

Antioxidant and Hepatoprotective Activity of Lotus (*Nelumbo Nucifera*) Seed Extract

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Abstract

Background: Lotus the National flower of India is commonly called as Pink lotus belonging to the species *Nelumbo nucifera*. The seeds of the mature lotus are of great importance and are extensively used in traditional Chinese medicine for their free radical scavenging effects. Hence the present study was undertaken to assess the Antioxidant and Hepatoprotective activity of methanol, ethanol and acetone extracts of lotus (*Nelumbo nucifera*) seeds by in vitro method.

Methods: The 3 extracts of Lotus seed were tested for its antioxidant potential using DPPH, FRAP Reducing power activity and H₂O₂ scavenging activity. All the analysis was carried out using UV - Visible spectrophotometer. The Hepatoprotective activity of lotus seed extract was analysed on HepG2 cell lines using MTT assay. This was carried out in 96 well plates using methanolic extract of lotus seed at concentration range 25µl, 50 µl and 75 µl for 48 hours which showed a dose dependent cytotoxic effect.

Results: The results of the present study revealed that DPPH activity was greater in Acetone extract (86.6 per cent), the percentage inhibition in FRAP was found to be high in methanolic extract with 93.28 per cent. Among the 3 extracts, aqueous extract of lotus seed showed highest reducing power with 81.2 per cent. Hydrogen peroxide scavenging activity was found to be high in methanolic extract with the highest inhibition of 67.52 per cent. From this methanolic extract was found to be the best than the others thus hepatoprotective activity was tested in methanolic extract. On evaluation it was observed that the least concentration of extract exhibited more pronounced activity than the other concentration which was evident from the Formazan formation.

Conclusion: Thus lotus seeds are medicinally versatile and can be used as a nutraceutical for delivering bio active components.

Keywords: Hepatoprotective activity; Formazan; Nutraceutical

Introduction

Plants have been part of our lives since the beginning of time. Numerous products from plants are obtained of which, most of them are not only good and beneficial for health, but are also crucial to our existence. In the recent years, there has been an increasing interest in certain compounds having enormous health effects which include antioxidants and phytochemicals. According to Ncube et al. [1] medicinal plants are the richest bio-resource of modern medicines, traditional medicine, nutraceuticals, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs. Plants contain wide variety of bioactive molecules including Terpenoids, steroids, phenols, and flavonoids. Approximately 80% of the world's population has employed traditional medicine for health care, which is based predominantly on plant materials. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs. Plant derived phenolic, flavonoid and polyphenolic compounds are considered to contribute to the prevention of diseases associated with oxidative stress.

Cancer is one of the major causes of death and globally the numbers of cases of cancer are highly increasing. Hepato Cellular Carcinoma (HCC) is the sixth most common cancer worldwide, accounting for 9.1% of all cancers and an estimated incidence of 746,000 new cases every year. It is considered to be the third cause of cancer related deaths (692,000 cases). The highest incidence rates of HCC around 85 per cent of cases are present in East Asia and sub-Saharan Africa [2]. HCC has nearly doubled over the last decade among patients with Chronic Liver Disease (CLD) [3]. Using chemo-preventive agents that have low toxicity and high efficiency in inhibiting tumour growth and are involved in decrease of carcinogenic agents effects is very promising target for cancer therapy [4].

Recently, attention has been increased to identify the naturally occurring anticancer agents, particularly those present in dietary and medicinal plants due to their bioactive substances [5]. In many developed countries herbal medicines are achieving attractiveness as alternative and courtesy therapies [6]. *Nelumbo nucifera*, (2n = 16) commonly known as Lotus 'The National Flower of India' is a naturally occurring anticancer agent

and an aquatic perennial plant belonging to family Nelumbonaceae [7].

The use of lotus seeds in treating various ailments were stressed in a review done by Khare [8] which mentioned that the seeds and fruits are used as a health food in Asia and to treat many ailments, including palpitations, poor digestion, chronic diarrhoea, enteritis, insomnia, spermatorrhoea, dermatopathy, halitosis, menorrhagia, leprosy, leucorrhoea, tissue inflammation, cancer, fever and heart complaints and as an antiemetic, poisoning antidote, diuretic and refrigerant [9]. Cytotoxicity, antioxidant activity, anti-inflammatory activity [10], are considered to be some of the possible mechanisms through which the lotus seed extract may mediate its anti-hepatocarcinogenic action.

Hence the present study is a small footprint in the path of innovation with the following objectives to,

1. Conduct quantitative analysis of phytochemicals and,
2. Evaluate the therapeutic effect of Lotus seed extract against Hepato Cellular Carcinoma (HepG2) cell line.

Materials and Methods

The dried seeds of lotus (*Nelumbo nucifera*) flower needed for the present study were collected from flower market, locality in Coimbatore, Tamilnadu, India. From the purchased seeds mature ones alone were picked up for carrying out the study. Initially the dried lotus seeds were broken into pieces using an electric grinder, since the seed coat was very hard. The broken seeds were then crushed to produce fine powder using mortar and pestle and stored in an air tight container.

Preparation of Extracts

Methanol, acetone and aqueous extracts of lotus seed sample was prepared by soaking 10 gram of powdered lotus seed in 100ml of methanol, acetone and distilled water each separately. This was mixed well and covered tightly using Polythene paper to prevent evaporation of solvent added. This was then placed in a shaking incubator for 24 hours at room temperature to collect the extract. The sample was then filtered through Whatman No.1 filter paper. The extract obtained was thereafter stored in closed bottle at room temperature.

Quantitative Analysis of Phytochemical Constituents

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay: As illustrated by Blois (1958) [11] 100µl of lotus seed extract was taken in a eppendorf tube. To that 100µl of 0.1 molar DPPH and 400µl (0.4ml) 50m Molar Tris HCl was added. This was then incubated at room temperature for 30 minutes and measured in spectrophotometer at 517nm. The capability of scavenging DPPH radical was calculated using the following equation (Ara and Nur, 2009).

$$\text{Percentage Scavenged} = (A_{\text{control}} - A_{\text{test}}) / (A_{\text{control}}) \times 100$$

Where “A control” is the absorbance of the control reaction and “A test” is the absorbance of the sample of the extracts.

Ferric ion Reducing Antioxidant Power (FRAP): One ml of extract was taken and added one ml of Phosphate buffer solution. Mixed well and added one ml of 0.1 per cent potassium ferric cyanide. This was incubated at 50°C for 20 minutes. To that added one ml of 10% Tri Chloro Acetic Acid (TCA). Mixed well and added one ml of distilled water. To this 0.5 ml of 0.1 per cent ferric chloride was added and measured spectrophotometrically at 700 nm wavelength. The inhibition percentage of FRAP was calculated using the given equation:

$$\text{Inhibition rate (\%)} = (A_{\text{control}} - A_{\text{test}}) / (A_{\text{control}}) \times 100$$

Where “A control” is the absorbance of the control reaction and “A test” is the absorbance of the sample of the extracts.

Reducing power activity: Two ml of extract was taken and added two ml of Phosphate buffer solution. Mixed well and added two ml of 0.1 per cent potassium ferric cyanide. This was incubated at 50°C for 20 minutes. To that added two ml of 10 per cent Tri Chloro Acetic Acid (TCA). Mixed well and added two ml of distilled water. To this 0.5 ml of 0.1per cent ferric chloride was added and measured in spectrophotometer at 700 nm wavelength. The inhibition rate was calculated using the following equation:

$$\% \text{ Reducing power} = (A_{\text{control}} - A_{\text{test}}) / (A_{\text{control}}) \times 100$$

Where “A control” is the absorbance of the control reaction and “A test” is the absorbance of the sample of the extracts.

H₂O₂ scavenging activity: To 0.5 ml of extract, added 2 ml of 20mM H₂O₂. This was mixed well and added 0.9 ml of ethanol. This was incubated at room temperature for 10 to 15 minutes and the absorbance of the sample is measured in spectrophotometer at 230nm wavelength. The inhibition rate of H₂O₂ was calculated using the equation given below:

$$\text{H}_2\text{O}_2 \text{ scavenging effect (\%)} = (A_{\text{control}} - A_{\text{test}}) / (A_{\text{control}}) \times 100$$

Where “A control” is the absorbance of the control reaction and “A test” is the absorbance of the sample of the extracts

Evaluating the effect of lotus seed extract on apoptosis of HepG2 cell line by MTT assay Cell culture

Cell lines used in the present study was HepG2 cell line. HepG2 is a perpetual cell line consisting of human liver carcinoma cells, derived from the liver tissue of a 15-year-old Caucasian male who had a well-differentiated hepatocellular carcinoma [12]. HepG2 cell lines were obtained from CBNR (Centre for Bioscience and Nanoscience Research) laboratory, Eachanari, Coimbatore. The culture was maintained in DMEM (Dulbecoo's Modified Eagels Medium, high glucose), supplemented with 10% Fetal Bovine Serum (FBS) (Hi Media, Mumbai), and 10µl Ampicillin (Hi Media, Mumbai) and incubated at 37°C in a 5% CO₂ atmosphere.

MTT Assay

Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4, 5-dimethylthiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg. isopropanol) and the released, solubilised formazan reagent is

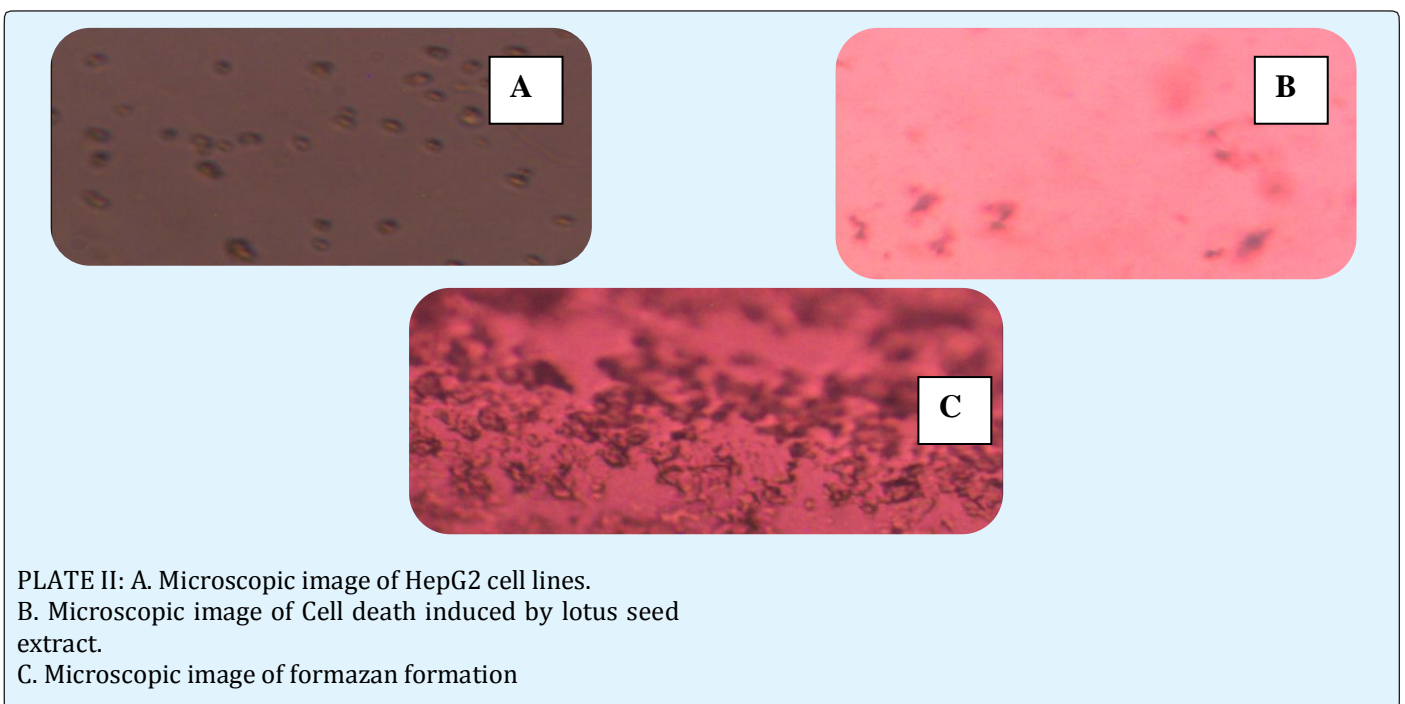
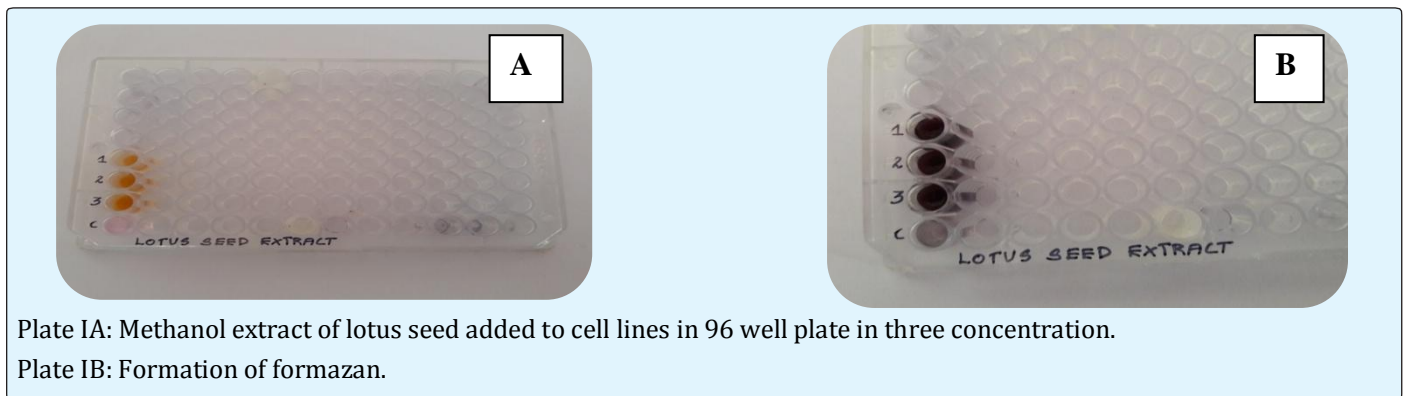
measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells [13].

In this study MTT assay was followed according to the method described by Akinpelu and Onakoya [14]. In a 96 well plate methanolic extract of lotus seed was added in three different concentrations as 25 μ l, 50 μ l and 75 μ l respectively. To which the HepG2 cells were added at a concentration of 100 μ l per well after incubating the cells for 24hrs. Controls were performed in which only cells were added at a concentration of 200 μ l. After addition the plate was then incubated at 37°C in a 5% CO₂, 95% air atmosphere. The cell culture suspension was washed with 1 x PBS (Phosphate Buffered Saline) and then added with 200 μ l MTT [3-(4, 5-Dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium Bromide] solution to the culture. It is then incubated at 37°C for 3 hours, removed all MTT solution,

washed with 1 X PBS and added with 300 μ l DMSO to each culture flask and incubated at room temperature for 30 minutes until all cells get lysed and homogenous colour was obtained. The solution was then transferred to centrifuge tube and centrifuged at top speed for 2 minutes to precipitate cell debris. Debris was dissolved using DMSO. OD (optical density) was measured at 540 nm using DMSO blank. Then the percentage viability was calculated using the percentage of viability formulated.

Percentage of viability = OD of Sample / OD of Control X 100

The percentage of cell death was calculated using the percentage cell death formula,
Percentage of cell death = OD of control - OD of test / OD of control X 100



Results and discussion

Quantitative analysis of phytochemical constituents

DPPH radical scavenging assay: The data on percentage inhibition of DPPH radical scavenging assay for three different extracts of Lotus seed is presented in Table I. In the present study the DPPH assay for three extracts of Lotus seeds were estimated by Blois method. The percentage inhibition for methanolic extract was 80.2, for acetone extract it was 86.6 and for aqueous extract it was found to be 75.8. Among the three extracts acetone

extract was found to be the most efficient solvent for scavenging activity of Lotus seed and had the highest percentage of inhibition. The obtained results are in accordance with the study carried out by Mohadjerani and Pakzad (2013) [15] proved that the acetone extract contained the highest amount of total phenolics, and was found to be the most active radical scavenger followed by methanol and water extracts. Sohn (2003) [16] reported that the organic solvent extracts of lotus seeds were reported to have high levels of DPPH radical scavenging activity and inhibited both the production of serum enzymes and cytotoxicity.

Extracts	DPPH scavenging activity (%) at 517nm	FRAP (%) at 700nm	Reducing Power Activity (%) at 700nm	H ₂ O ₂ scavenging activity (%) at 230nm
Methanol	80.2	93.28	54.6	67.52
Acetone	86.6	9.28	7.53	11.64
Aqueous	75.8	92.5	81.2	60.1

Key - nm (nanometre), OD (optical density)

Table 1: Quantitative analysis for phytochemical constituents of lotus seed extracts

Ferric Ion Reducing Antioxidant Power (FRAP): FRAP assay for lotus seed extract was carried out according to the method described by Lim and Murtijaya [17]. From the above table it is observed that the methanolic extract of lotus seed with percentage inhibition of 93.28 was found to be the highest followed by aqueous extract with 92.50 per cent and acetone extract with 9.28 per cent which was the least. Ahmed, 2015 in a study confirmed that the methanolic extract which had the highest phenolic and flavonoid content, found to display the highest reducing power. The correspondence alluded to the fact that phenolics and flavonoids possess high antioxidant potential.

Reducing Power Activity: The reducing power activity of various extracts was determined and the results are depicted in Table I. The aqueous extract of lotus seed showed the highest reducing power with 81.2 per cent followed by methanol extract with 54.6 while acetone extract with 7.53 per cent was least active. Thus, increase in absorbance of sample with concentrations indicates high reducing potential of the samples. According to Shi et al. [18] the higher absorbance of the extracts may be due to its strong reducing power potential. The reducing power of the extracts may be due to the biologically active compounds in the extract which possess potent donating

abilities. This assay further confirmed the antioxidant properties of the extracts.

H₂O₂ scavenging activity: Hydrogen peroxide scavenging activity of lotus seed extracts were evaluated and given in table I. The percentage inhibition for various extracts was calculated using the specified formula. From the values obtained it is evident that the methanol extract of lotus seed showed highest inhibition with 67.52 per cent, followed by aqueous extract with 60.1 per cent and at the least acetone extract with 11.64 per cent.

Effect of Lotus Seed Extract on Apoptosis of HepG2 Cell Line

Table 2 gives the percentage of cell death and cell viability. The effects of methanol extract of lotus seed on cell growth was assessed by the MTT assay after 48 hours of incubation with seed extract. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. It was observed from the present study that HepG2 cell line when suspended in lotus seed extract obtained using methanol significantly increased HepG2 cell death in dose-dependent manner which was evident by the colour

change from pink to purple (shown in plate I and II), this was because viable cells with active metabolism converted MTT into a purple coloured formazan product with an absorbance maximum at 570 nm. When cells die,

they lose the ability to convert MTT into formazan, thus colour formation serves as a useful and convenient marker of the viable cells [19].

Samples	Concentration of Methanol Extract of lotus seed	HepG2 Cell lines	OD value	OD value of control	Percentage of cell death	Percentage of cell viability
Sample 1	25µl	100µl	0.106	0.829	87.2	12.78
Sample 2	50µl	100µl	0.495		40.28	59.71
Sample 3	75µl	100µl	0.764		7.84	92.16

Table 2: Effect of lotus seed extract on HepG2 cell line

Treatment of extract by concentrations range of 25-75 µl for 48 hours showed a significant dose-dependent cytotoxic effects on HepG2 cell line with maximum elimination of cells which was about 87.2 per cent at a concentration of 25 µl followed by 40.28 per cent at a concentration of 50 µl and to the least 7.84 per cent for 75 µl respectively. Lotus seed extract showed an outstanding decrease in cell viability which was 12.78 per cent at a concentration of 25µl, followed by 59.71 per cent for a concentration of 50µl and 92.16 per cent for 75µl respectively. It is possible that the decrease in cell viability by lotus seed treatment as determined by MTT assay could be due to either cell growth arrest or cell death.

The results obtained are in correspondence with the experiment carried out by Dhanarasu *et al.*, 2013[20] which relayed that Lotus seed extracts exhibited hepatoprotective effects against production of serum enzymes and cytotoxicity caused by carbon tetrachloride extract also protected against the genotoxic and cytotoxic effects of aflatoxin B1.

Conclusion

The results of the study form a basis for using lotus seed in traditional medicine to manage ailments and disorders. It also contains some biologically active constituents worthy of further investigations. Lotus seed may offer new alternatives to the limited therapeutic options that exist at present in the treatment of liver diseases or their symptoms and they should be considered for future studies. Through generating awareness regarding usage of lotus seed and exploring natural product properties, healthcare professionals can play significant clinical roles as knowledge resources for masses.

References

1. Ncube NS, Afolayan AJ, Okoh AI (2008) Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology* 7(12): 1797-1806.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, et al. (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN. *International Journal of Cancer* 136(5): E359-E386.
3. Abdel-Hamid N (2009) Update to risk factors of HCC. *International Journal of Medicine and Medical Sciences* 1(3): 38-43.
4. Sun J, Hai Liu R (2006) Cranberry phytochemical extracts induce cell cycle arrest and apoptosis in human MCF-7 breast cancer cells. *Cancer Letters* 241(1): 124-134.
5. Sultan AS, Sherif ZA (2011) Over expression synergizes with mistletoe (*Viscum album*) extracts, and/or diallyl trisulfide (DATS) to inhibit breast cancer stem cell characteristics, epithelial-mesenchymal transition, and invasion potential. *Molecular Cancer Therapeutics* 10 (11): B42.
6. Omobuwajo OR, Abdu A, Igbeneghu OA, Agboola IO, Alade GO (2011) Preliminary investigation of herbal soap incorporating *Cassia senna* (L) Roxb leaves and *Ageratum conyzoides* linn whole plant powders. *Continental Journal of Pharmaceutical Sciences* 5: 1-10.

7. Sheikh SA (2014) Ethno-medicinal uses and pharmacological activities of lotus (*Nelumbo nucifera*). *Journal of Medicinal Plants Studies* 29(6): 42-46.
8. Khare CP (2004) *Indian Herbal Remedies: Rational Western Therapy, Ayurvedic, and Other Traditional Usage*. Botany 1st edn. USA: Springer 326-327.
9. Anon (1982) *Pharmacognosy of Indigenous Drugs*. New Delhi: Central Council for Research in Ayurveda and Sidhha 2: 806.
10. Lin JY, Wu AR, Liu CJ, Lai YS (2006) Suppressive effects of lotus plumule (*Nelumbo nucifera* Geartn.) supplementation on LPS-induced systemic inflammation in a BALB/c mouse model. *Journal of Food and Drug Analysis* 14(3): 273-278.
11. Blois, MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature* 181(4617): 1199-1200.
12. <http://www.hepg2.com>
13. Van Meerloo J, Kaspers G, Cloos J (2011) Cell sensitivity assays: the MTT assay. *Methods Mol Biol* 731: 237-245.
14. Akinpelu DA, Onakoya TM (2006) Antimicrobial activities of medicinal plants used in folklore remedies in South-Western. *African J Biotech* 5(11): 1078-1081.
15. Maryam Mohadjerani, Khatereh Pakzad (2013) Evaluation of total phenolic content and antioxidant activity of *Nelumbo nucifera* seed from north of Iran. *Journal of Applied Chemistry* 7(25).
16. Sohn DH (2003) Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera*. *Phytomedicine* 10(2-3): 165-169.
17. Lim YY, Murtijaya J (2007) Antioxidant properties of *Phyllanthus amaruse* extracts as affected by different drying methods. *LWT- Food Science and Technology* 40(9): 1664-1669.
18. Feng Shi, Xiaobin Jia, Chenglei Zhao, Yan Chen (2010) Antioxidant Activities of Various Extracts from *Artemisia selengensis* Turcz (LuHao). *Molecules* 15(7): 4934-4946.
19. Terry L Riss, Richard A Moravec, Andrew L Niles, Helene A Benink, Tracy J Worzella, et al. (2015) *Cell Viability Assays* 1-3.
20. Dhanarasu S, Al-Hazimi A (2013) Phytochemistry, Pharmacological and Therapeutic Applications of *Nelumbo nucifera*. *Asian Journal of Phytomedicine and Clinical Research* 1(2): 123-136.