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Research Article

FATTY ACID COMPOSITION AND ANTIDERMATOPHYTIC AND ANTIDIARRHEAL ACTIVITY OF NELUMBO NUCIFERA SEED OIL

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ABSTRAT

The seeds of *Nelumbo nucifera* were collected from different localities of Coimbatore District. Extraction of oil from plant seed was performed using Soxhlet's procedure. Based on the physicochemical screening the lotus seed was found to be an excellent source of crude oil. The crude fat content of the ground seeds of *N. nucifera* was determined by GC-MS and identified by spectral library comparison and by the mass spectral fragmentation pattern. HPTLC analysis showed the presence of flavonoids. The hexane extract of the *N. nucifera* seed oil showed 66% inhibition of the radical DPPH. Whereas the standard ascorbic acid showed IC50 value of 15 ± 1.0 µg/ml. ABTS activity of N. *nucifera* seed oil (IC50 = 124 ± 1.0 µg/ml) was observed as the strongest activity. The antidiarrhoeal activity of *N.nucifera* seed oil was evaluated and it strongly inhibited the strains namely *Shigella* sp., *Salmonella* sp., *Klebsiella* sp., *Escherichia Coli*., *Pseudomonas* sp., and *Staphylococcus aureus* by disk diffusion method. The inhibitory activity of *N.nucifera* seed oil was pronounced against the dermatophytes like *Malassezia furfur, Trichophyton rubrum* and *Trichophyton mentagrophytes* with the extract concentration of 25µg/mL. This study systematically supports the usage of seed as a medicine for superficial bacterial and fungal infections, alternative source of nutrition and as well as renewable resources.

Keywords: *Nelumbo nucifera*, GC-MS, HPTLC, Antioxidant, Antidiarrhoeal & antidermatophytic activity, Agar disc diffusion.

INTRODUCTION

Nelumbo nucifera Gaertn (family: *Nelumbonaceae*) commonly known as Indian lotus, one of the oldest perennial aquatic herb consumed throughout Asia and commonly cultivated in Australia, China, India and Japan. Almost all parts of it, such as root, rhizome, leaf stalk, flower stalk, flower, pollen, stamen, pod's skin, young leaf, mature leaf, seed and embryo were consumed as vegetables[1]. Pharmacological studies of the plant revealed that the whole plant possess ant diabetic, antipyretic, anti-inflammatory, anti cancerous, antimicrobial and anti-obesitic properties[2,3]. *Nelumbo nucifera* flower has considerable reputation as a potent adjunct in the treatment of various ailments such as cancer, hypertension, diarrhea, fever, weakness, infection and body heat imbalance[4]. The seeds are used as an application in leprosy and other skin infections and are eaten by various people because of its caloric value.

Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical importance[5]. The suitability of oil for a particular purpose, however, is determined by its characteristics and fatty acid composition. Fatty acids are utilized in a wide variety of end-use industries that include food, medicine, rubber, plastics, detergents and cosmetics[6]. However, the sources of oil and fats are diminishing, implying that there is the growing need for new sources of oil to supplement the existing ones[7]. Much is known about the traditional use of lotus seeds; however, little information regarding its lipid composition and functional characteristics is available. Lotus oil can be added to an aromatic bath or a massage blend, or diffused in the air as a way to recharge energies and clear away unwanted thoughts. Lotus oil used gently perfuming the room and it softens the skin and protects the skin from effects of the weather. It is also said to offer protection from harmful UV rays[8].

As part of interest, the study was under taken to analyze physicochemical properties and fatty acid composition of *Nelumbo nucifera* seed oil in order to establish their potential value in medicine for skin diseases, nutritional value in the food, cosmetic industry and its contribution as the renewable resources. Further the functional characteristics of *Nelumbo nucifera* seed oil like antioxidant, antidermatophytic and antidiarrhoeal activity was determined to provide an advanced knowledge for its uses in the preparation of different pharmaceutical products.

MATERIAL AND METHODS

Collection of seed material

The seeds of *N. nucifera* were collected from different localities of Coimbatore District and authenticated by Botanical Survey of India (BSI) in "Tamil Nadu Agriculture University" Coimbatore, Tamil Nadu, India. Seed samples were sun dried for one week before drying at 105°C till constant weight in an oven. After drying, the seeds were shelled by hand to remove the kernels which were crushed to produce fine seed flour, from which oil samples were extracted.

Extraction of oil from plants seeds

The seeds of *N. nucifera* were dried in oven at 105°C crushed in to fine powder. Oil from the flour was extracted using the Soxhlet's procedur[9], by repeated washing with Hexane (boiling point 40 to 60°C). After 8 h, the Soxhlet extraction flask containing oil and solvent mixture was removed from Soxhlet apparatus. The oil dissolved in hexane was filtered using filter paper (Whitmann No. 1) and the solvent evaporated under vacuum in a rotary evaporator. The remaining solvent traces were removed by heating the flask containing oil in a water bath at 90°C. The oil obtained, was thereafter stored in closed bottles and kept at 37°C till further analyses[6].

Lipid class assays for the crude oil

Important physicochemical properties of the crude oil, concerning acid value, saponification value, peroxide value, iodine value, free fatty acid content and phospholipids content were characterized according to the AOCS recommended method[10].

Analysis of fatty acids (FAs)

The FA composition of *N. nucifera* seed oil as well as lipid classes was determined by converting into FA methyl esters (FAMEs) followed by Gas Chromatography Mass Spectroscopy (GC- MS).

Analysis of hexane extract of *N. nucifera* seed oil using HPTLC

HPTLC analysis of *N. nucifera* seed oil was conducted in order to determine phytochemical compounds. High performance thin layer chromatography was performed on 10 cm X 10 cm silica gel 60 F_{254} HPTLC plates, particle size 5-40µm and layer thickness 0.2mm, from Merck (Darmstadt, Germany). The hexane extract of *N. nucifera* seed oil were applied as narrow 8 mm bands by means of Linomat 5

(Camag, Switzerland) equipped with a 100µL Camag syringe, 3 bands were applied to the plate. Plates were then developed at 25°C in a twin trough chamber containing mobile phase I/II to a distance of 80 mm. The development distance was 70mm; before development the plates and chamber were pre saturated with Whatmann No: 1 filter paper soaked in mobile phase for 10 min. After development the plate was dried for 2 min with a hair dryer and recorded the chromatograms under UV 254 nm and UV 366 nm light with the help of Reprostar 3 (Camag, Switzerland).

Antioxidant activity

DPPH radical scavenging assay

The scavenging activity was estimated by the method described by Sanchez-Moreno[11]. Different concentrations (125µg/mL, 250µg/mL, 500µg/mL and 1000µg/mL) of hexane extract of *N. nucifera* seed oil was taken in test tubes and the volume was adjusted to 1 mL with DMSO. 0.5 ml extract was added to 3 ml of DPPH (2, 2-diphenyl-2-picrylhydrazyl) solution (0.004%) and the tubes were shaken vigorously. After incubation of 30 min at room temperature in darkness, the absorbance was read against blank at 517 nm by UV-visible spectrophotometer. Inhibition of the free radical by DPPH (*I* %) was calculated using the following equation described by Kartal *et al.,* [12]:

$$I\% = [(A\text{blank} - A\text{sample}) / A\text{blank}] \times 100$$

Where *A*blank is the absorbance of the blank (containing all reagents except the extract or standard), and *A*sample is the absorbance of the extract or standard. Experiments were carried out in triplicate and ascorbic acid was used as standard antoxidants[13]. Based on this IC_{50} value was calculated (concentration of the sample required to scavenge 50% DPPH free radicals).

ABTS radical scavenging assay

The ability of the hexane extract of lotus seed to scavenge ABTS was described by the method of Re *et al.*,[14]. In this assay, ABTS was dissolved in water at 7 mM concentration. ABTS radical cation (ABTS+) was produced by reacting ABTS stock solution with 2.45 mM potassium per sulfate (final concentration) and allowed the mixture to stand in dark at room temperature for 12 h before use. Absorbance of 2mM ABTS solution in potassium per sulfate was recorded at 734 nm by spectrophotometer. 0.1 ml of the extracts was added to 1 ml of ABTS solution and absorbance change of ABTS solution was recorded after 4 min. The scavenging ability of ABTS was determined as above said. Ascorbic acid was used as comparative standard and the samples were run in triplicate[13].

Antidiarrhoeal activity assay

A panel of enteric bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli* and *Enterobacter aerogenes* analyzed in this study was obtained from clinical laboratories.

Antidiarrhoeal activity of the crude hexane extract of *N. nucifera* seed oils was determined against enteric bacteria by an agar disc diffusion method[15]. Eighteen hours old culture of the test organisms from the nutrient broth was transferred to sterile Nutrient Agar plates to make bacterial lawn. Stock solutions (3 mg/ml) of the test samples were prepared in sterile DiMethyl Sulfoxide (DMSO, less than 1%). The crude hexane extract at the concentration of 25, 50 and 75 µl were spotted onto the filter paper discs (6 mm diameter) placed in Nutrient agar plates and incubated at 37°C for 2 days. Commercial disc of streptomycin (30µg) was used as positive control and DMSO (less than 1%) were used as negative control. Antidiarrhoeal activity was evaluated by measuring the diameter of the zones of inhibition (in mm) against the tested bacteria and the experiment was done thrice[16].

Antidermatophytic activity

Microbial strains

The cultures of dermatophytes *Malassezia furfur* (MTCC), *Trichophyton mentagrophytes* and *Trichophyton rubrum* purchased from Kovai Medical College Hospital, Coimbatore maintained on PDA medium at RT was used for the present study.

Antidermatophytic activity was determined using the agar disc diffusion method described by Parekh and Chanda[17]. Each fungal inoculum was incubated in 2.5 ml Potato dextrose broth and incubated at room temperature for 5 days. After incubation, every inoculum was spread over plates containing Potato Dextrose Agar. Five millimeter discs containing different concentrations of extract (25, 50, 75 and 100 µg/mL) were placed on cultured fungi on agar plates and incubated at room temperature for 7 days. At the end of incubation the diameter of the zone of inhibition was measured, experiment was done thrice. The disc dipped in Fluconazole antibiotic was used as positive control and in DMSO was used as negative control.

Statistical analysis

Data, unless otherwise specified, were expressed as means ± standard deviation of triplicate experiments.

RESULTS

In the present study fatty acid composition of *Nelumbo nucifera* seed oil and functional characteristic were determined in order to establish their potential value in medicine and its contribution as the renewable resources. The seeds of *N. nucifera* were collected from different localities of Coimbatore District., oil was extracted using Hexane in Soxhlet's apparatus.

Physicochemical properties of oil

The lotus seed was found to be an excellent source of crude oil *i.e.* 3.52 ± 0.12 g/100 g dry weight. The physicochemical characteristics of the lotus seed oil was investigated (Table 1) and observed as acid value (26.66 ± 1.05 mg KOH), saponification value (210.43 ± 2.97mg KOH), peroxide value (10.60± 2.00 g/100 g), iodine value (6.00 ± 1.52) and free fatty acid content of 3.50 ± 2.01 as per standard methods. The physicochemical characteristics suggested that the lotus seed with oil can be utilized for human consumption.

Table 1: Physicochemical properties of oil extracted from lotus seeds

Properties	Values*
Acid value (mg KOH)	26.66 ± 1.05
Saponification value (mg KOH)	210.43 ± 2.97
Peroxide value (g/100 g)	10.60± 2.00
Iodine value	6.00 ± 1.52
Free fatty acid	3.50 ± 2.01

*Means ± Standard Deviation of three replicates

GC-MS analysis of fatty acid extracted from *N. nucifera* seed

The crude fat content of the ground seeds of *N. nucifera* was extracted by Soxhlet with hexane as 38.6%. The fatty acid methyl esters (FAMEs) were assayed by GC-MS, fatty acid profile of the oil is shown in Fig. 1. The identification of the individual fatty acid methyl esters was carried out by spectral library comparison and by the mass spectral fragmentation pattern as Capric Acid Methyl Ester (C10:0), Lauric Acid Methyl Ester (C12:0), Tridecanoic Acid Methyl Ester (C13:0), Palmitic Acid Methyl Ester (C16:0), Pentadecanoic Acid MethyEster (C15:0) . Cis-10- pentadecanoic Acid Methyl Ester (C15:1), Palmitic Acid Methyl Ester (C16:0), Palmitooleic Acid Methyl Ester (C16:1) cis-10-Heptadecenoic Acid Methyl Ester (C17:1) and Lignoolenic Acid Methyl Ester (C19:1) were identified as a prominent fatty acid. The above analysis resulted that the *N. nucifera* seed oil might have contribution as renewable resources of fatty acids.

HPTLC analysis of hexane extracts of *N. nucifera* seed

HPTLC analysis of *N. nucifera* seed oil was conducted in order to determine phytochemical compounds. The plates developed in Mobile phase II (Ethyl acetate – Methanol - Water, 10:1.35:1 Polar**)** and visualized under UV 366 nm/white light showed nearly 10 thin bands were seen in the plate, after derivatisation one major band was visualized on the plates. The major band was scrubbed from the plate and subjected for phytochemical quality analysis. The fraction

detected consists of flavonoids (Fig. 2a) suggested that flavonoids might have contributed in antioxidant activity.

The plates developed in Mobile phase I (Toluene - ethyl acetate (9.3 + 0.7), Non polar**)** showed nearly early 8 thin bands in the plate, after derivatisation three major bands and three thin bands were visualized on the plate. The three major bands were scrubbed from the plate and subjected for phytochemical quality analysis. The fraction detected consists of sterols, steroids and bile acids (Fig. 2b).

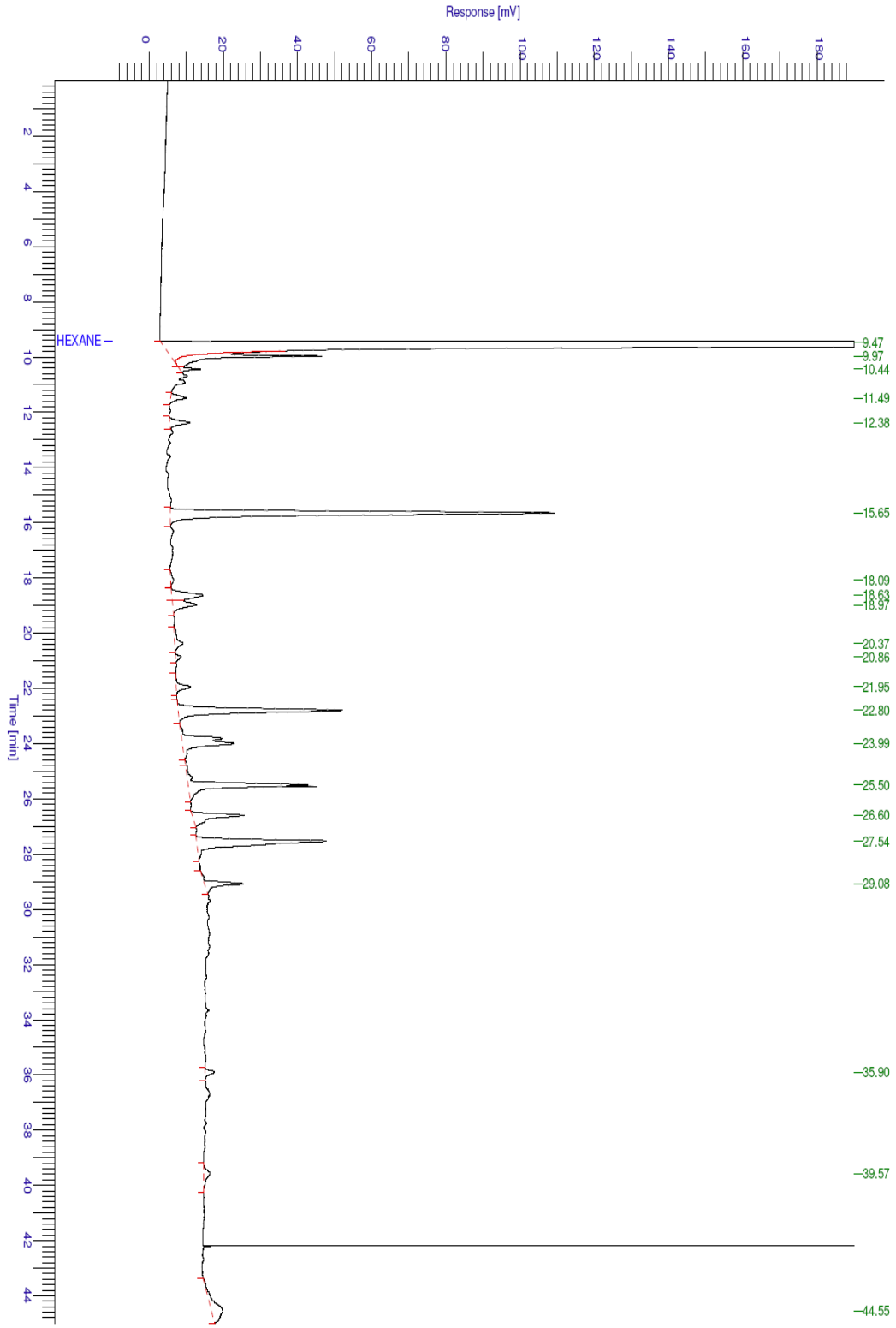


Fig 1: GC-MS chromatogram of *N. nucifera* seed oil

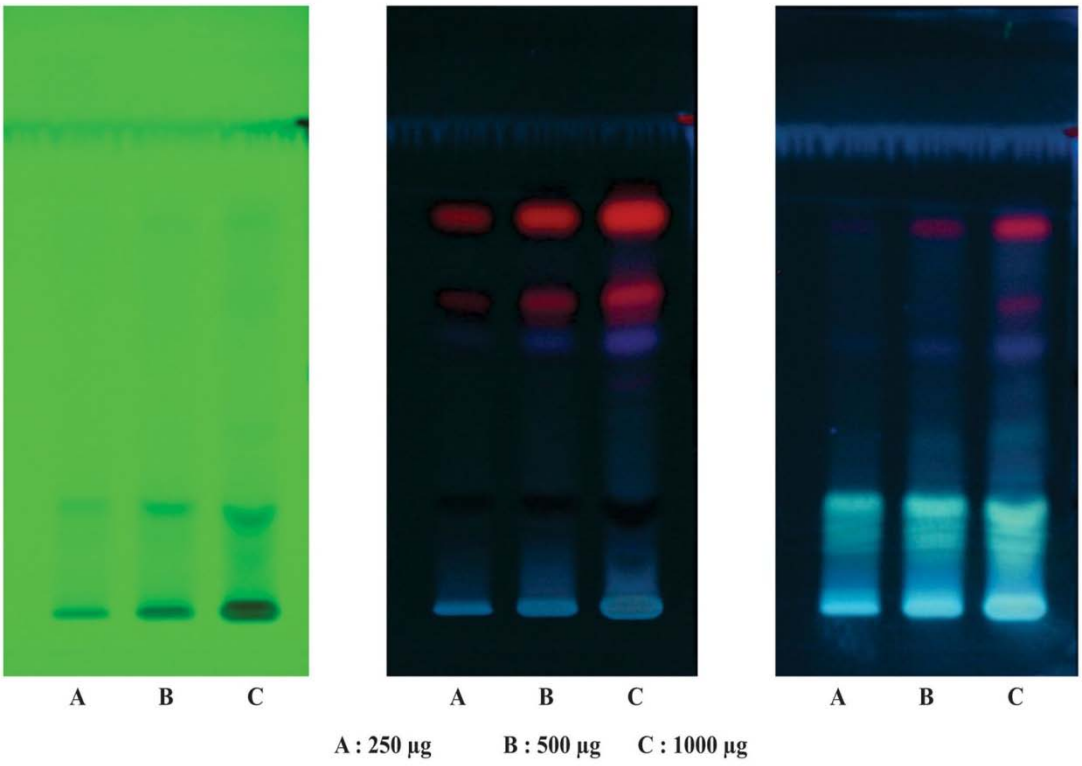
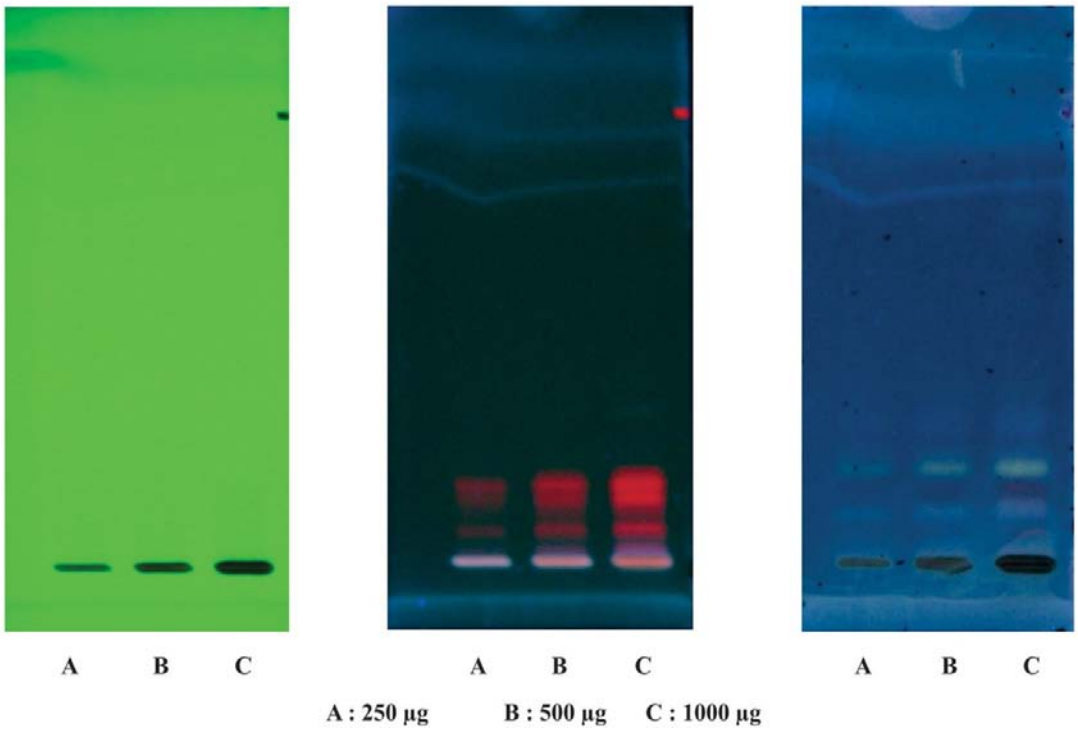


Fig 2a: HPTLC fractions of *N. nucifera* seed oil using polar solvent



b: HPTLC fractions of *N. nucifera* seed oil using non polar solvent

***Invitro* antioxidant study**

DPPH is used as a free radical to evaluate antioxidant activity of some natural compounds. The degree of its discoloration is attributed to hydrogen donating ability of test compounds. The DPPH assay was carried out in hexane extract of the *N. nucifera* seed oil with six different concentrations. The results of the anti-oxidant activity were recorded in Table 2. The hexane extract of the *N. nucifera* seed oil showed 66% inhibition of the radical DPPH.

The least concentration of the extract showed the least percentage of inhibition while the highest concentration showed the highest percentage of inhibition, were as the standard ascorbic acid showed IC50 value of 15 ± 1.0 µg/ml.

Table 2: DPPH assay of hexane extract of *N. nucifera* seed oil

Concentration (µg/mL)	% Inhibition	IC 50 (µg/mL)
1000	76.56 ± 1.4	15±1.0
500	42.78 ± 2.3	
250	31.41 ± 2.9	
125	23.13 ± 2.5	
62.5	19.05 ± 7.2	
31.25	14.62 ± 1.2	
Control : Ascorbic Acid	11.24 ± 0.2	

The radical scavenging activities of lotus extract were evaluated using the method of ABTS followed by DPPH which have been widely used to test radical scavenging activity. Result of the evaluation of extract at various concentrations along with reference compound were showed in Table 3, it was observed that *N. nucifera* seed oil (IC50 = 124 ± 1.0 µg/ml) had the strongest activity. The hexane extract of *Nelumbo nucifera* seed oil exhibited high antioxidant activity by scavenging DPPH and ABTS radicals. The good antioxidant activity of might be attributed due to the presence of flavonoids that was proved by HPTLC analysis. The findings of this study confirmed the therapeutic potency of *N. nucifera* seed oil in traditional medicine.

Table 3: ABTS assay of hexane extract of *N. nucifera* seed oil

Concentration (µg/mL)	% Inhibition	IC 50 (µg/mL)
1000	346.34 ± 2.1	124 ± 1.0
500	282.87 ± 1.3	
250	161.44 ± 3.0	
125	84.23 ± 1.5	
62.5	49.15 ± 3.2	
31.25	54.42 ± 2.2	
Control : Ascorbic Acid	31.14 ± 1.2	

***In Vitro* antidiarrhoeal activity of *N.nucifera* seed extract**

The antidiarrhoeal activity of the hexane extract of *N.nucifera* seed oil extracts were evaluated at three different concentrations against five diarrhea causing bacterial strains by disk diffusion method and the results were summarized in Table 4. The antidiarrhoeal activity of *N.nucifera* seed extract was found to be increased in dose dependent manner.

The maximum zone of inhibition was exhibited by *N.nucifera* seed oil against *Shigella* sp., (14.6±0.58) at the concentration of 75µg/ml followed by *Staphylococcus aureus* (12.3±0.6) and *Klebsiella sp.,* (11.3±0.12) sp. whereas moderate activity was observed against *Salmonella* sp., (10±0.1), lowest zone of inhibition was observed against *Escherichia Coli* (0.67±0.06). The results revealed that gram negative bacteria were more susceptible to the *N.nucifera* seed extract than gram positive bacteria. The antidiarrhoeal activity of standard antibiotic streptomycin was found to be similar as *N.nucifera* seed extract against all the tested bacterial strains, were as in control no zone formation was observed (Table 4). The hexane extract of *N. nucifera* seed oil posses' powerful antidiarrhoeal activity against enteric organisms, stands as a scientific support for the usage of this lotus seed for treating diarrhea and in traditional medicine.

Table 4: Antidiarrhoeal activity of *N. nucifera* seed extract

Test Organisms	Diameter of Zone Of Inhibition(mm)				
	Control	Ab	25 µg/ml	50 µg/ml	75 µg/ml
Shigella sp.,	NZ	15.3±0.58	9.0±1.0	10.0±1.0	14.66±0.58
Salmonella sp.,	NZ	10.4±0.58	3.34±0.58	4.66±0.58	10.0±1.0
Klebsiella sp.,	NZ	11.3±1.52	8.33±0.58	10.0±1.0	11.3±1.16
Escherichia Coli	NZ	7.0±1.74	2.33±0.58	5.33±0.58	6.67±0.58
Pseudomonas sp.,	NZ	6.4±1.53	3.0±1.0	4.66±1.15	6.0±1.0
Staphylococcus Aureus	NZ	13.6±1.15	7.3±0.58	10.0±1.0	12.33±0.58

NZ – No Inhibition Zone; Values are Means ± Standard Deviation of three parallel replicates.

***In Vitro* antidermatophytic activity of *N.nucifera* seed extract**

The maximum concentration of *N.nucifera* seed extract used in the present study was < 75 µg/mL and it was found to be effective in controlling the tested dermatophytes (Table 5). With all the concentrations used, the inhibitory activity of the hexane extract of *N.nucifera* seed oil was found to be very significant. The dermatophyte *M. furfur* showed inhibition of 17.33±3.06mm, 20.67±3.21mm, 24.67±3.51mm with the extract concentrations of 25, 50 and 75µg/mL. The inhibition produced by the antibiotic was found to be 20.67±1.15mm.

Table 5: Antidermatophytic activity of *N. nucifera* seed extract

Test Organisms	Diameter of Zone of Inhibition (mm)				
	Control	Ab	25 µg/ml	50 µg/ml	75 µg/ml
Malassezia furfur	NZ	22.33±2.51	17.33±3.06	20.67±3.21	24.67±3.51
Trichophyton mentagrophytes	NZ	20.67±1.15	22.33±3.05	22.00±2.0	22.33±2.08
Trichophyton rubrum	NZ	21.66±1.52	19.66±3.51	22.33±2.51	23.67±1.52

NZ – No Inhibition Zone; Values are Means ± Standard Deviation of three parallel replicates.

The next sensitive organism was *T. mentagrophytes* sterile colony with 22.33±2.51 mm inhibition in antibiotic treatment. The hexane extract inhibited the growth of *T. mentagrophytes* by 22.33±2.08 mm with the increasing concentration 75 µg/mL. The growth of *T. rubrum* was inhibited by the antibiotic and the inhibition was 21.66±1.52 mm, while the extract produced 22.33±2.51 mm, 23.67±1.52mm inhibition when used in the concentrations of 50, 75 µg/mL, respectively. These findings suggested that seed oil could be used for preparation of cream, ointment and lotion as per the requirement of the treatment.

DISCUSSION

The physicochemical characteristics of the *Nelumbo nucifera* seed oil were estimated as acid value (26.66 ± 1.05 mg KOH), saponification value (210.43 ± 2.97mg KOH), peroxide value (10.60 ± 2.00 g/100 g), iodine value (6.00 ± 1.52) and free fatty acid content of 3.50 ± 2.01 was observed as per standard methods. The acid value and saponification value was found to be higher as compared to those reported by Hamed *et al.,*[18] revealed iodine value (90.0), acid value (3.70) and saponification value (175.8) of lotus seed oil. Similarly acid value and saponification value of the present study was found to be higher as compared to those reported by Bi *et al*., [19] for lotus plumule oil and for rhizomes of lotus reported by Shad *et al*., [20].

The fatty acid methyl esters (FAMEs) of *N. nucifera* seed oil was assayed by GC-MS, appreciable fatty acid profile was observed. The identification of the individual fatty acid methyl esters was carried out by spectral library comparison and by the mass spectral fragmentation pattern. The FA profiles of lotus seed oil of the current study was supported by the earlier works reported by Hamed *et al.,*[18] and Bhat and Sridhar,[21].

Analyzing the solvent extract of plant samples by HPLC will help to know its composition and promote biological properties[22]. Various phytoconstituents from plants were reported to be responsible for cardioprotective activity including carotenoids, triterpenes, flavonoids, cardiac glycosides, alkaloids, saponins and terpenoids[23]. In this study, flavonoids compounds were observed in *N. nucifera* seed extract qualitatively by HPTLC method might have contributed in antioxidant activity and also cardioprotective activity. Similarly the identification of natural product, including flavonoids and phenolic compounds which are attributed to antioxidant activity of the soybean by HPTLC method was reported by Hubert *et al.,*[24], Malencic *et al.,*[25] and Maltas *et al.,*[13].

The antioxidative phytochemicals in grains, vegetables and fruits have received increasing attention recently for their potential role in prevention of human diseases as well as in food quality improvement[26]. Lotus seed has been used both as vegetables and medicine in eastern Asia, particularly in China. DPPH and ABTS is frequently used free radical to determine the antioxidant ability of some natural compounds. In the present study antioxidant activity of lotus seed extract was carried out as per the above said assays. The maximum antioxidant capacity of *N.nucifera* seed extract was found to be 76.56 ± 1.4 and 346.34 ± 2.1 percent inhibition at 1000µg/ml concentration in DPPH and ABTS assay. Likewise the antioxidant activity of leaves of *N. nucifera* had been reported earlier by Saengkhae *et al.,*[4] followed by Gayathri *et al.,*[27]and for rhizomes of *N. nucifera* has been reported by Dongmei-Yang *et al.,*[26] and Shad *et al.,*[20].

The antidiarrhoeal activity of *N. nucifera* seed oil against five clinical microorganisms such as *S. aureus, E. coli, Shigella* sp., *salmonella* sp. and *klebsiella* sp was determined. The disc diffusion method for antidiarrhoeal activity showed significant reduction in microbial growth of all the tested organisms in terms of zone of inhibition. Similar work by Rogger *et al*.,[28] reported that antibacterial effect of *Nelumbo nucifera, Tithonia diversifolia* against *S. aureus, P. aeruginosa and Escherichia coli* suggesting that the plant can be used in the treatment of gastrointestinal infection and diarrhea in human. Likewise essential oil extracted from *N. nucifera* pollen grains showed significant inhibition of *S. typhimurium* ATCC14028 and *E.coli*ATCC25922 among all the tested gram positive organisms. In contrast antibacterial activity of the hydroethanolic extract of both white and pink *N.nucifera* flower extracts were evaluated by Brindha and Arthi, [16]. The maximum zone of inhibition was exhibited by both white and pink *N.nucifera* flowers against *E.coli* (16mm & 14mm), *B.Subtilis* (15mm & 13mm) and *S.aureus* (13mm & 11mm). In such a way, methanolic extract of whole plant of *nelumbo nucifera* gaertn showed appreciable antimicrobial activity[29].

In the course of all the concentrations tested, the inhibitory activity of the hexane extract of *N.nucifera* seed oil was pronounced against the dermatophytes like *Malassezia furfur, Trichophyton rubrum* and *Trichophyton mentagrophytes* with the extract concentration of 25µg/mL. Similarly the antimalarial and antifungal activity of *N. nucifera* extract has been reported by Agnihotri *et al.*[30], Sakthi-Abirami *et al.,*[29]. The findings of this study (antioxidant, antidermatophytic and antidiarrhoeal activity) confirmed the therapeutic potency of *Nelumbo nucifera* seed oil in preventing the progress of diseases and can be used in alternative medicine. Future work is therefore under progress to identify and elucidate the bioactive compounds that are responsible for free radical scavenging activity to establish its potential as therapeutic medicine in animal demonstration.

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