioxidant Activity Measurement: The radical scavenging action of the stable DPPH free radical was used to test the antioxidant activity of the plant extracts and standard [18]. The different concentrations of the fractions of the hydroethanolic extract in different solvents (benzene, chloroform, n-butanol, ethyl acetate) were taken (10µl to 300µl).

The volume was made up to 1 ml with methanol. Then 1.5 ml solution of freshly produced DPPH in methanol was added. After 30 min of incubation at room temperature in the dark, the absorbance of each solution was measured with a spectrophotometer at 517nm. The equivalent blank reading and ascorbic acid were taken, which was used as a standard, in various concentrations ranging from 20µl to120µl. The antioxidant activity was calculated using Equation 3.

Inhibition of DPPH activity (%) = A-B ×100 / A

Optical density of the control (A) and Sample (B)

FRAP in Determination of Antioxidant Activity: The activity of antioxidants was measured

spectrophotometrically using the FRAP assay [19]. The FRAP reagent was made by combining 300mM acetate buffer, 10mM TPTZ in 40mM HCl and 20mM $FeCl_3$. $6H_2O$ in a 10:1:1 ratio at 37°C.The freshly made FRAP reagent was mixed well with plant extracts.

After 30 min of incubation at 37°C, a strong blue colour complex developed and absorbance was measured at 593nm against blank. All the tests were carried out in triplicates. The FRAP values were computed in mg of ascorbic acid equivalent/ gm of plant material.

Compound Characterization by FTIR: The ethyl acetate fraction (mg/ml) was subjected to IR spectroscopy which showed the presence of functional groups. The transverse frequencies of infrared waves in wave number cm were used to identify the functional categories. Each functional group's transverse frequencies differed from one another. A Perkin-Elmer FT-IR spectrophotometer was used to evaluate the samples' FT-IR spectra.

GCMS Analysis: Using a Perkin Elmer GC clarus 500 system with an AOC-20i autosampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument, the following parameters were used to perform GC-MS analysis: Column TG 5MS, 5 percent phenyl methyl polysiloxane, operating in electron impact mode at 70 eV; carrier gas was helium (99.999 percent) at a constant flow of 1 ml/min, with an injection volume of 1µl (split ratio of 10:1); injector temperature 200°C; ion source temperature 200°C.

The oven temperature was programmed to climb gradually from 110°C to 200°C (isothermal for 2 min), then 5°C/min to 280°C, with a 9-min isothermal at 280°C.

With pieces spanning from 40 to 70 eV, the mass spectrum was obtained at 70 eV with a 0.5 s scan interval. The temperature of the MS transfer line is 280°C.

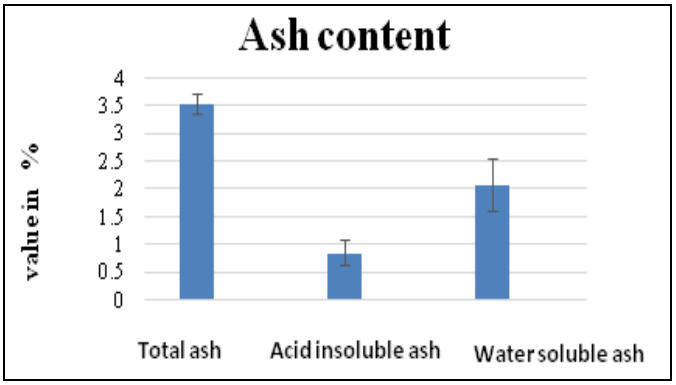
RESULT:

Ash Content Determination: The total ash content of *N. nucifera* seeds was found to be higher than the acid-insoluble and water-soluble ash values in this investigation **Table 1**. The Total ash (3.56±0.36), acid insoluble ash (0.86±0.68) and water-soluble ash (2.09±0.88) values were estimated to be below the standard values.

TABLE 1: PHYSICO-CHEMICAL EVALUATION OF THE SEEDS OF *N. NUCIFERA*

Parameters (% w/w)	*N. nucifera* (value in %)
Total ash	3.56±0.36
Acid insoluble ash	0.86±0.68
Water soluble ash	2.09±0.88

(Values expressed as Mean ±SD where n=3).



GRAPH 1: VALUE OF ASH CONTENT IN % (W/W) OF *N. NUCIFERA* SEEDS

Assessment of the Extractive Value: The active components in plant material, when extracted with different solvents, is determined by extractive value. When compared to other extracts, non-polar extracts had a higher extraction yield.

TABLE 2: EXTRACTIVE VALUES OF SEEDS OF *N. NUCIFERA* IN DIFFERENT SOLVENTS

Solvent	Extractive value (%)
Benzene	7.42±0.273
Chloroform	0.53±0.020
Ethyl acetate	0.37±0.025
n- butanol	3.5±0.251
Aqueous	2.29±0.083

(Values expressed as Mean ±SD where n=3)

Fluorescence Characteristics Analysis: Fluorescence is mostly an inherent and natural property of some natural compounds when exposed to different light conditions, generally the visible daylight. Herbal plants often exhibit fluorescence when their cut surface or powdered form is exposed to UV light.

However, certain compounds which do not fluoresce under the normally visible daylight can produce fluorescence under UV light. Moreover, non-fluorescent substances also get converted into their fluorescent derivatives on treatment with different reagents [20].

TABLE 3: DIFFERENT FLUORESCENCE CHARACTERISTICS OF THE SEED POWDER OF *N. NUCIFERA*

S. no.	Treatments	UV short (254nm)	UV long (366nm)	Visible
1	Powder	Creamish white	Fluorescent	Whitish cream
2	Powder + distilled water	Light green	Fluorescent black	Whitish cream
3	Powder + glacial acetic acid (GAA)	Light green	Fluorescent cream	Whitish cream
4	Powder + 1N HCl	Light green	Black	Creamish yellow
5	Powder +1N H_2SO_4	Dark green	Fluorescent black	Brownish yellow
6	Powder + conc. HNO_3	Dark green	Black	Dark yellow
7	Powder + $FeCl_3$ (5%)	Green	Black	Yellowish orange
8	Powder + iodine solution 5%	Brownish green	Fluorescent black	Dark brown
9	Powder + ammonia solution	Dark green	Fluorescent black	Dark Creamish,
10	Powder + 1N NaOH	Dark green	Black	Creamish peach
11	Powder + potassium dichromate	Green	Black	Yellowish orange
12	Powder + HNO_3 + NH_3 solution	Dark green	Black	Brownish yellow
13	Powder + ethanol	Fluorescent light green	Fluorescent black	Creamish brown
14	Powder + methanol	Fluorescent light green	Fluorescent black	Creamish brown
15	Powder + Toluene	Dark green	Black	Creamish white

Phytochemical Screening: Phytochemical screening is a useful method in the study of bioactive compounds.

It is a rapid, low-cost, and straightforward process that demonstrates the numerous types of phytochemicals found in plants. Phytochemicals indicate that the plant could be a potential source of precursors for synthetic medicine production. Phytochemicals are physiologically active molecules found in trace levels in plants that are not recognized as nutrients but contribute greatly to preventing degenerative diseases.

Phytochemical testing was completed to screen the existence of phytochemicals in different fractions of the current study's hydroethanolic extracts of N. nucifera seeds.

TABLE 4: PHYTOCHEMICAL SCREENING OF DIFFERENT FRACTIONS OF HYDROETHANOLIC EXTRACTS OF THE SEEDS OF *N. NUCIFERA*

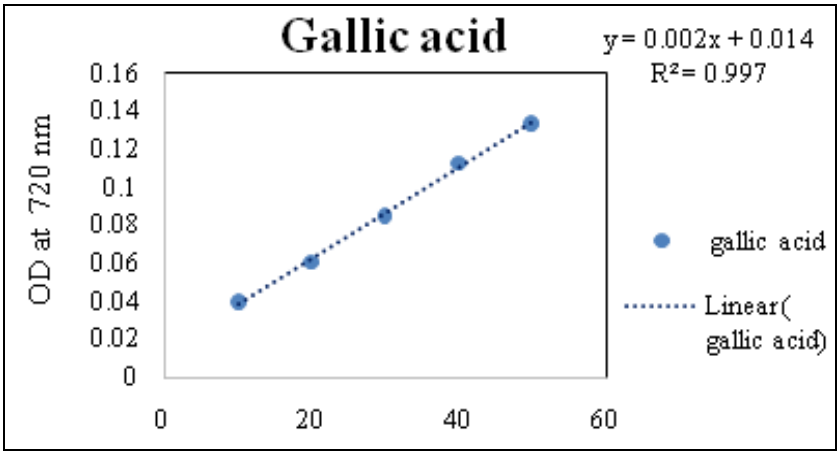
S. no.	Secondary metabolites	Phytochemical tests	Benzene	Chloroform	Ethyl acetate	N-butanol	Aqueous
1.	Tannins	Ferric chloride test	-	-	-	-	-
2.	Saponins	Foam test	-	-	-	-	-
3.	Quinones	Sulphuric acid test	+++	++	++++	+++	+
4.	Terpenoids	Sulphuric acid test	-	-	-	-	-
5.	Flavonoids	Sodium hydroxide test	+	+	+++	++	-
6.	Phenols	Potassium per magnet test	+	+	+	+	+
7.	Steroids	Liebermann- burchard test	-	-	-	-	-
8.	Coumarins	Sodium test	-	-	-	-	-
9.	Anthocyanin	Sodium hydroxide test	-	-	-	-	-
10.	Carbohydrates	Fehling's test Benedict's	-	-	-	-	-
		test	-	-	-	-	-
11.	Proteins	Millon's test Biuret test	+++	+	+	+++	++
			-	+	+++	++++	-

(Where, + = slightly, ++ = moderate, +++ = significant and – = negative).

Total Phenolic Content: The phenolic compounds have oxidation-reduction potential, allowing them to function as antioxidants.

The free radical scavenging activity is due to the hydroxyl group. Whereas the flavonoids are the secondary metabolites present in the plants, the antioxidant activity depends on the presence of free OH groups. The total phenolic content of the different extracts of seeds of *N. nucifera* ranged from 56.66±2.25mg GAE/ g^{-1} dry weight of dried sample for the benzene extract to 366.6±10.77mg GAE/ g^{-1} dry weight of dried sample for the ethyl acetate extract.

total flavonoids content of the different solvent extracts of seeds of *N. nucifera* ranged from 124.4±3.02mg RE/ g^{-1} dry weight in the aqueous extract to 344.4±3.71mg RE/ g^{-1} dry weight in the ethyl acetate extract.

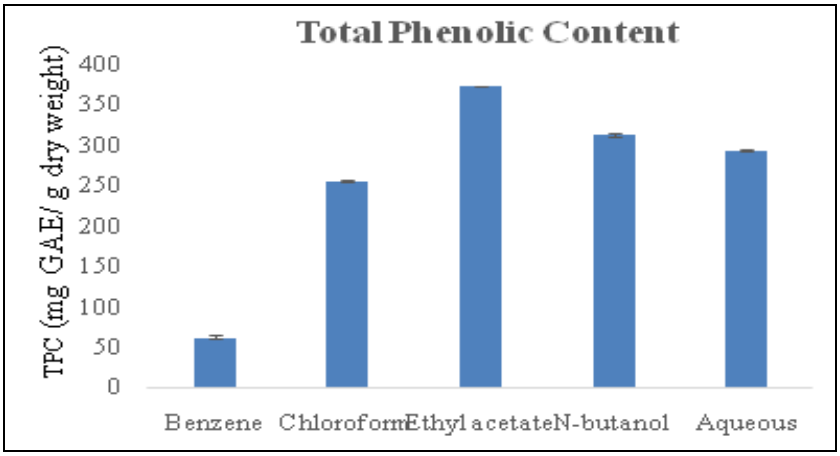


GRAPH 2: STANDARD CURVE FOR THE ASSESSMENT OF TOTAL PHENOLIC CONTENT

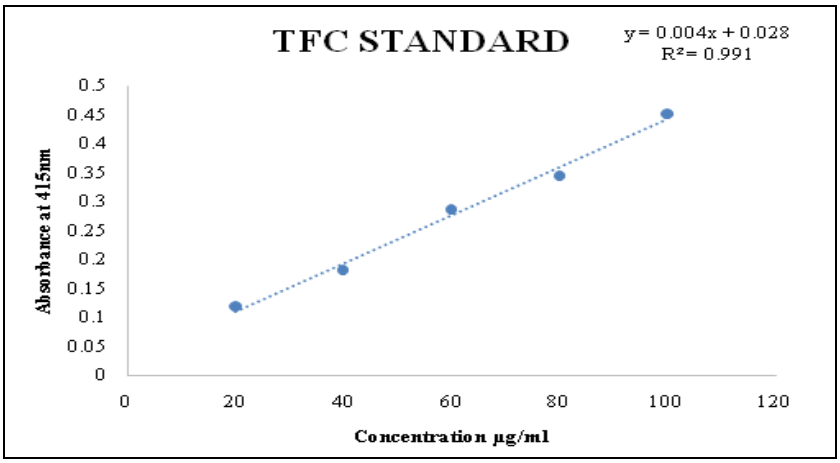
TABLE 5: TOTAL PHENOLIC AND FLAVONOID CONTENT IN THE DIFFERENT FRACTIONS OF THE HYDROETHANOLIC EXTRACT OF SEEDS OF *N. NUCIFERA*

Extracts of *N. nucifera* seeds in different solvents	Total phenolic content (mg GAE/ g^{-1} dry weight)	Total flavonoid content (mg RE/ g^{-1} dry weight)
Benzene	56.66±2.25	146±0.57
Chloroform	116.6±5.13	173±1.33
Ethyl acetate	366.6±10.77	344.4±3.71
n-Butanol	156.6±2.08	162±1.07
Aqueous	176.6±3.68	124.4±3.02

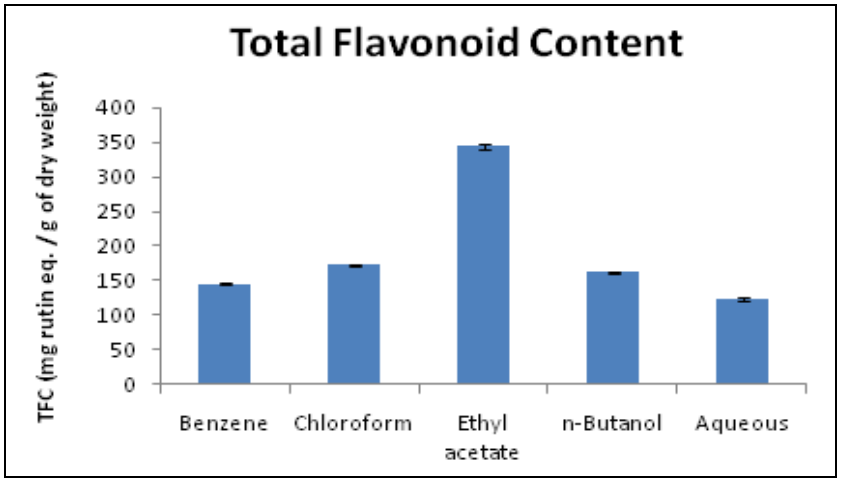
(Values expressed as Mean ±SD where n=3)



GRAPH 3: TOTAL PHENOLIC CONTENT OF DIFFERENT FRACTIONS OF THE HYDROETHANOLIC SEED EXTRACTS OF *N. NUCIFERA*



GRAPH 4: STANDARD CURVE FOR THE ASSESSMENT OF TOTAL FLAVONOID CONTENT



GRAPH 5: TOTAL FLAVONOID CONTENT OF DIFFERENT FRACTIONS OF THE HYDROETHANOLIC EXTRACT OF THE SEEDS OF *N. NUCIFERA*

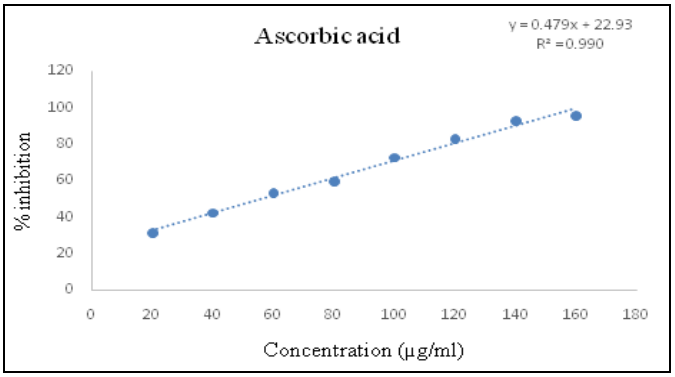
Determination of Antioxidant Activity by DPPH Assay: The principle of the DPPH assay depends on the hydrogen-donating ability of the natural antioxidant compounds present in each plant.

The lowest the IC_{50} value, the higher the inhibition potential of the extract. The lowest IC_{50}value was determined in Ethyl acetate (105.30 ± 12.06µg/ml), and the highest was determined in N-butanol (555.63 ± 7.56µg/ml) in comparison to the standard ascorbic acid 56.40±23.63µg/ml

TABLE 6: % INHIBITION OF DIFFERENT FRACTIONS OF THE HYDROETHANOLIC EXTRACT OF THE SEEDS OF *N. NUCIFERA*

Solvent	IC_{50} value
Standard	56.40±23.63
Benzene	537.17 ± 9.27
Chloroform	245.5 ± 17.51
Ethyl acetate	105.30 ± 12.06
N-butanol	555.63 ± 7.56
Aqueous	237.29 ± 14.54

(Values expressed as Mean ±SD where n=3).

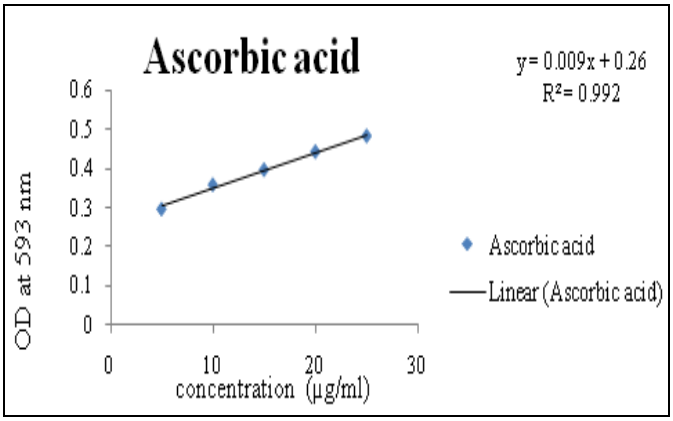


GRAPH 6: STANDARD CURVE FOR DPPH ASSAY

Determination of Antioxidant Activity by FRAP: The reducing power assay of the isolated fractions of the seed extract determined by the ferricyanide method also confirmed significant antioxidant activity by the extracts.

The highest value was determined in the Ethyl acetate fraction (142.2±0.51 mg $AAgm^{-1}$) and the lowest value was reported in the chloroform (34.099±0.39mg $AAgm^{-1}$).

The non-polar fractions showed the lowest value as compared to the polar fractions.

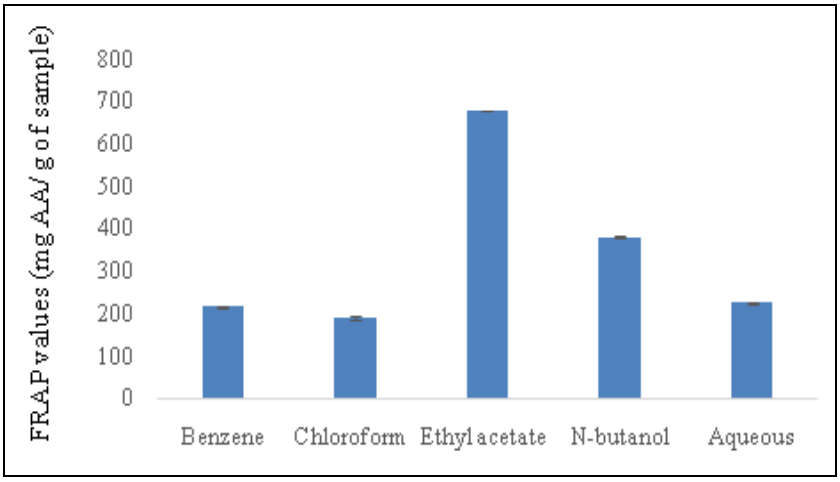


GRAPH 7: STANDARD CURVE OF THE FRAP ASSAY

TABLE 7: FRAP VALUES OF DIFFERENT FRACTIONS OF HYDROETHANOLIC EXTRACT OF THE SEEDS OF *N. NUCIFERA*

Different fractions of the hydroethanolic extract of the seeds of *N. nucifera*	FRAP values (mg AA/ gm)
Benzene	40.72±0.46
Chloroform	34.099±0.39
Ethyl acetate	142.2±0.51
n-Butanol	122.9±2.01
Aqueous	74.09±0.44

(Values expressed as Mean ±SD where n=3).

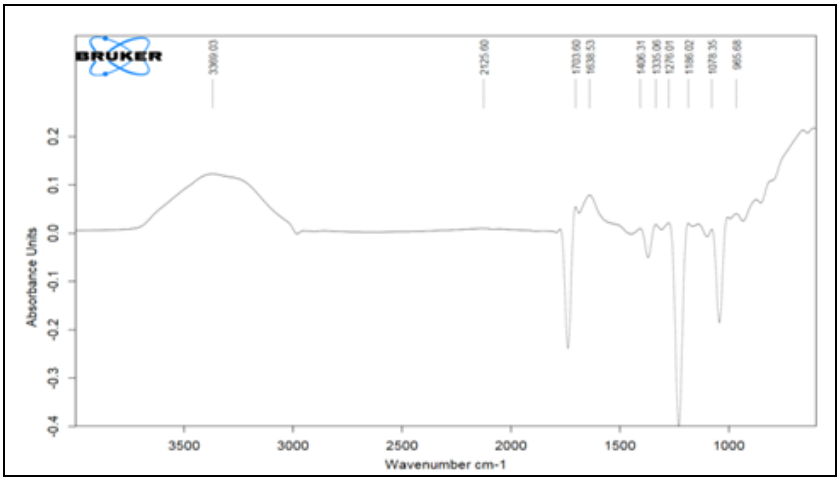


GRAPH 8: VALUES OF FRAP IN MG AA/G OF SAMPLE IN DIFFERENT FRACTIONS OF THE HYDROETHANOLIC EXTRACT OF THE SEEDS OF *N. NUCIFERA*

Compound Characterization by FTIR: FTIR (Fourier Transform Infrared Spectroscopy) is a high-resolution analytical method that may be used to identify chemical components and interpret structural compounds. To fingerprint plant extracts or powders, FTIR provides a quick and non-destructive method [21]. The frequency of functional groups found in the Ethyl acetate fraction is shown in **Table 8.**

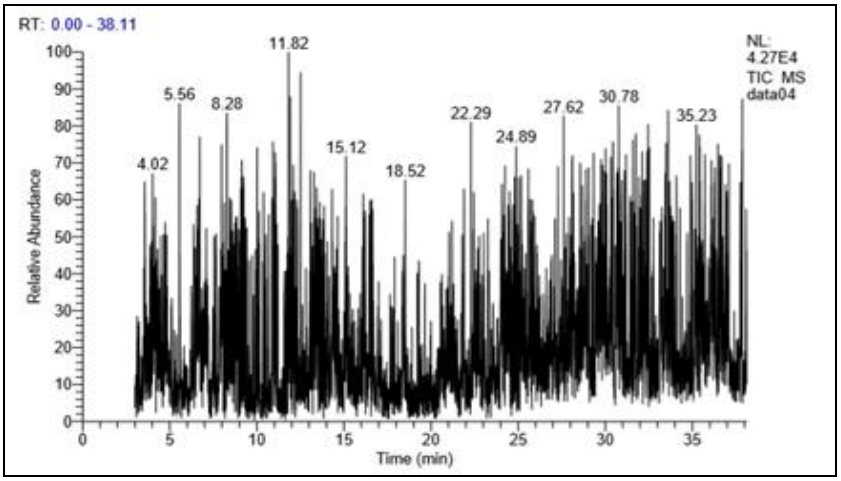
TABLE 8: FTIR COMPOUND PEAKS OF ETHYL ACETATE FRACTION OF THE SEEDS OF *N. NUCIFERA*

S. no.	Wave number cm^{-1} (Test sample)	Wave number cm^{-1} (Reference article)	Functional groups	Phytocompound identified
1.	3369.03	3400-3300	N-H stretching	Aliphatic primary amine
2.	2125.60	2140- 2100	C=C stretching	Alkyne
3.	1703.60	1710- 1680	C=O stretching	Conjugated acid
4.	1638.53	1648- 1638	C=C stretching	Alkene
5.	1406.31	1410-1380	S=O stretching	Sulfonyl chloride
6.	1335.06	1350- 1250	C-N stretching	Sulphone
7.	1276.01	1310- 1250	C-O stretching	Aromatic ester
8.	1078.35	1085- 1050	C-O stretching	Primary alcohol
9.	965.68	980 - 960	C=C stretching	Alkenes



GRAPH 9: FTIR SPECTRA OF THE ETHYL ACETATE FRACTION OF THE SEEDS OF *N. NUCIFERA*

GCMS Analysis: The presence of numerous peaks in the GCMS chromatogram analysis **Fig. 9** of the ethyl acetate extract of *N. nucifera* seeds suggests the presence of many phytochemical constituents. The thirty-eight phytocompounds were described and identified by comparing their mass spectra with Wiley 9 and the NIST library, and their retention time (RT) and molecular weight were calculated.



GRAPH 10: GCMS CHROMATOGRAM OF ETHYL ACETATE FRACTION OF *N. NUCIFERA* SEEDS

TABLE 9: GCMS ANALYSIS OF ETHYL ACETATE FRACTION OF *N. NUCIFERA* SEEDS

S. no.	Compounds	RT	Formula	Probability	MW	Biological activity
1	Tungsten, di carbonylbis (η-4-2-methylene cycloheptanone)η-4-2-methylenecycloheptanone)	11.05	$C_{18}H_{24}O_4W$	16.12	488	Antibacterial
2	Bis(η-4-2-methylenecycloheptanone)tributylstannyl)acetylene	11.05	$C_{26}H_{54}Sn_2$	10.73	606	Anticancer
3	Palladium, dichloro(η-4-2-methylenecycloheptanone)1,2,3,4-tetraphenyl-1,3-cyclobutadiene)	13.38	$C_{28}H_{20}Cl_2Pd$	19.35	532	Antibacterial
4	Brucine	15.12	$C_{23}H_{26}N_2O_4$	13.60	394	Anti-tumor Anti-inflammatory Analgesic
5	Tungsten(η-4-2-methylenecycloheptanone)0), tetracarbonyl-(η-4-2-methylenecycloheptanone)phenylmethoxycarbene)(η-4-2-methylenecycloheptanone)η-2-E-cyclooctene)		$C_{20}H_{22}O_5W$		526	Antibacterial
6	Carda-16,20(η-4-2-methylenecycloheptanone)22)-dienolide, 3-[(η-4-2-methylenecycloheptanone)6-deoxy-3,4-O-methylenehexopyranos-2-ulos-1-yl)oxy]-5,11,14-trihydroxy-12oxo-, (η-4-2-methylenecycloheptanone)3β,5α,11α	24.08	$C_{30}H_{38}O_{11}$	11.49	547	Hepatoprotectant, Antioxidant Cholesterol antagonist
7	4H-1-Benzopyran-4-one, 6,7-bis(η-4-2-methylenecycloheptanone)acetyloxy)-3-hydroxy-5-methoxy-2-[4-[(η-4-2-methylenecycloheptanone)2,3,4-tri-O-acetyl-6-deoxy-α-L-mannopyranosyl)oxy]phenyl]	24.08	$C_{32}H_{32}O_{16}$	27.57	672	Antimicrobial
8	Pyrazine,2,5-bis(η-4-2-methylenecycloheptanone)p-fluorophenyl)-3,6-diphenyl	25.07	$C_{28}H_{18}F_2N_2$	42.69	420	Hepatoprotective Antimicrobial Anti-thrombogenic
9	2-Ethyl-acridone	25.07	$C_{15}H_{13}NO$	11.17	223	Anti-tumor Antiviral Antimicrobial
10	Tungsten, dicarbonylbis(η-4-2-methylenecycloheptanone)η-4-2-methylenecycloheptanone)	33.16	$C_{18}H_{24}O_4W$	14.99	488	Antibacterial

DISCUSSION: The results clearly show that the seed is the most effective medication activity. The water-soluble ash value is an important factor when evaluating a drug as it indicates the inorganic compounds present. The ash value accepted should be below the adequate limit 14% recommended by the European Pharmacopoeia [22]. The less ash value indicates purity and less contamination in the drug as the higher value indicates the presence of organic matter and impurities [23]. The phytochemical analysis shows that the extracts include a high concentration of non-polar molecules such as sugars, proteins, glycosides, organic acids, tannins, salts, and mucus can all be extracted from plant material using non-polar solvents. The varying polarity of different chemicals found in the seeds may account for significant variances in extraction yield. The polarity of the solvents utilized also influenced it. The higher the water-soluble extractive value, the more will be the moisture content leading to the deterioration of drug [23].

The phenomenon of fluorescent analysis can be efficiently used to assess the pharmacognostical parameters of any crude drug qualitatively and could also help in its identification. As the compounds present in the drugs and fluorescent behavior are correlated. These distinct and reproducible colour changes can be well attributed to the solvent properties of the specific phytochemicals [14].

These extracts were made from the plant's various solvent fractionates, including benzene, chloroform, ethyl acetate, n-butanol, and the aqueous. Quinones, flavonoids, phenols, and glucose were found in high concentrations in the several extracts of *N. nucifera* seed power, while tannins, terpenoids, and saponins were absent as shown in **Table 4**. These findings backed up claims that its seeds contain active ingredients like carbohydrates, flavonoids, and phenolic compounds that have antioxidant, anti-diabetic, anti-obesity, and anti-fungal properties [24, 25, 26, 27, 28, 29]. In epidemiological investigations, the phytochemical components have strong antioxidant activities and produce numerous biological effects. They effectively reduce the number of diseases such as cardio-vascular diseases, and hypercholesterolemia, and possess antibiotic properties. The polarity of the solvents employed for extraction affects the concentration of phenols and flavonoids. Plants' antioxidant capabilities are closely connected to their phenolic and flavonoid content. Phenolic compounds can act as reducing agents, hydrogen donors, and scavengers of free radicals. The presence of phenolics in substantial amounts contributes greatly to the antioxidant capabilities. Phenolic substances have a wide range of antioxidant responses depending on their chemical composition [17]. *N. nucifera* has extensively been demonstrated to have a good antioxidant potential largely due to the synergistic effects of various bioactive compounds found in it. The results exhibited by the ethyl acetate extract show the highest activity among all the solvent fractionates used. Its lower IC_{50} value of 105.30± 12.06µg/ml clearly resembled a good antioxidant activity attributed to its high DPPH radical scavenging compared to values obtained for the ascorbic acid used as standard (56.40±23.63µg/ml).

The DPPH test, among many others, is one of the most practical techniques for evaluating plant antioxidant potential. The methanolic DPPH solution is decreased owing to the production of non-radicals when antioxidant compounds containing hydrogen-donating groups, such as flavonoids and phenols, are present [30].

The antioxidants act as reductants and contribute to the reducing properties of the plant by acting as hydrogen donors, thereby breaking the free radical chain. These also prevent peroxidation by reacting with the precursors of peroxide. In the FRAP assay, the potent antioxidant activity was indicated by the reducing capabilities of the antioxidants (reductants), based on the ability to reduce and transform Fe^{3+}/ferric cyanide complex to ferrous (Fe^{2+}) form. The colour change measures this transformation at 680nm, directly related to the associated reducing power. The results exhibited by the ethyl acetate extract showed the highest activity among all the solvent fractionates used (142.2±0.509 mg /AA gm). The antioxidants are essential molecules that can provide protection from harm caused by oxidative stress produced by free radicals. The polyphenols function as antioxidants and reducing agents in the body. Their hydroxyl groups have a hydrogen-donating property. The FTIR studies were conducted to

confirm the presence of phytocompounds in seeds extract. The spectra showed the presence of the functional groups (C=O, NO, CH, C=N, RX, and OH group), which significantly contribute to the pharmaceutical activity of the plant. The C=O stretching is predominantly found in the seeds of the plant. Carboxylic acid, found in medicinal plants, is the primary pharmaceutical product used to treat ulcers, jaundice, and liver discomfort[31]. The absence of absorbance in the range 2220-2260 cm-1 shows the absence of cyanide group and, therefore, toxic substance. As a result, the functional group is in favor of hepatoprotective action.

Only a few of the compounds have been reported to have biological activity due to the different functional groups. **Table 9** lists the numerous phytochemicals that contribute to the therapeutic properties of *N. nucifera* seed. Among all the compounds Pyrazine, 2, 5-bis(η-4-2-methylenecycloheptanone) p-fluorophenyl)-3,6-diphenyl (Probability 42.69) and Carda-16,20(η-4-2-methylenecycloheptanone)22)-dienolide, 3-[(η-4-2-methylenecycloheptanone) 6-deoxy-3,4-O-methylenehexopyranos – 2 – ulos – 1 - yl) oxy]-5, 11, 14 – trihydroxy – 12 oxo -, (η-4-2-methyl-enecycloheptanone)3β,5α,11α (Probability 11.49) shown in the **Table 9** are interesting findings as because of their hepatoprotective role. These phytochemical constituents can be isolated and can be potential novel drugs against various hepatoprotective disorders.

CONCLUSION: The findings of this study backed up the traditional use of *N. nucifera* seeds, which contain bioactive chemicals that could be employed as active agents in novel medicines to treat a variety of metabolic issues. The nutritional composition data analysis revealed that seed could be used as a significant source of protein and carbohydrates for humans and livestock and contain significant amounts of phytochemicals, suggesting that lotus seeds may have scientific support for their use in traditional medicine for the treatment of metabolic disorders. Because of the low ash content and the presence of medicinal compounds, the seeds have been discovered to be useful against various ailments. Antioxidant properties are enhanced by the presence of phenolic chemicals in significant levels. When compared to the standard, the antioxidant activity was determined to be high, indicating that it provides protection against free radicals. The presence of functional groups (C=O, NO, CH, C=N, RX, and OH) contribute considerably to the plant's medicinal activity. The presence of Pyrazine, 2,5-bis (-4-2-methylene cycloheptanone) p-fluorophenyl)-3,6-diphenyl (Probability 42.69) in the crude extract was found by GC-MS. These are noteworthy discoveries because of their hepatoprotective effect. These phytochemical ingredients can be extracted and used to develop new medications to treat various hepatoprotective conditions. Finally, the findings show that ethyl acetate in *N. nucifera* seeds is the most efficient against hepatic ailments and a variety of other illnesses.

ACKNOWLEDGEMENT: Author’s take this opportunity to express their gratitude and thanks to the Former Hon’ble VC Prof. Aditya Shastri, Banasthali Vidyapith, Banasthali, Rajasthan, for providing us the research facilities, encouragement, and support. Author’s also thank Hon’ble Vice-Chancellor, Prof. Ina Shastri, Banasthali Vidyapith, Banasthali, Rajasthan, for being a consistent support source providing the necessary research facilities. The authors also thank Prof. Dipjyoti Chakraborty, Head of the Department of Bioscience and

Biotechnology, Banasthali Vidyapith, Banasthali, for supporting and encouraging the research work. The authors would like to acknowledge DST for providing networking support through the FIST program at the Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali.

CONFLICTS OF INTEREST: The authors have no conflicts of interest regarding this investigation**.**

REFERENCES:

1. Tanaka N and Kashiwada Y: Phytochemical studies on traditional herbal medicines based on the ethnopharmacological information obtained by field studies. Journal of Natural Medicines 2021; 75(4): 762-83.
2. Bhatt ID, Rawat S and Rawal RS: Antioxidants in medicinal plants. In Biotec for Med Plants 2013; 295-326.
3. Avneet G, Pal SM and Siddhraj SS: Ash value and extractive value comparison between pet ether and ethanolic extracts of *Bacopa monnieri*, *Evolvulus alsinoides* and *Tinospora cordifolia*. Journal of Pharmacy Research 2018; 12(4): 477.

4. Abdulrahman M, Hussain UG, Shaaban S, Yushau S, Abubakar A, Abubakar MZ and Shamunu AI: Phytochemical Screening and Antioxidant Activity of the Leaves Extract of *Nymphaea lotus*. International J of Re and Innovation in Applied Science 2020; 5(5): 97-101.
5. Hasanuzzaman M, Bhuyan MH, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M and Fotopoulos V: Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants 2020; 9 (8): 681.
6. Liu Z, Ren Z, Zhang J, Chuang CC, Kandaswamy E, Zhou T and Zuo L: Role of ROS and nutritional antioxidants in human diseases. Frontiers in Physiology 2018; 17(9): 477.
7. Pal I and Dey P: A Review on Lotus (*N. nucifera*) Seed. International J of Science Res 2015; 4 (7): 1659-1666.
8. Lee DB, Kim DH and Je JY: Antioxidant and cytoprotective effects of lotus (*Nelumbo nucifera*) leaves phenolic fraction. Prev Nut and Food Sci 2015; 20(1): 22.
9. Ruvanthika PN, Manikandan S and Lalitha S: A comparative study on phytochemical screening of aerial parts of *Nelumbo nucifera* gaertn by gas chromatographic mass spectrometry. International Journal of Pharmaceutical Sciences and Research 2017; 8 (5): 2258.
10. Zaidi A and Srivastava K: Nutritional and therapeutic importance of *Nelumbo nucifera* (Sacred lotus). Era's Journal of Medical Research 2019; 6(2): 98-102.
11. Amir M, Zafar A, Ahmad R, Ahmad W, Sarafroz M, Khalid M, Ghoneim MM, Alshehri S, Wahab S, Ahmad S, and Mujeeb M: Quality control standardization, contaminant detection and *in-vitro* antioxidant activity of *Prunus domestica* Linn. Fruit Plants 2022; 11(5): 706.
12. Jesse AO: Fourier transform infrared spectroscopy analysis of *Allium sativum* L. and *Nymphaea lotus* L. Asian Journal of Applied Chemistry Research 2020; 6: 7-24.
13. Preethika KK, Arul P and Karpagam S: Comparative phytochemical analysis of white and pink flower of *Nelumbo nucifera*. Inter J of Science and Res 2018; 7: 8.
14. Fardiyah Q, Kurniawan F, Ersam T and Slamet A: Preliminary Phytochemical Screening and Fluorescence Characterization of Several Medicinal Plants Extract from East Java Indonesia. In IOP Conference Series: Materials Science and Engineering 2020; 833(1): 012008.
15. Gul R, Jan SU, Faridullah S, Sherani S and Jahan N: Preliminary phytochemical screening, quantitative analysis of alkaloids and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. The Scientific World Journal 2017.
16. Islam MR, Roy D and Naher J: Antioxidant potential of aquatic plant *Scirpus mucronatus* found in water bodies of Dinajpur district, Bangladesh. Journal of Pharmacognosy and Phytochemistry 2021; 10(6): 56-60.
17. Phuyal N, Jha PK, Raturi PP and Rajbhandary S: Total phenolic, flavonoid contents and antioxidant activities of fruit, seed and bark extracts of *Zanthoxylum armatum* DC. The Scientific World Journal 2020.
18. Guo Z, Li P, Hung W, Wang J, Liu Y and Liu B: Antioxidant and anti-inflammatory caffeoyl phenylpropanoid and secoiridoid glycosides from *Jasminum nervosum* stems, a Chinese folk Medicine. Phytochemistry 2014; 106: 124-133.
19. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, and Koirala N: Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants 2019; 8 (4): 96.
20. Singh N and Tripathi M: Fluoride toxicity in fresh water fishes and aquaculture: A Review. Indian Journal of Life Sciences 2015; 4(2): 115-124.
21. Fadlelmoula A, Pinho D, Carvalho VH, Catarino SO and Minas G: Fourier Transform Infrared (FTIR) Spectroscopy to Analyse Human Blood over the Last 20 Years: A Review towards Lab-on-a-Chip Devices. Micromachines. 2022; 13(2): 187.
22. Vaikosen EN and Alade GO: Evaluation of pharmacognostical parameters and heavy metals in some locally manufactured herbal drugs. Journal of Chemical and Pharmaceutical Research 2011; 3(2): 88-97.
23. Ayurvedic Pharmacopoeia of India. Tests and Determinations. Part 1. 1st Ed. Vol. 9. Pharmacopoeia Commission for Indian Medicine and Homoeopathy. India 2016; 113-114.
24. Chen GL, Fan MX, Wu JL, Li N and Guo MQ: Antioxidant and anti-inflammatory properties of flavonoids from lotus plumule. Food Chemistry 2019; 277: 706-12.
25. Sujitha R, Bhimba BV, Sindhu MS and Arumugham P: Phytochemical evaluation and antioxidant activity of *Nelumbo nucifera*, *Acorus calamus* and *Piper longum*. International Journal of Pharmacy and Chemical Scence 2013; 2: 1573-8.
26. Rajput MA, Zehra TA, Ali FI and Kumar GU: Assessment of analgesic activity of *Nelumbo nucifera* fruit ethanol extract. Inter J of Pharmaceutical Sciences 2019; 11: 1-5.
27. Sim WS, Choi SI, Cho BY, Choi SH, Han X, Cho HD, Kim SH, Lee BY, Kang IJ, Cho JH, and Lee OH: Anti-obesity effect of extract from *Nelumbo nucifera* L., *Morus alba* L, and *Raphanus sativus* mixture in 3T3-L1 adipocytes and C57BL/6J obese mice. Foods 2019; 8(5): 170.
28. Harishkumar R, Manjari MS, Rose C and Selvaraj CI: Protective effect of *Nelumbo nucifera* (Gaertn.) against H2O2-induced oxidative stress on H9c2 cardiomyocytes. Molecular Biology Reports 2020; 47 (2): 1117-28.
29. Arooj M, Imran S, Inam-ur-Raheem M, Rajoka MS, Sameen A, Siddique R, Sahar A, Tariq S, Riaz A, Hussain A and Siddeeg A: Lotus seeds (*Nelumbinis semen*) as an emerging therapeutic seed: A comprehensive review. Food Science & Nutrition 2021; 9(7): 3971-87.
30. Tyagi T and Agarwal M: Antioxidant properties and phenolic compounds in methanolic extracts of *Eichhornia crassipes*. Reserarch J of Phytochem 2017; 11(2): 85-89.
31. Ezzat MI, Okba MM, Ahmed SH, El-Banna HA, Prince A, Mohamed SO and Ezzat SM: In-depth hepatoprotective mechanistic study of *Phyllanthus niruri*: *In-vitro* and *in-vivo* studies and its chemical characterization. PloS one 2020; 15(1): 0226185.

How to cite this article:
Tyagi S, Shukla A, Jain P and Tripathi R: Characterization and antioxidant activity of seed extract of *N. nucifera*. Int J Pharm Sci & Res 2022; 13(11): 4608-19. doi: 10.13040/IJPSR.0975-8232.13(11).4608-19.