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A COMPARATIVE STUDY ON PHYTOCHEMICAL SCREENING OF AERIAL PARTS OF *NELUMBO NUCIFERA* GAERTN BY GAS CHROMATOGRAPHIC MASS SPECTROMETRY

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Keywords:

Nelumbo nucifera, Phenols, Quercetin, Terpenoids, Phytochemical screening.

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ABSTRACT: *Nelumbo nucifera* Gaertn a perennial aquatic plant also known as sacred lotus. The Rhizome, flower, seeds and leaves all are edible and have been proved for their medicinal value. Study about its phyto-nutrients in the aerial parts are scanty. In the present study a phytoscreening of aerial parts of *Nelumbo nucifera* seeds and seed pod was done to assess and compare their phytoconstituents. Total phenolic content in *Nelumbo nucifera* seed pod was 93.45mg Gallic Acid Equivalents and 10.5mg/g in the seeds, the total flavonoid content was 295.312mg/g Quercetin Equivalents in NN seedpod and 28.125mg/g in the seeds, tannin content is 508.7mg/g Tannin Equivalents in *Nelumbo nucifera* seedpod and 69.637mg/g in seeds. Gallic acid, Quercetin and Tannin Equivalents were taken as standard for phenol, flavonoid and tannin content respectively. Phytochemical constituents analyzed by Gas Chromatography – Mass Spectrometry analysis of aerial parts *Nelumbo nucifera* can be used for routine quality control analysis. The data obtained emphasize, the potential of traditional medicine *Nelumbo nucifera*, whose phytoconstituents form a source of natural antioxidant which could prevent many free radical mediated diseases.

INTRODUCTION: *Nelumbo nucifera* Gaertn a common perennial aquatic plant, was first brought into horticulture in the year 1787 in Western Europe. It is a common herb grows throughout India, known even from Himalayan lakes at altitude up to 1400 m [1]. Lotus seeds sold in the Indian markets (‘kamal gatta’) as vegetable or raw material for Ayurvedic drug preparation [2]. All parts of *Nelumbo nucifera* like seed, rhizome, and flower are edible and are used in numerous ways in oriental cuisine and for medicinal purposes [3].

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Medicinal plants due to their abundant biologically active phytoconstituents are a source of synthetic and herbal drugs.

In recent years herbal products are becoming more popular because phytochemistry had made a rapid progress [4]. *Nelumbo nucifera* contains abundant functional components including polyphenols, flavonols, procyanidins, polysaccharides and alkaloids which relate to its pharmacological activities, such as anti-inflammatory, anti-obesity, anti-cancer, anti-aging and anti-cardiovascular. Flavonoids and alkaloids forms the most important functional components of *Nelumbo nucifera* and their physiological properties have been extensively reported [5]. The various flavonoids include Flavonols, flavone, gallic acid, proanthocyanidins, anthocyanins.

These components are analyzed by phytochemical screening and exhibits wide range of pharmacological activities like antioxidant [6], antipyretic [7], hepatoprotective [8], hypoglyceamic, lipolytic [9] and the alkaloids include nuciferine, pronuciferine, dauricine, lotusine, liensinine, isoliensinine, neferine which possesses pharmacological activities like anti-oxidant, anticancer [10], anti-fertility [11], anti-arrhythmic [12], anti- obesity [13]. All parts of *Nelumbo nucifera* have many medicinal uses the rhizome, leaf, seed, and flower are traditionally used in the treatment of many diseases including cough, haematuria, metorrhagia, spermatorrhoea, leucoderma, smallpox, dysentery, lipidaemia, fever, cholera. Extracts of different parts of *Nelumbo nucifera* have shown antioxidant, anti-cancer, anti-diaorrheal, anti-viral, anti-pyretic, hepatoprotective, anti-obesity, anti-ischaemic, lipolytic, anti-inflammatory activities [14, 15].

In a recent study, it has been reported that the seedpod of *Nelumbo nucifera*, which is inedible part of the plant is discarded as a waste, and is found to possess higher levels of polyphenols. Antioxidants are radical scavengers which protect the human body against oxidative stress related diseases such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion [16]. There are many studies showing the relationship between antioxidant activity and phenolic content of plant extracts [17, 18].

Phytochemical analysis in the seedpod of the plant is scanty though many activities of different parts of *Nelumbo nucifera* have been reported [19]. This lacuna formed the basis of this present study. A phytochemical screening of seed and seed pod was performed to evaluate bioactive contents of aerial parts of *Nelumbo nucifera* by Gas chromatogram and mass spectrometric method (GC-MS). This may be a best source for natural antioxidant.

MATERIALS AND METHODS:

Collection of Lotus: *Nelumbo nucifera* seed and seedpod were collected from a pond in Avadi, Chennai, Tamil Nadu, India. The voucher specimen was authenticated and deposited in the herbarium in National institute of Siddha, Tambaram, Chennai. (Authentication No: NISMB1442014).

Preparation of the plant extract: Extracts were prepared according to a combination of methods [20, 21]. About 15g of dried fine powder of *Nelumbo nucifera* seedpod were extracted with 150 ml ethanol (75%), chloroform, acetone and aqueous extract for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evaporator at 40 °C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in below 10 ºC.

Phytochemical screening of extracts of *Nelumbo nucifera* seed and seedpod: The phytochemical screening of extracts of *Nelumbo nucifera* Seed and Seedpods were assessed by standard methods [22-24]. Phytochemical screening was carried out in the extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids.

Phytochemical analysis:

Test for Tannins: To 1ml of plant extract, 1ml of 5% ferric chloride was added. Formation of dark blue or greenish black colour indicates the presence of tannins [25].

Test for Saponins: To 2ml Plant extract, 2ml of distilled water was added and shaken in graduated cylinder for 15 min lengthwise; formation of 1cm layer of foam indicates presence of saponins [25].

Test for Quinones: To 1ml Plant extract, 1ml of concentrated sulphuric acid was added. Formation of red colour indicates the presence of Quinones [25].

Test for Flavonoids: To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Formation of yellow colour indicates the presence of flavonoids [25].

Test for Alkaloids: To 2ml of Plant extract, 2ml of concentrated Hydrochloric acid was added. Then few drops Mayer's reagent is added**.** Presence of green color or white precipitate indicates the presence of alkaloids [25].

Test for Glycosides: To 2ml of plant extract, 3ml of chloroform and 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides [25].

Test for Cardiac glycosides: To 0.5 ml of plant extract, 2 ml of glacial acetic acid and few drops of 5 % ferric chloride were added. This is under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides [25].

Test for Terpenoids: To 0.5 ml of the plant extract, 2 ml of chloroform along with concentrated Sulphuric acid was added. Formation of red brown colour at the interface indicates the presence of Terpenoids [25].

Test for Phenols: To 1ml of plant extract, 2ml of distilled water followed by few drops of 10 % ferric chloride was added. Formation of blue/ green colour indicates the presence of phenols [25].

Test for Steroids: To 0.5 ml of plant extract, 2 ml of chloroform and 1 ml of Sulphuric acid was added. Formation of reddish brown ring at interface indicates the presence of steroids [25].

Test for Coumarins: To 1 ml of plant extract, 1 ml of 10 % sodium hydroxide was added. Formation of yellow colour indicates the presence of coumarins [25].

Test for Anthocyanin and Beta cyanin: To 2ml of the plant extract, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100 °C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin [25].

General reactions of the analysis revealed the presence or absence of these compounds in the seed and seedpod extracts tested are summarized in **Table 1** and **2**.

TABLE 1: PHYTOCHEMICAL SCREENING OF *NELUMBO NUCIFERA* SEED EXTRACTS

Phytochemicals Tested	Aqueous	Acetone	Chloroform	Ethanol
Tannins	+	++	-	++
Saponins	-	-	+	++
Flavonoids	+	+	-	++
Quinones	+	++	+	++
Glycosides	+	+	-	+
Cardiac glycosides	++	+	+	+
Terpenoids	+	++	+	++
Phenol	+	++	+	++
Coumarins	+	+	-	+
Steroids	+	++	+	+
Alkaloids	+	+	-	++
Antho cyanin	-	-	-	-
Beta cyanin	+	+	-	+

Key: ++: Strong positive, +: Positive, −: Negative

TABLE 2: PHYTOCHEMICAL SCREENING OF *NELUMBO NUCIFERA* SEED POD EXTRACT

Phytochemicals Tested	*NN* Seed Pod Extracts			
	Aqueous	Acetone	Chloroform	Ethanol
Tannins	-	++	-	++
Saponins	-	-	+	+
Flavonoids	+	++	+	++
Quinones	+	++	++	++
Glycosides	-	-	-	-
Cardiac glycosides	-	++	-	++
Terpenoids	+	++	++	++
Phenol	++	++	+	++
Coumarins	-	++	+	+
Steroids	+	++	+	++
Alkaloids	++	+	-	+
Antho cyanin	-	-	-	-
Beta cyanin	+	+	+	+

Key: ++: Strong positive, +: Positive, −: Negative

Estimation of Total phenol content in Ethanolic extracts of *Nelumbo nucifera* seed and seed pod: Total phenolic content in the ethanolic extracts of *Nelumbo nucifera* seed and seed pod were determined by the Folin Ciocalteau colorimetric method [26]. The contents were analyzed by adding, 0.5 ml of ethanolic extract of *Nelumbo nucifera* seed and seedpod to 0.1 ml of Folin- Ciocalteau reagent (0.5N) and contents of the flask were mixed thoroughly. Followed by 2.5 ml of Sodium carbonate was added and then the mixture was allowed to stand for 30 min after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolic contents are expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of Total Flavonoid Content in Ethanolic extracts of *Nelumbo nucifera* seed and seed pod: Total flavonoids content in ethanolic extracts of *Nelumbo nucifera* seed and seedpod powder was determined by aluminium chloride colorimetric method [27]. 0.5 ml of *Nelumbo nucifera* seed and seedpod extracts at a concentration of 1mg/ ml was taken and the volume was made up to 3ml with methanol. Then 0.1ml of 10% aluminium chloride, 0.1ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample (QE/g).

Estimation of Tannins in ethanolic extracts of *Nelumbo nucifera* seed and seed pod: Tannins content in ethanolic extracts of *Nelumbo nucifera* seed and seedpod was estimated using standard method [28]. 1 ml of extract was mixed with 0.5ml of Folin-Ciocalteau's reagent followed by 1ml of saturated sodium carbonate solution and 8ml of distilled water. The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-Visible Spectrophotometer.

Different concentrations of standard tannic acid were prepared and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content is expressed as mg tannic acid equivalent (TAE)/gram of the sample.

Gas Chromatography Mass Spectrometry (GC-MS) analysis: GC- MS analysis of ethnolic extract of aerial parts of *Nelumbo nucifera* were performed using GC-MS -5975C agilent system comprising an auto sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument, employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm ID × 1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; helium (99.999%) is used as carrier gas at a constant flow of 1.51 ml/min and an injection volume of 1µl was employed (split ratio of 10:1) injector temperature 240 ºC, ion-source temperature 200 ºC. The oven temperature was programmed from 70 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 300 ºC/min, ending with a 9 min isothermal at 300 ºC. Mass spectra were taken at 70eV; with a scan range 40-1000 m/z. Solvent cut time was 5 min; MS start time being 5 min; MS end time being 35 min; Ion source temperature set to 200 ºC and interface temperature being 240 ºC.

Identification of Bioactive Components: The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the component of the test materials were identified. The peaks in GC-MS of ethanolic extract of aerial parts *Nelumbo nucifera* showed the presence of secondary phytochemical compounds mostly fatty acids and its esters as summarised in **Table 3** and **4**.

TABLE 3: BIOACTIVE COMPOUNDS OF *NELUMBO NUCIFERA* SEEDS IDENTIFIED BY GAS CHROMATOGRAPHIC MASS SPECTROMETRY (GC-MS)

S.NO	RT (min)	Compound Name	Compound nature	Molecular formula	MW	Therapeutic use
1	9. 892	n-Tetradecanoic acid	Myristic acid	$C_{14}H_{28}O_2$	228	Antioxidant, Cancer preventive, Nematicide,

						Lubricant Hypocholesterolemic
2	10. 042	Hexadecanoic acid	Fatty acid	$C_{18}H_{36}O_2$	284	Anti-inflammatory, Hepatoprotective, Antieczemic, Antiacne
3	10. 700	9,12 Octadecadienoic acid	Linoleic acid	$C_{18}H_{32}O_2$	280	Hepatoprotective antihistaminic, hypocholesterolemic, antieczemic, antioxidant and anticancer properties
4	10. 733	11-Hexadecenoic acid	fatty acid	$C_{18}H_{34}O_2$	282	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic
5	12. 033	Di-n-octyl phthalate	Plasticizer compound	$C_{24}H_{38}O_4$	390	Antimicobial, antifouling
6	13. 892	1-Heptadecanol	Aliphatic alcohol	$C_{17}H_{36}O$	256	Anti microbial
7	14. 350	1-Monolinoleoyl glycerol trimethylsilyl ether	Steroid	$C_{27}H_{54}O_4Si_2$	498	Anti microbial Antiasthmatic,
8	14. 650	Ergost-25-ene-3,5,6,12-tetrol, (3.beta.,5.alpha.,6.beta. ,12.beta).	Sterol	$C_{28}H_{48}O_4$	448	Cancer preventive
9	14. 725	Vitamin A aldehyde	Vitamin compound	$C_{20}H_{28}O$	284	Antioxidant
10	14. 900	4,22-Stigmastadiene-3-one	Steriod	$C_{29}H_{46}O$	410	Antimicrobial
11	14. 942	Acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl ester	Acetic acid compound	$C_{17}H_{26}O_3$	278	Antimicrobial
12	15. 050	Ergost-25-ene-3,5,6,12-tetrol, (3.beta.,5.alpha.,6.beta. ,12.beta.)	Phytosterol	$C_{28}H_{48}O_4$	448	anti-oxidant activity, anti-inflammatory activity, anti-arthritis, anti-rheumatoid, anti-auto-immune disease, anti-allergy, anti-platelet aggregation, hypoglycemic
13	15. 200	6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl-	Essential oil	$C_{15}H_{25}I$	332	anti-inflammatory activity, antimicrobial, anticancer

RT=Retention time, MW=Molecular weight

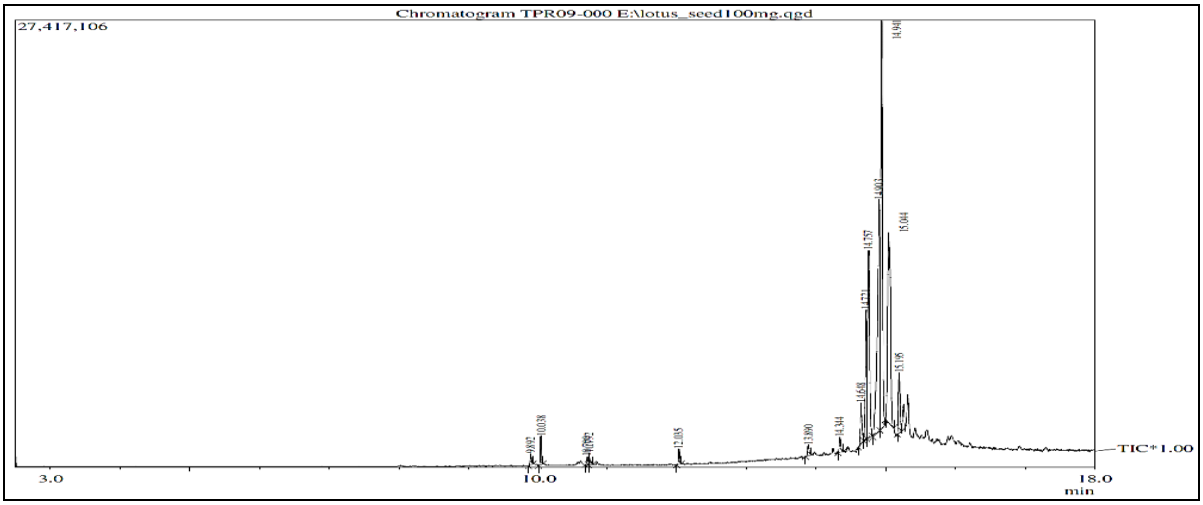
TABLE 4: BIOACTIVE COMPOUNDS OF *NELUMBO NUCIFERA* SEED POD IDENTIFIED BY GAS CHROMATOGRAPHIC MASS SPECTROMETRY (GC-MS)

S. NO	RT (min)	Compound Name	Compound nature	Molecular formula	MW	Therapeutic use
1	9. 884	Tetradecanoic acid	Saturated fatty acid	$C_{14}H_{28}O_2$	228	Antioxidant, Cancer preventive, Nematicide,
2	10.037	Hexadecanoic acid	Saturated Fatty acid	$C_{18}H_{36}O_2$	284	Lubricant, Hypocholesterolemic Anti-oxidant, Hypocholesterolemic, Nematicide, Pesticide,

3	10. 575	11,14-Eicosadienoic acid	Fatty acid	$C_{21}H_{38}O_2$	322	Antiandrogenic, Hemolytic anti-inflammatory activity
4	10. 601	13-Tetradecenal	Fatty acid	$C_{14}H_{26}O$	210	Antibacterial and antioxidant
5	10. 700	Linoleic acid ethyl ester	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	Hypocholesterolemic, Nematicide, Anti-arthritic, Hepato-protective Anti-androgenic, Hypo-cholesterolemic
6	10. 730	9-Octadecenoic acid ethyl ester	Linoleic acid ester	$C_{20}H_{38}O_2$	310	Hypocholesterolemic, Nematicide, Anti-arthritic, Hepatoprotective, Antiandrogenic, Hypocholesterolemic
7	12. 261	Docosanoic acid	saturated fatty acids	$C_24H_{48}O_2$	368	5-Alpha reductase inhibitor, Anti-histaminic, Anti-coronary, Insectifuge, Anti-eczemic, Anti-acne Anti-cancer, anti-oxidant, anti-inflammatory, cardioprotective

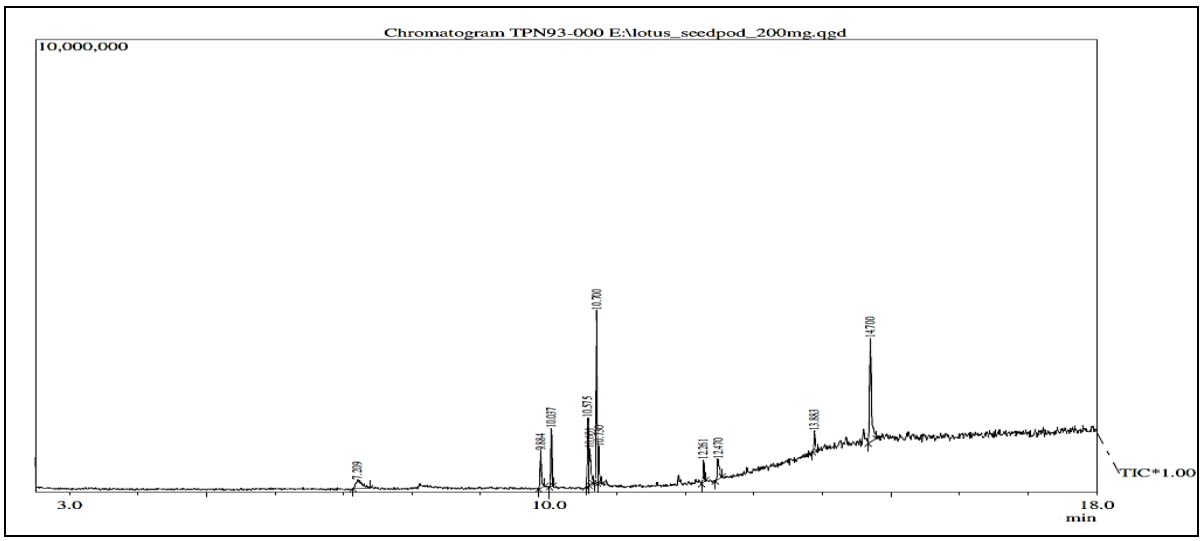
RT=Retention time, MW=Molecular weight

RESULTS: Preliminary phytochemical analysis of ethanolic extracts of *Nelumbo nucifera* seed and seed pod are shown in (**Tables 1** and **2**) respectively. The ethanolic extract of *Nelumbo nucifera* seeds showed fourteen peaks (**Fig. 1**) and extracts of *Nelumbo nucifera* seedpods showed eleven peaks (**Fig. 2**). These peaks indicated the presence of various bioactive constituents in *Nelumbo nucifera* seeds and seedpod. Among various extracts, the ethanolic seed were rich in secondary metabolites such as Tannins, Saponins, Quinones, Terpenoids, Phenols, Steroids and alkaloids when compared with seedpod which had other secondary metabolites such as flavonoids, quinones, cardiac glycosides, terpenoids, phenol, coumarins, steroid and alkaloids. Total phenol, flavonoid and tannin contents were estimated quantitatively. Total phenolic content measured by Folin-Ciocalteau method was 93.45mg/g GAE/g in seedpod of *Nelumbo nucifera* and 10.5 mg/g in the seeds, the total flavonoid contents was measured by aluminium chloride method in seedpod of *Nelumbo nucifera* was 295.312 mg/ QE/g and 28.125mg/g in the seeds, tannin content in seedpod of *Nelumbo nucifera* is 508.7mg/g and 69.637mg/g TAE/g in seeds (**Table 5**). It was observed that the seedpod of *Nelumbo nucifera* possessed highest level of Phenolic, flavonoid and Tannin contents when compared with seeds (**Fig. 3**). The phytoconstituents of seed and seedpod of *Nelumbo nucifera* with their medicinal activities are shown in (**Tables 3** and **4**).



Time (min)

FIG. 1: GC-MS CHROMATOGRAM OF ETHANOLIC EXTRACT OF *NELUMBO NUCIFERA* SEED



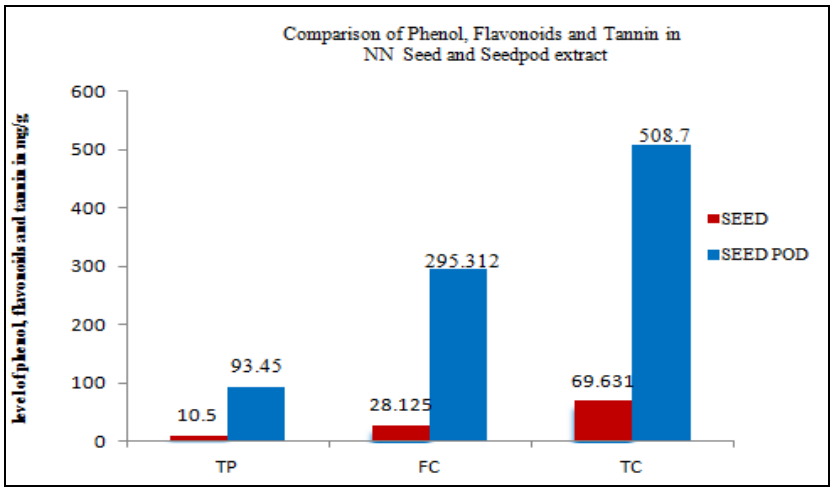
Time (min)

FIG. 2: GC-MS CHROMATOGRAM OF ETHANOLIC EXTRACT OF *NELUMBO NUCIFERA* SEEDPOD

TABLE 5: CONTENTS OF TOTAL PHENOLS, FLAVONOIDS AND TANNINS IN ETHANOLIC EXTRACTS OF *NELUMBO NUCIFERA* SEED AND SEED POD

Parts of NN	Total Phenol content (mgGAE/g)[a]	Flavonoid content (mg QE/g)[b]	Tannin content (mgTAE/g)[c]
Seed	10.5mg/g	28.125mg/g	69.637mg/g
Seed Pod	93.45mg/g	295.312mg/g	508.7mg/g

[a]GAE: Gallic acid equivalents, [b] QE: Quercetin equivalents, [c] TAE: tannin acid equivalents



TP-Total phenol, FC-flavonoids content, TC- Tannin content

FIG. 3: SHOWING THE COMPARISON OF TOTAL PHENOL, FLAVONOIDS AND TANNIN IN *NELUMBO NUCIFERA* SEED AND SEEDPOD EXTRACTS

DISCUSSION: The phytoconstituents which are present in abundant in various herbs forms a basis for curative properties of medicinal plants [29]. Their composition varies significantly in different parts of the plant. Among the bioactive constituents obtained, thirteen compounds of seed and seven compounds of seedpod were reported for their biological activities. They are n-Tetradecanoic acid, Hexadecanoicacid, 9,12-Octadecadienoic acid, 11- Hexadecenoic acid , Di-n-octyl phthalate, 1- Heptadecanol, 1- Monolinoleoylglycerol trimethylsilyl ether , Ergost-25-ene-3, 5, 6, 12-tetrol beta., 5.alpha., 6.beta., 12.beta., vitamin A aldehyde, 4,22-Stigmastadiene-3-one , Acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl 2, 3, 4, 4a, 5, 6, 7, 8-octahydronaphthalen-2-yl ester, Ergost-25-ene-3, 5, 6, 12- tetrol, (3.beta., 5.alpha., 6.beta., 12.beta.), Ergost- 25- ene- 3, 5, 6, 12- tetrol, (3.beta., 5.alpha., 6.beta., 12.beta.) 6.beta. Bicyclo[4.3.0]nonane, 5.beta.- iodomethyl- 1.beta.- isopropenyl- 4.alpha., 5.alpha.- dimethyl-, are components of seeds of *Nelumbo nucifera.*

Tetradecanoic acid, Hexadecanoic acid, 11, 14-Eicosadienoic acid, 13-Tetradecenal, Linoleic acid ethyl ester, 9-Octadecenoic acid ethyl ester, Docosanoic acid, 3.alpha.-(Trimethylsiloxy) cholest-5-ene are components of seedpods. Wu *et al*., [30] in their study had proved that the seedpod of *Nelumbo nucifera* possessed high levels of phytonutrients when compared to other parts of the plant which well correlated with our study and they were reported for various biological activities such as antioxidant, antiviral, antibacterial, anti-inflammatory and cardioprotective activities. In a previous study by Moustafa *et al*., [31] have shown that the seeds of *Nelumbo nucifera* possessed thirty eight compounds, but in our study we obtained only fourteen compounds. This shows that the bioactive components of the different parts of the plant may vary according to their growing regions. The phenolic, flavonoids and tannin contents were significantly higher in the seedpods of *Nelumbo nucifera* when compared with the seeds (**Fig. 3**), and this is the first study to show this difference in bioactive components between seed and seedpod in the white lotus in Chennai, Tamil Nadu, India. Several studies have reported the antioxidant activity of plants which is due to the presence of abundant phenolic bioactive constituents. Hence the presence of phenolic component in a plant forms important search for natural antioxidants. As plants are always natural heritage to mankind it becomes important to identify the plants which are really the rich source of these natural bioactive constituents.

CONCLUSION: In this present study a comparison between the ethanolic extracts of seeds and seedpod of *Nelumbo nucifera* revealed that the seedpod possesses higher percentage of bioactive constituents. It also shows the importance of this part of plant which is usually discarded as a waste. These results necessitate the need for phytochemical screening of plant materials so that their bioactive constituents can be detected, as a source for natural antioxidants. Further comparative analysis on the antioxidant activity of the aerial parts of *Nelumbo nucifera* and isolation of the active components is under process.

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CONFLICT OF INTEREST: Nil.

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