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GC-MS analysis of phytocomponents in the ethanolic extract of *Nelumbo nucifera* seeds from Russia

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ABSTRACT

The present study was designed to determine the phytocomponents of the ethanol extract of *N. nucifera* seed using GC-MS. The phytochemical screening of the ethanolic extract of *N. nucifera* seed was performed using Perkin-Elmer Gas Chromatography - Mass Spectroscopy. GC-MS chromatogram of the ethanolic extract of *N. nucifera* seed showed thirty-eight peaks which indicating the presence of thirty-eight phytochemical constituents. The major phytochemical constituents are lupeol (22.95%), octadecatrienoic acid-2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy] methyl]ethyl ester (Z,Z,Z) (7.86%), 2,3-dihydroxypropyl--cis-13-docosenoate (5.35%), oleanolic acid(5.19%), tristrimethylsilyl ether derivative of 1,25-dihydroxyvitamin D2 (3.36%) ,trimethylsilyl derivative of 2-monoolein (3.26%), lucenin 2 (3.02%), lupanol (2.95%), tetraneurin-a-diol (2.78%), carotene-1,1',2,2'-tetrahydro-1,1'-dimethoxy (2.32%), 1-oxo-forskolin (1.99%), oleic acid-3-hydroxypropyl ester (1.78%), oleic acid trimethylsilyl ester (1.69%), betulin (1.61%), à-amyrin-trimethylsilyl ether (1.49%), stigmast-5-en-3-ol-(3á,24S)(1.43%), flavone-4'-OH,5-OH,7-di-O-glucoside (1.11%). Among thirty-eight compounds identified, only twelve were reported to have biological activities. Our results indicate that *N. nucifera* seed contain various bioactive components.

INTRODUCTION

Nelumbo nucifera (N. nucifera), a widely used plant in folk medicine, belongs to the family Nymphaeaceae. It is heavily cultivated on the banks of Volga river delta in the south of Russia especially in Astrakhan region. All parts of this plant are eaten such as leaves, seeds, flowers and rhizomes and used as traditional Chinese herbs (Sridhar and Bhat, 2007). *N. nucifera* has many pharmacological activities including antidiabetic, antipyretic, anti-inflammatory, anticancerous, antimicrobial, antiviral and anti-obesity properties (Kashiwada *et al.,* 2005). Several bioactive compounds have been derived from *N. nucifera* parts belonging to different chemical groups, including alkaloids, flavonoids, glycosides, triterpenoid, vitamins etc., which all have their own therapeutic impact (Mukherjee *et al.,* 2009). There are variations in antioxidant activity and concentration of phytocomponents in the ethanol extracts of lotus seeds and based on the growing region and dryness (Zhao *et al.*, 2014).

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Linoleic acid, palmitic acid, and oleic acid are the main fatty acid components in *N. nucifera* seeds and most of them are in ester form, while phytosterols existed mainly in the free form rather than in steryl-fatty acid ester form (Zhao *et al.*, 2013).

The seeds of *N. nucifera* contain 2-3% oil comprised of myristic acid (0.04%), palmitic acid (17.32%), oleic acid(21.91%), linoleic acid (564.17%) and linolinic acid (6.19%) (Gangrade and Iushal, 1966; Dhar and Munjal,1972). Several studies have been reported the medicinal significance of different extracts of *N. nucifera* seeds and phytocomponents isolated from them.

The ethanol extract of *N. nucifera* seed reduces adipose tissue weights, ameliorates blood lipid profile, and modulates serum leptin level in rats fed a high-fat diet (You *et al.*, 2014). Embryo of *N. nucifera* seed has a neuropreventive and memory improving effects (Kim *et al.*, 2014). A petroleum ether extract of seed has been reported to possess anti-fertility activity in female albino mice at the dose of 3 mg/kg (Mazumder *et al.*, 1992). Methanol extract of *N. nucifera* seed contain neferine which has various therapeutic effects such as induction of sedation, hypothermia, antifever effects, and anxiolytic effects comparable with those of diazepam but with a different mechanism (Sugimoto *et al.*, 2008).

Sivasankari et al. evaluated the hypoglycemic activity of inorganic constituents in *N. nucifera* seeds on streptozotocin-induced diabetes in rats (Sivasankari *et al.,* 2010).

Several alkaloids have been isolated from the seed embryo of *N. nucifera* as liensinine and isoliensinine (Guo and Chen, 1984). Isoliensinine isolated from the seeds reduced bleomycin-induced pulmonary fibrosis in mice (Xiao *et al.,* 2005).

Hydroalcoholic extract of *N. nucifera* seed changed total and differential white blood cell counts, improved phagocytosis, and potentiated immune inflammatory reactions (Mukherjee *et al.,* 2010). However, there have been not much reports on the phytochemical screening of the ethanolic extract of *N. nucifera* seeds.

MATERIALS AND METHODS

Collection and processing of plant material

Seeds of *Nelumbo nucifera* were collected from lotus flowers which cultivated on the banks of Volga river in the region of Astrakhan in the south of Russia. Seeds were washed under tap water to remove dust particles, then they were dried and seed coat was removed and discarded. Finally, seeds without coats were pulverized to powder by using of a mechanical grinder.

Preparation of the extract

20 g of the powder of seeds were soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatman filter paper No. 41 along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. The filtrate was then concentrated by rotator evaporator. 2 µl of filtrate was used for GC-MS analysis.

GC-MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column TG 5MS composed of 5% phenyl methyl polysiloxane, operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1 µl was employed (split ratio of 10:1) injector temperature 200°C; ion-source temperature 200 °C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10 °C/min, to 200 °C, then 5 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 650 Da. MS transfer line temperature 280 °C.

Identification of phytocomponents

Identification of compounds was conducted using the database of Wiley9 library combined with the National Institute of Standards and Technology (NIST) library. The name, molecular weight, molecular formula, and area under peak of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

GC-MS chromatogram analysis of the ethanolic extract of *N. nucifera* seed [Figure 1] showed thirty-eight peaks which indicating the presence of thirty-eight phytochemical constituents. On comparison of the mass spectra of the constituents with Wiley9 and NIST library, the thirty-eight phytocompounds were characterized and identified and their retention time (RT), molecular weight (MW), molecular formula and concentration (peak area %) are presented in table 1.

Among the compounds, twelve compounds were reported to have biological activities. The various phytochemicals which contribute to the medicinal activities of *N. nucifera* seed were shown in table 2.

Of thirty-eight compounds identified, the most prevailing components were lupeol (22.95%), octadecatrienoic acid-2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy] methyl]ethyl ester (Z,Z,Z) (7.86%), 2,3-dihydroxypropyl-cis-13-docosenoate (5.35%), oleanolic acid(5.19%), tristrimethylsilyl ether derivative of 1,25-dihydroxyvitamin D2 (3.36%), trimethylsilyl derivative of 2-monoolein (3.26%), lucenin 2 (3.02%) and lupanol (2.95%), tetraneurin-A-diol (2.78%), carotene-1,1',2,2'-tetrahydro-1,1'-dimethoxy (2.32%),1-oxo-forskolin (1.99%), oleic acid-3-hydroxypropyl ester (1.78%), oleic acid trimethylsilyl ester (1.69%), betulin (1.61%), à-amyrin-trimethylsilyl ether (1.49%), stigmast-5-en-3-ol-(3á,24S)(1.43%), flavone-4'-OH,5-OH,7-di-O-glucoside (1.11%).

The chemical constituents identified in the ethanolic extract of *N. nucifera* seed belongs to different chemical groups. The most prevailing component in the extract, lupeol (22.95%), is a triterpene was reported to possess several pharmacological activities including anticancer, antiprotozoal, anti-inflammatory, antimicrobial and chemopreventive properties (Gallo and Sarachine, 2009).

Lucenin 2 is a flavonoid possesses antibacterial activity (Basile *et al.,* 1999). Stigmast-5-en-3-ol is a phytosterol has an antidiabetic property (Sujatha *et al.,* 2010).

CONCLUSION

According to our results, it could be concluded that the seed of *N. nucifera* has various bioactive compounds and further studies will be needed to isolate phytocomponents of *N. nucifera* seed and investigate their biological activities. Therefore, it is recommended as a plant seed of pharmaceutical importance.

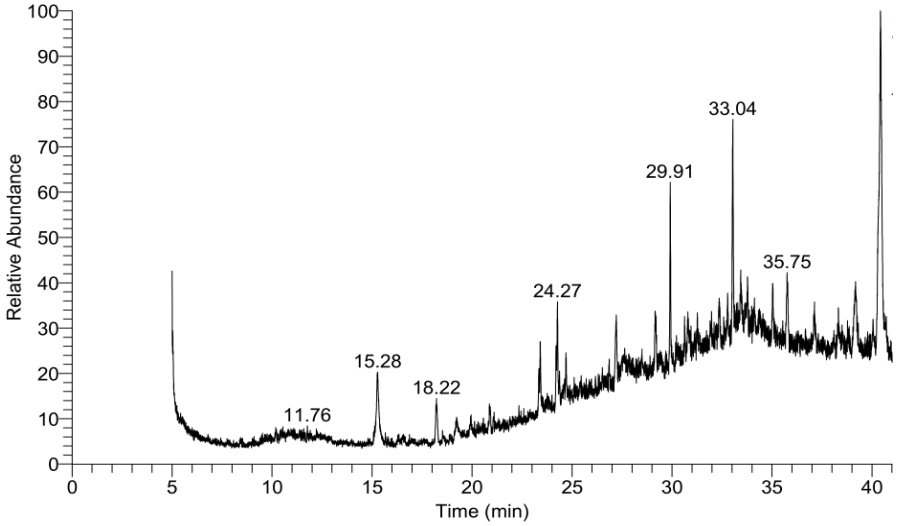

Fig. 1 : GC-MS chromatogram of the ethanolic extract of *N. nucifera* seed.

Table 1: Phytocomponents identified in ethanolic extract of *N. nucifera* seed by GC-MS.

No.	RT (min)	Name of the compound	Molecular formula	MW	Peak area (%)
1	15.28	Tristrimethylsilyl ether derivative of 1,25- dihydroxyvitamin D2	$C_{37}H_{68}O_3Si_3$	644	3.36
2	18.22	Silane,[(3,7,11,15-tetramethyl-2-hexadecenyl)oxy]trimethyl	$C_{23}H_{48}OSi$	368	2.32
3	19.24	Oleic acid trimethylsilyl ester	$C_{21}H_{42}O_2Si$	354	1.69
4	19.94	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-9,9a-diacetate,[1aR (1aà,1bá,4aà,5á,7aà,7bà,8à,9á,9aà)]-	$C_{26}H_{36}O_8$	476	0.74
5	20.91	4-O-Methylphorbol-12,13-didecanoate	$C_{41}H_{66}O_8$	686	1.40
6	23.34	7,10,13-Eicosatrienoic acid methyl ester	$C_{21}H_{36}O_2$	320	1.10
7	23.41	Oleic acid-3-hydroxypropyl ester	$C_{21}H_{40}O_3$	340	1.78
8	24.20	9,12,15-Octadecatrienoic acid-2,3-dihydroxypropyl ester, (Z,Z,Z)	$C_{21}H_{36}O_4$	352	1.46
9	24.27	Tetraneurin-A-diol	$C_{15}H_{20}O_5$	280	2.78
10	24.62	Anodendroside E 2 monoacetate	$C_{32}H_{40}O_{12}$	616	0.88
11	25.41	Spiro[9,9']difluorene-2,2'-dicarboxylic acid-7,7'-dinitro	$C_{27}H_{14}N_2O_8$	494	0.92
12	26.86	9,12,15-Octadecatrienoic acid-2-phenyl-1,3-dioxan-5-yl ester	$C_{28}H_{40}O_4$	440	0.97
13	27.21	Trimethylsilyl derivative of 2-monoolein	$C_{27}H_{56}O_4Si_2$	500	3.26
14	27.63	Isocolchifoline	$C_{22}H_{25}NO_7$	415	0.68
15	28.50	1-Acetoxy-[2-[(p-chlorophenyl)oxy]-5-methylphenyl]-1,2-diphenyl-ethene	$C_{29}H_{23}ClO_3$	454	1.12
16	29.16	Lupanol	$C_{30}H_{52}O$	428	2.95
17	29.91	2,3-Dihydroxypropyl-cis-13-docosenoate	$C_{25}H_{48}O_4$	412	5.35
18	30.23	6-Azacholest-4-en-7-one-6-benzyl-3à-hydroxy	$C_{33}H_{49}NO_2$	491	1.07
19	30.63	Flavone-4'-OH,5-OH,7-di-O-glucoside	$C_{27}H_{30}O_{15}$	594	1.11
20	30.79	1-Oxo-forskolin	$C_{22}H_{32}O_7$	408	1.99
21	30.87	Glycocholic acid methyl ester	$C_{36}H_{69}NO_6Si_3$	695	0.78
22	31.27	1,3,4,7-Tetraphenylthieno[3,4-c]pyridine	$C_{31}H_{21}NS$	439	1.11
23	32.36	Silane,[[(3á,5à,11á,20S)-pregnane-3,11,17,20,21-pentayl] pentakis (oxy)] pentakis [trimethyl-	$C_{36}H_{76}O_5Si_5$	728	1.94
24	32.59	Cholestan-3-one,cyclic 1,2-ethanediylaetal, (5á)	$C_{29}H_{50}O_2$	430	0.79
25	32.78	Betulin	$C_{30}H_{50}O_2$	442	1.61
26	33.04	9,12,15-Octadecatrienoic acid-2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester,(Z,Z,Z)	$C_{27}H_{52}O_4Si_2$	496	7.86
27	33.44	Carotene-1,1',2,2'-tetrahydro-1,1'-dimethoxy	$C_{42}H_{64}O_2$	600	2.32
28	33.78	Stigmast-5-en-3-ol-(3á,24S)	$C_{29}H_{50}O$	414	1.43
29	34.38	17-Pentatriacontene	$C_{35}H_{70}$	490	0.84
30	35.04	Lucenin 2	$C_{27}H_{30}O_{16}$	610	3.02
31	35.76	2,2,4a,6a,8a,9,12b,14a-Octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,12,12a, 12b, 13, 14,14a,14b-eicosahydropicene	$C_{30}H_{50}$	410	4.02
32	37.12	(5á)Pregnane-3,20á-diol,14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-diacetate	$C_{28}H_{43}NO_6$	489	1.89
33	38.09	Phenol-4-[[5-(2-methyl-3-benzofuranyl)-1H-pyrrol-2-yl][5-(2-methyl-3-benzofuranyl)-2H-pyrrol-2-ylidene]methyl]	$C_{33}H_{24}N_2O_3$	496	0.91
34	38.32	Tetradecanoicacid-9a-(acetyloxy)-1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-1H-cyclopropa[3,4]benz[1,2-e]azulen-9-yl ester	$C_{36}H_{56}O_8$	616	1.53
35	38.78	Phytofluene	$C_{40}H_{62}$	542	0.91
36	39.17	Oleanolic acid	$C_{30}H_{48}O_3$	456	5.19
37	40.05	à-Amyrin-trimethylsilyl ether	$C_{33}H_{58}OSi$	498	1.49
38	40.43	Lupeol	$C_{30}H_{50}O$	426	22.95

RT=Retention time, MW=Molecular Weight

Table 2: Bioactivity of phytocomponents identified in the ethanolic extract of *N. nucifera* seed by GC-MS.

No.	RT (min)	Name of the compound	Biological Activity*
1	19.24	Oleic acid trimethylsilyl ester	Cancer-Preventive, Anti-inflammatory, Hypocholesterolemic
2	19.94	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 9,9a-diacetate,[1aR (1aà,1bá,4aà,5á,7aà,7bà,8à,9á,9aà)]-	Antiallergic, Antibacterial, Antihistaminic, Antiinflammatory, Hepatoregenerative , Antiulcer
3	24.27	Tetraneurin-A-diol	Antifeedant ,Pesticide
4	30.63	Flavone-4'-OH,5-OH,7-di-O-glucoside	Analgesic, Antiaflatoxin, Antibacterial, Antileukemic, Antimutagenic, Antiproliferant, Fungicide, Hepatoprotective
5	30.79	1-Oxo-forskolin	Antiasthmatic,Antibronchitic, Anti-inflammatory, Antimetastatic, Antimitotic, Antithrombotic, Prolactinogenic, Mast-Cell-Stabilizer, Cardiotonic, Bronchodilator
6	32.78	Betulin	AntiHIV, Anticarcinomic, Antiflu, Antiinflammatory, Antitumor, Antiviral, Cytotoxic, Hypolipemic
7	33.78	Stigmast-5-en-3-ol-(3á,24S)	Antidiabetic
8	35.04	Lucenin 2	Antibacterial
9	38.09	Phenol-4-[[5-(2-methyl-3-benzofuranyl)-1H-pyrrol-2-yl][5-(2-methyl-3-benzofuranyl)-2H-pyrrol-2-ylidene]methyl]	Antioxidant ,Analgesic, Anesthetic, Antibacterial, Antiviral, Cancer-Preventive, Vasodilator, Fungicide
10	39.17	Oleanolic acid	Abortifacient, AntiHIV, Antibacterial, Antiviral Antiatherosclerotic, Anticarcinomic, Anticariogenic, Antiedemic, Antifertility, Antihepatotoxic, Antioxidant, Antiperoxidant, Anti-inflammatory, Antimalarial, Antitumor, Antihyperlipidemic, Hypotensive, Immunomodulator
11	40.05	à-Amyrin-trimethylsilyl ether	Antitumor
12	40.43	Lupeol	Antiangiogenic, Antiedemic, Antiflu, Antihyperglycemic, Antioxidant, Anti-inflammatory, Antimalarial, Antioxidant, Antitumor, Antiviral, Cytotoxic, Pesticide

*Source: Dr. Duke's: Phytochemical and Ethnobotanical databases.

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