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RESEARCH ARTICLE

Phytochemical Analysis and Anticancer Activity of *Nelumbo nucifera* Floral Receptacle Extracts in MCF-7 Cell Line

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Abstract

Nelumbo nucifera floral receptacle methanolic extracts were evaluated for its phytochemical constituents and anticancer activity. Preliminary phytochemical analysis of the extract revealed the presence of saponins, phlobotannins, flavonoids, tannins, sterols and phenols but with few differences in receptacles of white and pink flower. The methanolic extract of the receptacles showed equally good antioxidant activity as determined by DPPH radical scavenging activity assay where *N. nucifera* white receptacle had a better antioxidant activity of 54.14%. Quantitative analysis of compounds was performed using GC-MS in which 9-octadecadienoic acid and 2,2,4-Trimethyl-3-[3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl]-cyclohexanol was found to be the potential compounds that may be responsible for producing anticancer activity in human breast cancer cell line (MCF-7). Anticancer activity of methanolic receptacle extract by MTT assay showed good activity against MCF-7.

Keywords: *Nelumbo nucifera* receptacle, phytochemical analysis, GC-MS analysis, MTT assay.

Introduction

Cancer is the 2nd largest killer disease threatening the world and it is not controlled properly due to the resistance developed in the body on prolong administration of modern medicine. This has forced the researchers to search for an effective eco-friendly, natural control and cure for cancer. Natural drugs are drugs of choice, especially those derived from plants started in the last decades. “Green Medicine” is healthy, safer and is highly potent, cost-effective and eco-friendly. *Nelumbo nucifera,* a monogeneric plant belongs to the family Nymphaeaceae, has numerous common names namely Indian lotus, Chinese water lily and sacred lotus. There are two varieties of ‘kamala’: one has white flowers and is commonly called ‘pundarika’ or ‘sveta kamala’; the other has pink or reddish-pink flowers and is called ‘rakta kamala’. Different classes of phytoconstituents have been isolated from various parts of *N. nucifera*. The most important classes include alkaloids, steroids, triterpenoids, flavonoids, glycosides and polyphenols (Wang *et al.,* 1991; Wu *et al.,* 2004; Chen *et al.,* 2007). Extracts of different parts have shown antioxidant (Hu *et al.,* 2002; Jung *et al.,* 2003; Hyun *et al.,* 2006), anticancer (Liu *et al.,* 2004), antiviral (Kuo *et al.,* 2005), antiobesity (Ono *et al.,* 2006), hypocholesterolaemic, antipyretic, hepatoprotective (Rao *et al.,* 2005), hypoglycemic, antidiarrhoeal, antifungal, antibacterial, anti-inflammatory and diuretic activities (Mukherjee *et al.,* 1995). Keeping the above facts in view, the main objective of the present study was to analyze the phytochemical constituents and anticancer activity of *N. nucifera* floral receptacles.

Fig. 1. *Nelumbo nucifera* pink flower with receptacle in the centre.

Materials and methods

Chemicals and reagents: Analytical grade chemicals supplied by Hi-Media was used in the study namely methanol, phosphate buffer saline (PBS), 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Dulbecco’s modified eagle’s medium (DMEM), fetal bovine serum (FBS), dimethyl sulfoxide (DMSO) and trypsin-EDTA.

Collection and solvent extraction of N. nucifera flower receptacles: The flowers of *N. nucifera* (white and pink) were collected from Sri Raghavendra Mutt, Srirangam, Tiruchirapalli, TN between December 2015 to January 2016 (Fig. 1).

The botanical identity of the plant was authenticated by RAPINAT herbarium, Dept. of Botany, St. Joseph College, Tiruchirapalli. The receptacles were shade dried and powdered using mechanical pulverizer and subjected for extraction. The dried powder of both pink and white *N. nucifera* receptacle (20 g each) was extracted successively with methanol (60°C) using Soxhlet apparatus for 48 h and the solvent was removed at 40-50°C under reduced pressure in a rotary evaporator.

Phytochemical analysis: Phytochemical analysis of flavonoids, alkaloids, tannins, terpenoids, saponins and glycosides was performed for according to Evans (2002).

DPPH radical scavenging activity assay for antioxidants: The free radical scavenging activity of the fractions was measured *in vitro* by 2,20-diphenyl-1-picrylhydrazyl (DPPH) assay (Deng *et al.,* 2011). The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and stored at 20°C until required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.62±0.02 at 515 nm using the spectrophotometer. A 2 mL aliquot of this solution was mixed with 50 µL of the sample. The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 515 nm. Control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

Scavenging effect (%) = Control absorbance-Sample absorbance/Control absorbance X 100

Determination of total phenolic content: Concentration of phenolics in plant extracts was determined using spectrophotometric method. Ten milligram methanolic extract was dissolved in 200 µL distilled water and used for the analysis. The reaction mixture was prepared by mixing 200 µL extract, 1 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 1 mL 7.5% $NaHCO_3$. Blank was concomitantly prepared**,** containing 200 µL distilled water, 1 mL 10% Folin-Ciocalteu's reagent dissolved in water and 1 mL of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at λmax 765 nm. The samples were prepared in duplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Gas Chromatography-Mass Spectrometry (GC-MS): An Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15 m Alltech EC-5 column (250 µ I.D., 0.25 µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. Oven temperature program was programmed to start at 35˚C, hold for 2 min, then ramp at 20˚C per min to 300˚C and hold for 5 min. The helium carrier gas was set to 2 mL/min flow rate (constant flow mode). A JEOL GCmate-II bench top double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-20001 software was used for analysis. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 sec per scan with a 0.2 sec inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 sec per scan.

Mass spectrometry library search: Identification of the components of the purified compound was matched with their recorded spectra with the data bank mass spectra of NIST library V-11 provided by the software. The entire analysis was done in Sophisticated Analytical Instrument Facility (SAIF) available at IIT Madras, Chennai.

Cytotoxicity determination by MTT assay: In this study, MCF-7 human breast cancer cell line purchased from NCCS, Pune was used. The cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin. Cells were incubated at 37°C in a 5% CO_2 humidified incubator with pH 7. Methanol extract of receptacles were evaluated for anticancer activity. MCF-7 breast cancer cells were maintained DMEM supplemented with FBS and Penicillin/Streptomycin-L-glutamine and cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37°C in Thermo Hera Cell 150 incubator. Cells were seeded in 96 well plates at the density of 5000 cells/well in 100 µL of medium. Then various concentrations of the crude extract (Low concentration: 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/mL; High concentration: 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 µg/mL) were added to the cells in 100 µL medium. Cells were incubated for 24 h with test extract concentrations. Each concentration was tested in duplicate.

Microscopic methodology: The cells were observed under inverted microscope at 40X magnification for cell growth. MTT assay was used to determine cell viability. MTT assay measures changes in colour for measuring the activity of enzyme that reduce MTT to formazan, giving a purple colour. Yellow MTT (a tetrazole) reduce to purple formazan in living cells (Mosmann, 1983).

Table 1. Preliminary phytochemical screening of *N. nucifera* methanolic extract.

Phytochemicals	*N. nucifera* (White receptacle)	*N. nucifera* (Pink receptacle)
Saponins	+	+
Alkaloids	-	-
Phlobatannins	+	+
Glycosides	-	-
Flavanoids	-	+
Tannins	+	+
Terpenoids	-	-
Sterols	+	-

+ Present, - Absent

Table 2. Determination of total phenol content of *N. nucifera* receptacle extracts.

Sample	*N. nucifera* (White)	*N. nucifera* (Pink)
mg GAE/g of dry extract	386.4±0.141	146.7±0.212

After 24 h incubation, 10 µL of MTT was added to each well and incubated for additional 4 h. Then 100 µL of DMSO solution was added to each well to solubilize the formazan crystals. The plates were read for optical density at 570 nm using a plate reader. By using optical density, the percentage inhibition of MCF-7 cells was calculated. The percentage viability was calculated as follows:

Cell viability = OD of samples/OD of control × 100.

Statistical analysis: Mean, standard deviation, one-way ANOVA T-test was used to calculate the data obtained in the study.

Results and discussion

Preliminary phytochemical analysis of powdered methanolic extracts of *N. nucifera* receptacles revealed the presence of saponins, phlobotannins, flavonoids, tannins, sterols and phenols (Table 1). The content of phenolics in *N. nucifera* receptacle extracts was expressed in terms of gallic acid equivalent using spectrophotometric methods (Table 2). The phenoilc content was found to be 386.4 and 146.7 mg GAE/g of dry weight of white and pink receptacle extracts of *N. nucifera* respectively. The high content of phenolic compounds indicated that *N. nucifera* can be regarded as promising candidate for natural plant sources of antioxidants with high value.

The methanolic extract of the white and pink receptacles showed equally good antioxidant activity as determined by DPPH radical scavenging assay with standard ascorbic acid. *Nelumba nucifera* white receptacle extract had a better antioxidant activity of 54.14%. Yang and coworkers (2007) showed *N. nucifera* methanolic rhizome extract recording highest DPPH scavenging activity of 66.7%. This reveals that *N. nucifera* is capable of damaging oxidation byproducts and can remain as an effective antioxidant compound.

GC-MS analysis of methanolic receptacle extracts of *N. nucifera* reported the presence of 9-octadecadienoic acid peak (41.10%) (Fig. 2 and Table 3), 2,2,4-Trimethyl-3-[3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl]-cyclohexanol peak (16.82%) as shown in Fig. 3 and Table 4.

Fig. 2. The peak area percentage and peak area coverage of *N. nucifera* (White receptacle).

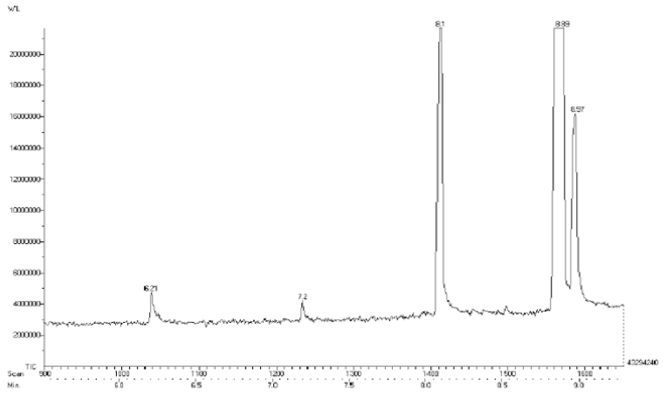


Table 3. The peak area percentage and peak area coverage of *N. nucifera* (White receptacle).

Peak No.	RT (Min.)	Compound name	Peak area	Peak area (%)
1	6.21	Dodecanoic acid, methyl ester	4758320	4.52
2	7.2	Tridecanoic acid, 12-methyl, methyl ester	4206912	3.99
3	8.1	Hexadecanoic acid, methyl ester	28210160	26.78
4	8.89	9-Octadecanoic acid [Z], methyl ester	43294240	41.10
5	8.97	Octadecanoic acid, methyl ester	24875216	23.61
Total			105344848	100.00

Table 4. The peak area percentage and peak area coverage of *N. nucifera* (Pink receptacle).

Peak No.	RT (Min.)	Compound name	Peak area	Peak area (%)
1	15.3	Nor-diazepam, 3-(Nhydroxymethyl) aminocarbonyloxy]	12956336	14.69
2	17.24	Cyclohexane, 1,1'-dodecylidenebis4-methyl	26086048	29.58
3	18.53	1H-Cyclopropal[3,4]benz[1,2-e]azulene-5,7b,9,9atetrol, 3[(acetyloxy)methyl] 1a,1b,4,4a,5,7a,8,9- octahydro-1,1,6,8-tetramethyl, 9,9a-diacetate, [1aR(1aa,1ba,4aa,5a,7aa,7ba,8a,9a,9aa)]	9253200	10.49
4	19.84	3,8,12-Tri-O-acetoxy-7-desoxyingol-7-one	9001072	10.21
5	21.49	1H-Cyclopropal[3,4]benz[1,2-e]azulene 5,7b,9,9atetrol,3[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl, 9,9a-diacetate, [1aR (1aa,1ba,4aa,5a,7aa,7ba,8a,9a,9aa)]	8407152	9.53
6	23.66	Acetic acid, 17-acetoxy-3-hydroxyimino-4,4,13-trimethyl hexadecahydrocyclopenta[a]phenanthren-10yl methyl ester	7655104	8.68
7	26.26	2,2,4-Trimethyl-3-[3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl]-cyclohexanol	14835600	16.82
Total			88194512	100.00

Table 5. Cytotoxicity test results (MTT Assay).

Methanol receptacle extract (µg/mL)	Mean OD value (570 nm)		Viable cells (%)		Death cells (%)	
	White	Pink	White	Pink	White	Pink
Control	0.760±0.002	0.744±0.001	100	100	-	-
200	0.615±0.003	0.629±0.002	80.99	83.40	19.01	16.6
300	0.544±0.002	0.574±0.003	71.58	77.22	28.42	22.78
400	0.406±0.002	0.364±0.004	54.42	48.99	45.58	51.01
500	0.270±0.002	0.407±0.002	35.59	54.70	64.41	45.3
600	0.215±0.003	0.260±0.002	28.36	34.95	71.64	65.05

***P<0.005 for all concentrations (Software: VassarStats).

Fig. 3. The peak area percentage and peak area coverage of *N. nucifera* (Pink receptacle).

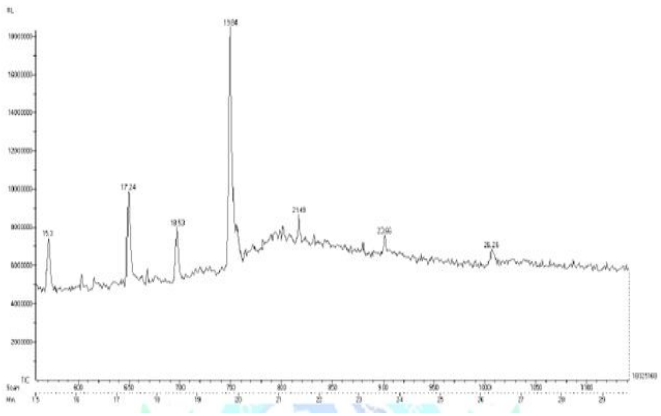
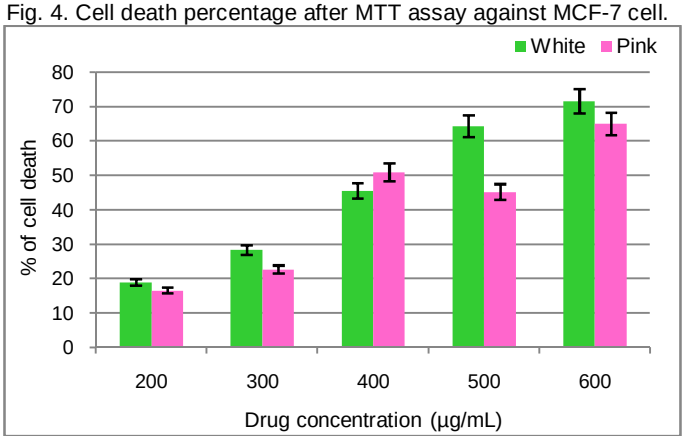


Fig. 4. Cell death percentage after MTT assay against MCF-7 cell.



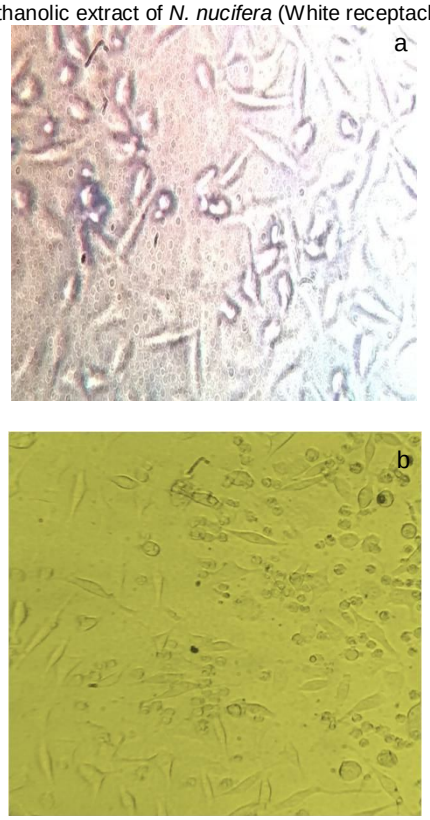
In vitro MTT assay revealed significant cytotoxic effects at the dose level of 600 µg/mL recording 71.64% and 65.05% of cell death as observed in white and pink *Nelumbo nucifera* receptacle extracts respectively (Fig. 4 and Table 5). The receptacle extracts were found to be potent as evident by the concentration at which 50% of cancer cell death occurred at 590 µg/mL (http://ic50.tk/IC50 Tool Kit). Weng *et al.* (2009) identified a compound called Armepavine in *Nelumbo nucifera* which showed immunosuppressive effects *in vitro.*

Methanol and acetone leaf extract of *N. nucifera* showed less anticancer activity against breast cancer (Arjun *et al.,* 2012) whilst *N. nucifera* receptacle extract showed good anticancer activity against human breast cancer MCF-7as shown in Fig. 5 and Fig. 6 in the present study.

Conclusion

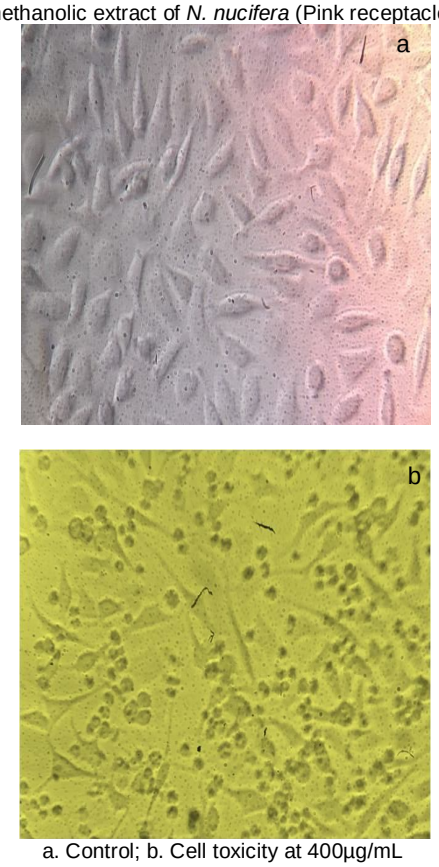
Preliminary phytochemical analysis of methanolic extracts of white and pink *Nelumbo nucifera* receptacles revealed the presence of saponins, phlobotannins, flavonoids, tannins, sterols and phenols but with few differences.

Fig. 5. Photomicrograph (40X) of MCF-7 cell treated with methanolic extract of *N. nucifera* (White receptacle).



a. Control; b. Cell toxicity at 400µg/mL

Fig. 6. Photomicrograph (40X) of MCF-7 cell treated with methanolic extract of *N. nucifera* (Pink receptacle).



a. Control; b. Cell toxicity at 400µg/mL

The methanolic extract also showed good antioxidant activity by DPPH radical scavenging activity assay. *In vitro* anticancer activities of the extracts were found effective against human breast cancer cell line MCF-7. Based on GC-MS analysis, 9-octadecadienoic acid in *Nelumbo nucifera* (White receptacle) and 2,2,4-Trimethyl-3-[3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl]-cyclohexanol in *N. nucifera* (Pink receptacle) were identified as potential compounds that may be responsible for producing anticancer activity in human breast cancer cell line MCF-7. To conclude, pharmacological studies of the active phytoconstituents may be considered and studied elaborately to treat various types of cancer.

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References

1. Arjun, P., Saranya Sivan, P.S., Mohana Priya, S., Krishnamoorthy, M. and Balasubramanian, K. 2012. Phytochemical analysis and anticancer activity of *Nelumbo nucifera* extracts. *J. Acad. Indus. Res*. 1(2): 81-85.
2. Chen, Y., Fan, G., Wu, H., Wu, Y. and Mitchell, A. 2007. Separation, identification and rapid determination of liensine, isoliensinine and neferine from embryo of the seed of *Nelumbo nucifera* Gaertn. by liquid chromatography coupled to diode array detector and tandem mass spectrometry. *J. Pharm. Biomed*. 43(1): 99-104.
3. Deng, J., Cheng, W. and Yang, G. 2011. A novel antioxidant activity index (AAU) for natural products using the DPPH assay. *Food Chem.* 125(4): 1430-1435.
4. Evans, W.C. 2002. Trease and Evans. WB Saunders Harcourt Publishers Ltd., pp.357-375.
5. Hu, M. and Skibsted, L.H. 2002. Antioxidative capacity of rhizome extract and rhizome knot extract of edible lotus (*Nelumbo nuficera*). *Food Chem*. 76(3): 327-333.
6. Hyun, S.K., Jung, Y.J., Chung, H.Y., Jung, H.A. and Choi, J.S. 2006. Isorhamnetin glycosides with free radical and ONOO−scavenging activities from the stamens of *Nelumbo nucifera*. *Arch. Pharmacal. Res*. 29(4): 287-292.
7. Kuo, Y.C., Lin, Y.L., Liu, C.P. and Tsai, W.J. 2005. Herpes simplex virus type 1 propagation in HeLa cells interrupted by *Nelumbo nucifera*. *J. Biomed. Sci*. 12(6): 1021-1034.
8. Liu, R.H. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J.Nutr.* 134(12): 3479S-3485S.
9. Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Meth.* 65(1-2): 55-63.

10. Mukherjee, K., Das, J.B.R.S., Balasubramanian, R., Kakali, S., Pal, M. and Saha, B.P. 1995. Antidiarrhoeal evaluation of *Nelumbo nucifera* rhizome extract. ***Ind. J. Pharmacol.*** 27(4): 262.
11. Ono, Y., Hattori, E., Fukaya, Y., Imai, S. and Ohizumi, Y. 2006. Anti-obesity effect of *Nelumbo nucifera* leaves extract in mice and rats. ***J. Ethnopharmacol***. 106(2): 238-244.
12. Rao, G.M.M., Pushpangadan, P. and Shirwaikar, A. 2005. Hepatoprotective activity of *Nelumbo nucifera* Geartn. Flower an Ethnopharmacological study. *Acta. Pharm. Turc*. 47: 79-88.
13. Saravanakumar, K., Vivek, R., Boopathy, N.S., Yaqian, L., Kathiresan, K. and Chen, J. 2015. Anticancer potential of bioactive 16-methylheptadecanoic acid methyl ester derived from marine *Trichoderma*. ***J. Appl. Biomed.*** 13(3): 199-212.
14. Stankovic, M.S. 2011. Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac. J. Sci*. 33: 63-72.
15. Wang, J., Hu, X., Yin, W. and Cai, H. 1991. Alkaloids of *Plumula Nelumbinis*. *Chinese Mater. Med.* 16(11): 673-675.
16. Weng, T.C., Shen, C.C., Chiu, Y.T., Lin, Y.L., Kuo, C.D. and Huang, Y.T. 2009. Inhibitory effects of armepavine against hepatic fibrosis in rats. *J. Biomed. Sci*. 16(1): 78-91.
17. Wu, S., Sun, C., Cao, X., Zhou, H., Zhang, H. and Pan, Y. 2004. Preparative counter-current chromatography isolation of liensinine and its analogues from embryo of the seed of *Nelumbo nucifera* Gaertn. using upright coil planet centrifuge with four multilayer coils connected in series. ***J. Chromatogr. A.*** 1041(1): 153-162.
18. Yang, D., Wang, M.E.Q., Leqin, B.E. and Jianmei, B.E. 2007. Antioxidant activities of various extracts of lotus (*Nelumbo nuficera* Gaertn) rhizome. ***Asia Pac. J. Clin. Nutr.*** 16: 158.