Investigation of composition extracts, biological activities and optimization of *Solanum nigrum* L. extraction growing in Iran

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Abstract: Raw materials including medicinal plants are gaining popularity for the production of reliable and safe medicines suitable for human. Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques used for solving the optimization problems. Using the RSM method, compounds, extracts, antioxidant and antibacterial agents of methanol, hexane, chloroform and water extracts of black nightshade (*Solanum nigrum*) found in Iran, had been studied for this research. GC and GC/MS had been used to determine the composition of the extracts from the aerial parts of *S. nigrum*. The major components of these varieties are alkane and alkane acid derivatives. The research has shown significant results that the maximum antioxidant activity was achieved in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, and significant differences were also observed between the extracts and solvents in this respect ($P<0.05$, $R^2_{adj}=99.97\%$). As regards the antibacterial activity, the chloroform extract has the largest zone of growth inhibition diameter in which the gram-positive bacteria were cultured.

Keywords: Response surface methodology, *Solanum nigrum*, biological activity, optimization.

INTRODUCTION

The genus *Solanum* is widespread in Europe, Asia and in few regions of Africa. In Iran, 13 species are present, among which 8 are the endemic ones (Podlech, 1996). The role played by free radicals in the development of various diseases has been well proved (Kumaran, 2006). Numerous biochemical reactions in the body produce active oxygen that can destroy biomolecules. Antioxidants can block this harmful effect of free radicals because they trap free radicals and detoxify them. Foods rich in antioxidants are known to play an important role in preventing cardiovascular diseases such as cancer (Kris-Etherton, Hecker *et al*., 2002) and degenerative diseases (Parkinson's disease and Alzheimer disease) (Matteo and Esposito, 2003). Since plants are being the main source of natural antioxidants, more research has been carried out on them (Kumaran, 2006). Plants, rich in antioxidants, can protect cells against oxidative stress (Ielpo, Basile *et al*., 2000). Black nightshade is a plant of the Solanaceae family which is extensively used in traditional medicine to treat various wounds and tumors (Son, Kim *et al*., 2003). It is apparent that substances such as alkaloids, steroid alkaloids, steroid saponins and glycoproteins cause these antitumor characteristics (Hsieh, Fang *et al*., 2008). In Indian traditional medicine, this plant is used to protect the liver (Fallah Huseini, Alavian *et al*., 2005). Extensive study has been conducted on the protective effects of black nightshade extract and the results of this study showed that it prevents liver damage (Hsieh, Fang *et al*., 2008). Moreover, it has been found that this extract is used in the treatment of psoriasis, inflammatory states, diarrhea and heart and eye diseases (Jainu and Devi, 2006) and black nightshade also contains tannins and polyphenolic compounds (Jainu and Devi, 2006). The methanol extract of the fruit of this plant has exhibited protective effects against ulcer resulting from the intake of aspirins (these effects have been considered as one of its antioxidant characteristics) (Jainu and Devi, 2004). The effect of this plant on the central nervous system (causing excitement and delirium) has been reported (Perez G, Perez L *et al*., 1998), so it has also its protective effect against gentamicin induced renal toxicity. Therefore, black nightshade can be of much interest as an antioxidant source (Son, Kim *et al*., 2003). In fact, it has been reported that the ethanol extract of its fruit exhibits antioxidant activities by trapping DPPH and hydroxyl radicals (Son, Kim *et al*., 2003). The method of trapping DPPH radicals is based on hydrogen donor ability, and is used to evaluate free radical activity. One of the advantages of this method is that it does not depend on the polarity of the sample. One of the main causes of food spoilage is destruction of fatty acids. Therefore, inhibition of fatty acid oxidation is an important process in food industry and antioxidants are mainly used for this purpose in industry. By using iron reduction method, the reductive power of materials in the sample can be assessed by the reduction of ferric iron to ferrous iron, which can result into an ability of these materials to donate electrons (Esmaeili and Khakpoor, 2012).

Methanol, n-hexane, chloroform and water extracts of black nightshade were used to determine the total content of phenolic compounds, to study the activity of trapping free radicals using the DPPH method, to investigate reductive power, to determine antioxidant activity using the FTC method and to study hydrogen peroxide scavenging.

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MATERIALS AND METHODS

Chemicals

Ferrozine, Gallic acid, trichloroacetic acid (TCA), DPPH and potassium fire cyanide were purchased from the Sigma Chemicals Co. (USA). Folin-Ciocalteu, sodium bicarbonate, ethanol, chloroform, n-hexane, methanol, other chemicals and regents were purchased from the Merck (Darmstat, Germany). All other unlabeled chemicals and reagents used were of analytical grade.

Plant material

The aerial parts of *S. nigrum* were collected from East of Tehran, near the Amol city in Mazandaran province, Iran. Voucher 90-205 deposited in the Herbarium of the Department of Botany Shahid Beheshti University, Eveen, Tehran. Aerial parts were dried at room temperature and coarsely ground before extraction. A known amount of each part was extracted at room temperature by percolation method using methanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal.

Analysis

GC analysis was performed on a Shimadzu 15A gas chromatograph equipped with a split/split less injector (250°C) and a flame ionization detector (250°C). N^sub 2^ was used as a carrier gas (1mL/min) and the capillary column used was DB-5 (50m x 0.2mm, film thickness 0.32µm). The column temperature was kept at 602°C for 3 min, heated to 220°C at a rate of 5°C/min and then kept constant at 220°C for 5min. Relative percentage amounts were calculated from the peak area using a Shimadzu C-R4A chromatopac without using correction factors.

For the GC-MS spectroscopy, a Hewlett-Packard 5973 equipped with HP 5MS column including 30m X 0.25 mm was used. The temperature of the oven was set at 60° C for 3min. The constituents of the oil were identified by comparing their mass spectra and retention indices with those given in the literature and the authentic samples (Aligiannis, Kalpoutzakis *et al*., 2001).

Qualitative and quantitative analyses

All the compounds of each extract were identified by GC and GC-MS and literature reference (Adams, 2012) compared with find retention indices in compounds. The *n*-alkane (C_8-C_{28}) was taken as standard for identification in RI compounds. The literature or stored in the Wiley 275, the NIST 02 and our own libraries, a comparison of MS on both columns with those compounds were reported.

Antioxidant activity

Determination of total phenolic compound

Total phenol content was determined by the Folin Ciocalteu regent (Ghasemi, Ghasemi *et al*., 2009). A dilute solution of *S. nigrum* extract (0.5mL of 1:10g/mL) or Gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (5mL, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4mL 1M). The mixture was allowed to stand for 15 min and the phenols were determined by the colorimetric method at 765nm. The standard curve was prepared by 50, 100, 200, 400, and 800mg/mL solutions of Gallic acid in methanol, n-hexane, chloroform and water (50:50 v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

DPPH radical-scavenging activity

The stable DPPH was used to determine free radical-scavenging activity of the extract (Yildirim, Mavi *et al*., 2001). Different concentrations of each extract were added, at an equal volume, to methanolic, n-hexane, chloroform and solutions of DPPH (100µM). After 15 min at room temperature, the absorbance was recorded at 517nm. The experiment was repeated for three times. Vitamin C was used as a standard control.

Reducing power determination

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action (Yu, 2001). The reducing power of *S. nigrum* was determined according to our recently published paper. Different amounts of each extract (25-800µg/mL) in water were mixed with phosphate buffer (2.5mL, 0.2, pH 6.6) and potassium ferricyanide [K_3 $Fe(CN)_6$] (2.5mL, 1%). The mixture was incubated at 50^0C for 20min. A portion (2.5mL) of tricholoacetic acid (10%) was added to the mixture to cease the reaction, which was then centrifuged at 3000 rpm for 10min. The upper layer of solution (2.5mL) was mixed with distilled water (2.5mL) and $FeCl_3$ (0.5mL, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as a positive control.

Determination of antioxidant activity by the FTC method

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specially, linoleic acid and arachidonic acid are the targets of lipid per oxidation (Esmaeili and Amiri, 2011). The inhibitory capacity of *S. nigrum* extracts was tested against oxidation of linoleic acid by FTC method. This method was adopted from (Osawa and Namiki, 1985). Twenty mg/mL of samples dissolved in 4mL of 95% (w/v) ethanol were mixed with linoleic acid 2.5% (v/v) in 99.5% (w/v) ethanol (4.1mL), 0.05 M phosphate buffer pH 7.0 (8mL), and distilled water (3.9mL) and kept in screw cap containers at 40^0C in dark. To 0.1mL of this solution, 9.7mL of 75% (v/v) ethanol and 0.1mL of 30% (w/v)

ammonium thiocyanate were added. Precisely 3min after the addition of 0.1mL of 20mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance at 500nm of the resulting red solution was measured, and then it was measured again after every 24 h till the absorbance of the control reached the maximum value. The percent inhibition of linoleic acid per oxidation was calculated as:

$$(\%)\text{Inhibition} = 100 - \frac{\text{Absorbance increase of the sample}}{\text{Absorbance increase of the control}} \times 100$$

All tests were carried out in duplicate, and analyses of all samples were performed in triplicate and averaged. Vitamin C used as a positive control.

Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to our recently published paper (Esmaeili, Mousavi *et al*., 2012). A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Extract (0.1-1mg/mL) in distilled water was added to a hydrogen peroxide solution (0.6mL, 40mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds, was calculated as follows:

$$(\%\ \text{Scavenged}\ [H_2O_2] = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard (27).

Antimicrobial assay the diffusion method in agar culture medium

In this method, the Mueller-Hinton agar culture (MHA) was used for bacteria. One-hundredth of a milliliter of the studied microorganism suspension was cultured on an MHA medium. In each of the three Erlenmeyer flasks, 7.5 g of nutrient agar for microbiology was added to 375mL of water and the flasks were put into an autoclave of 15 Pa atmospheric pressure for 10min. After completely disinfecting the working table with alcohol, the sterilized plates were filled with the agar in the Erlenmeyer flasks. After cooling the agar, the wells cut in agar were inoculated with bacteria in a sterile environment, 5 and 100 µL samples of various concentrations of the chloroform, methanol, n-hexane, and aqueous extracts were added to the surfaces of the plates inoculated with the bacteria, and the plates were then placed in an incubator at 37°C for 24h. The zones of growth inhibition diameters were then measured and reported in mm, the plates were examined with respect to bacterial growth and the results were determined considering the initial number of bacteria for each dilution series.

RESULTS

Identification of compounds analysis of extracts of S. nigrum

The chemical components of three extracts of *S. nigrum* are shown in table 1. Methanol extract had 6 compounds representing 90.15% and major compounds were logifolene (24.36%), hexadecanoic acid (25.15%) and octadecanoic acid (24.54%). In the hexane extract, 10 compounds were identified representing 81.61%. The main compounds were heptacosane (25.16%), tetracosane (26.07%) and eicosane (23.73%) and they were found in a large amount. In the chloroform extract, eicosane (30.28%) and nonadecane (17.05%) were found to be the main components among the 8 constituents isolated, representing 80.32% of the total components detected. fig. 4 show GC-MS chromatogram of three solvent.

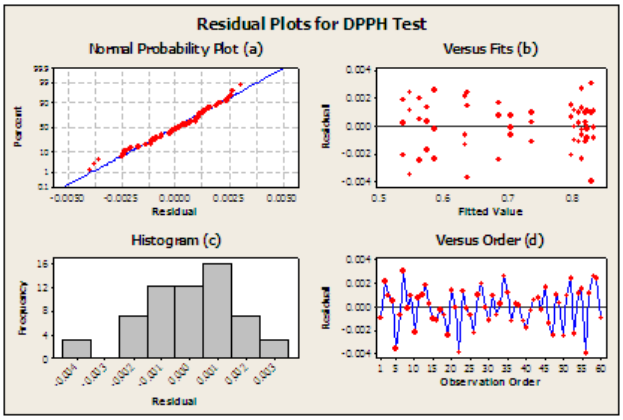

Fig. 1(A): Residual plots for extraction of S. nignam by DPPH. (a) residuals possibility diagram. (b) chronological diagram of the residuals. (c) histogram (d) diagram of the residuals based on the predicted of extraction percent efficiency.

DPPH Radical-Scavenging Activity

In this study, a two-factor full factorial design was used (one qualitative and one quantitative factor). In the DPPH test, light absorption by the factors of extract and solvent type and by the interaction between them, it could be explained that all four extracts had significant effects at α=5%. The power of the test was also high (R^2_{adj}=99.97%). Light absorption increased with a gentle slope when the quantity of the added extract was raised. The maximum absorption belonged to the 800mg/L extract, the chloroform and n-hexane solvents exhibited the maximum absorption and the water solvent the minimum (which was much lower than the other solvents). In the interaction diagram also, the n-hexane and chloroform solvents showed less sensitivity to the quantity of the extract compared to the other solvents (table 2a). Model adequacy was also examined. It had a normal range and the diagram of fitted values against residuals did not exhibit any special trend suggesting model inadequacy (fig. 1A).

Table 1: Chemical composition (%) of the aerial parts extract of methanol, hexane and chloroform of *S. nigrum*

Compound	RI[a]	Methanol	n-Hexane	Chloroform
Neodecanoic acid	1436	-	-	5.47
Spathulenol	1576	-	-	7.64
Acorone	1806	-	-	7.46
Hexadecane	1885	-	0.36	-
Octadecane	2331	-	0.27	-
Vetivone	2350	-	-	6.9
Eicosane	2740	-	23.73	30.28
Heneicosane	2931	-	1.85	3.17
Docosane	3115	-	0.74	-
Nonadecane	3001	-	2.75	17.05
Logifolene	1362	24.36		-
Hexadecanoic acid	2562	25.15	0.18	2.36
Octadecanoic acid	2755	24.54	-	-
Sclareol	3149	5.74	-	-
Manool	3175	1.78	-	-
Incensole oxide	3273	8.58	-	-
Heptacosane	3433	-	25.16	-
Tetracosane	3462	-	26.07	-
Pentacosane	3627	-	0.85	-
Total		90.15	81.61	80.32

[a]RI: relative retention indices as determined on a DB-5 column using the homologous series of n-alkanes

Table 2a: Analysis of variance for DPPH test

Source	Extract	Solvent	Extract (solvent)	Error	Total
df	4	3	12	40	59
Seq SS	0.017489	0.688249	0.011490	0.000159	0.717387
Adj SS	0.017489	0.688249	0.011490	0.000159	-
Adj MS	0.004372	0.229416	0.000958	0.000004	
F	1100.55	57746.27	241.02		
P	0.000	0.000	0.000		
S=0.00199320; R-Sq=99.98% R-Sq (adj)=99.97%					

Table 2b: Analysis of variance for scavenging of hydrogen peroxide test

Source	Extract	Solvent	Extract (solvent)	Error	Total
df	4	3	12	40	59
Seq SS	0.0114366	0.0166713	0.0001993	0.0004052	0.0287124
Adj SS	0.0114366	0.0166713	0.0001993	0.0004052	-
Adj MS	0.0028592	0.0055571	0.0000166	0.0000101	-
F	282.24	548.56	1.64	-	-
P	0.000	0.000	0.119		
S=0.00318282; R-Sq=98.59% R-Sq (adj)=97.92%					

Table 2c: Analysis of variance for folin test

Source	Extract	Solvent	Extract (solvent)	Error	Total
df	4	3	12	40	59
Seq SS	0.0180287	0.0690295	0.0311675	0.0001080	0.1183337
Adj SS	0.0180287	0.0690295	0.0311675	0.0001080	-
Adj MS	0.0045072	0.0230098	0.0025973	0.0000027	
F	1669.43	8522.68	962.02		
P	0.000	0.000	0.000		
S=0.00164312; R-Sq=99.91% R-Sq (adj)=99.87%					

Table 2d: Analysis of variance for FTC test

Source	Extract	Solvent	Extract (solvent)	Error	Total
df	4	3	12	40	59
Seq SS	0.094745	0.186196	0.015488	0.000781	0.297210
Adj SS	0.094745	0.186196	0.015488	0.000781	-
Adj MS	0.023686	0.062065	0.001291	0.000020	
F	1212.34	3176.73	66.06		
P	0.000	0.000	0.000		
S=0.00442012; R-Sq=99.74% R-Sq(adj)=99.61%					

Table 2e: Analysis of variance for reducing power determination test

Source	Extract	Solvent	Extract (solvent)	Error	Total
df	4	3	12	40	59
Seq SS	0.061162	0.181155	0.011875	0.000705	0.254897
Adj SS	0.061162	0.181155	0.011875	0.000705	-
Adj MS	0.015290	0.060385	0.000990	0.000018	
F	867.73	3426.85	56.16		
P	0.000	0.000	0.000		
S=0.00419776; R-Sq=99.72% R-Sq(adj)=99.59%					

Table 3: Antibacterial activity of *S. nigrum* extra methanol, n-hexan and chloroform

Bacterial species	Gram +/-	Methanol	n-Hexane	Chloroform
Staphylococcus aureus (ATCC 25923)	+	-	18	23
Staphylococcus epidermidis (ATCC 12228)	+	8	12	15
Staphylococcus saprophyticus (ATCC 12229)	+	-	10	15
Escherichia coli (ATCC 25922)	-	3	2	5
Pseudomonas aeruginosa (ATCC 27358)	-	10	-	3
Shigellaflexner(ATCC 25923)	-	1	10	16

[a]Values are the mean diameter of inhibitory zones (cm); non-growth zone diameter (NGZD).

Scavenging of hydrogen peroxide

In the H_2O_2 test, the two factors and the solvents had significant effects on absorption, but no significant interaction was observed between the factors (α=5%). Moreover, R^2_{adj} was 99.92%, indicating that 97.92% of changes in absorption could be explained by the two factors of extract and solvent types (table 2b). The main effects in the diagram also showed that absorption was very sensitive to extract and increased with a steep slope when the dose of the extract was raised. The solvent n-hexane had the least absorption in the experimental design. No particular differences were found between methanol and chloroform in absorption, and absorption by water was at an average level. The interaction diagram indicated that absorption by all solvents increased with the same slope using more extract and behaved similarly, but methanol and chloroform were more similar to each other in their behavior. The normality test of residuals suggested the data was normal and the residual versus fits plot did not show any differences in variance (fig. 1B).

Determination of total phenolic compound

In Folin's test, absorption of the water solvent considerably increased as compared to the other three solvents (which had almost similar effects on absorption), and the 400 and 800mg/L doses of the extract had greater effects on absorption. The power of the test (R^2_{adj}) was 99.87% (table 2c). The effects of both factors and those of their interaction were significant at α=5% type I error. Examination of model adequacy showed that the diagram of residuals against fitted values was centered on the two sides of the diagram (warning), but the residuals had a normal trend and the Anderson-Darling coefficient was 0.20 (fig. 2A).

Determination of antioxidant activity by the FTC method

The FTC test is shown in the diagrams of model adequacy. In the diagram of residuals versus fitted values, the residuals exhibited a relatively identical scatter and the error variance was constant. In the next diagram (residuals versus order), which shows residuals against time of execution, the residuals did not exhibit any specific trend and, therefore, the postulate of model adequacy was accepted. The section on data analysis showed that the quantity of the extract and the type of the solvent, and the interaction of these two were significant (α=5%). Moreover, the value of the R^2_{adj} index was 99.61%, which showed high power of the model (table 2d). This meant that 99.6% of absorption could be

explained by the quantity of the extract, by the solvent type and by the interaction between them.

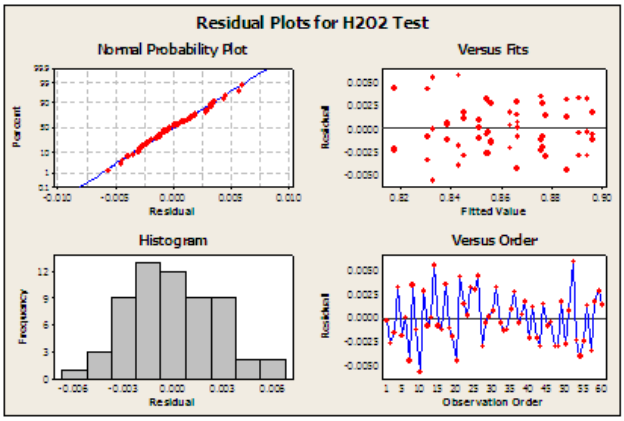

Fig. 1(B): Residual plots for extraction of S. nigrum by scavenging of hydrogen peroxide. (a) residuals normal possibility diagram. (b) chronological diagram of the residuals. (c) Histogram (d) diagram of the residuals based on the predicted amounts of extraction percent efficiency

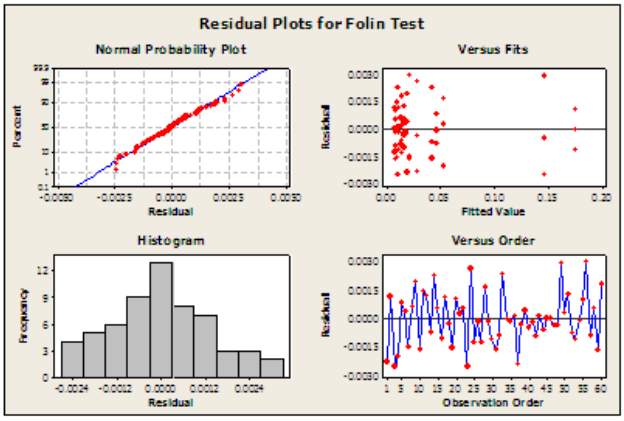

Fig. 2(A): Residual plots for extraction of *S. nigrum* by folin. (a) Residuals normal possibility diagram. (b) chronological diagram of the residuals. (c) Histogram (d) diagram of the residuals based on the predicted amount of extraction percent efficiency

The diagrams showing the interaction between extract and the type of the solvent indicated that the methanol solvent has the maximum absorption and the 50mg/L extract, and that water had lesser sensitivity to the quantity of the extract as compared to the other solvents. Water had the least absorption at all quantities of the extract, while chloroform was the best solvent, after methanol, with respect to absorption. However, examination of the residuals in relation to the quantities of the extract showed that the quantity of the 100mg/L extract had a lower scatter as compared to the other quantities of the extract (there was less error) and that the other quantities of the extract had variances (while variance in this diagram must be constant)(fig. 2B).

Reducing power determination

In the iron test, methanol had the maximum absorption at the 800mg/L dose of the extract and minimum absorption at 50mg/L, but at 200, 400 and 800mg/L doses, absorption increased with a gentler slope. Absorption by chloroform at 50 and 100mg/L doses of the extract was the same but jumped at 200mg/L and increased with a gentle slope at higher doses. n-hexane and water had the least absorption and these two solvents exhibited relatively similar behavior of absorption. In general, absorption was the highest at the 800mg/L dose among the various doses of the extract (the interaction plot for Fe^{2+} test). Moreover, absorption increased with a relatively steep slope when more extract was used (the diagram of main effects)

The ANOVA table showed that absorption at the level of R^2_{adj}=99.59% was dependent on factors, such as the quantity of the extract and the solvent type (table 2e). Moreover, the interaction between these two factors was significant (α=5%) and the diagram of residuals was normal with the AD index value of 0.289 (which indicated the hypothesis of normality of the data was not rejected). Furthermore, the diagram of residuals versus fitted values exhibited a constant variance, which indicated experimental data adequacy. Additionally, the diagram of residuals against time did not show any trend suggesting the presence of errors in the experiment (fig. 3).

Antimicrobial assay

In the section on antibacterial characteristics, the chloroform extract had the largest diameter of the zone of growth inhibition against the gram-positive bacteria aurous, and the corresponding diameters of other extracts against various bacteria are listed in the table below (table 3).

DISCUSSION

Three extracts isolated of *S. nigrum* are listed in table 1, in which the percentage and retention indices of components are given. Six constituents, representing 90.15% of the total components in the metanol extract of *S. nigrum*, were characterized by logifolene (24.36%), hexadecanoic acid (25.15%) and octadecanoic acid (24.54%). Ten compounds identified in the hexane extract representing 81.61% hadlogifolene (24.36%), hexadecanoic acid (25.15%), and octadecanoic acid (24.54%) as the main compounds. So, in the chloroform extract, eicosane (30.28%) and nonadecane (17.05%) were found to be the main components, representing 80.32% of the eight components in the extract. Alkane and acid derivatives were the most abundant components found in the methane, hexane and chloroform extracts (58.23%, 88.1% and 58.32, respectively). The amount of extracts of *S. nigrum* by Soxhlet extractor, based on dry weight was 0.1% (w/w) of the Solanaceae family in Iran

on the basis of essential oil content classified as being poor (<0.0002%). Essential oil was taken for five times. In each section, this plant gave very poor amount of volatile oil being less than 0.0002 and GC-MS spectra could not be identified for this work. Previous studies have shown that *Solanum* are poor in oil (Obeng-Ofori and Freeman, 2004). The chemical composition of methanol, hexane and chloroform extracts from *S. nigrum* is presented in table 1; and components are listed in order of their retention indices as determined on a DB-5 column. A comparison of components in *S. nigrum* with other species in other countries demonstrated monoterpene (0.7% hydrocarbon and 1.2% oxygenated); fatty acids were also present in an appreciable quantity (14.1%). Cycloalkanes were the major components of oil. Extracts of *S. nigrum* have shown anti-tumor and neuro-pharmacological properties as well as antioxidant and cancer chemo-protective matter(JI, WANG *et al*., 2004). Other research reported that *S. nigrum* have abundance of alkane compounds (UD-DIN, DIN KHAN *et al*., 2009). In the current study, alkane compounds were found as major constituents (fig. 4).

The antioxidant and antibacterial activities of plant species have countless applications in traditional medicine: In curing infectious diseases, protecting food, and producing soap, cosmetics, and insecticides and as antiparasitic drugs. In this research, the antioxidant and antibacterial powers of five samples of black nightshade were measured. Plant polyphenolic compounds play an important role in antioxidant activities, which probably results from the oxidative-reductive characteristics of these compounds. In this section, the antioxidant characteristics of the plant extract were examined employing the five methods of trapping the DPPH radical, determining the total content of phenolic compounds, examining the reductive power, studying the hydrogen peroxide scavenging activity, and determining the antioxidant activity using the FTC method. Absorption at each of the 50, 100, 200, 400 and 800mg/L concentrations of the extract in the n-hexane, methanol, chloroform, and water solvents was determined and the data were then optimized using the RSM method and Minitab 16. The optimized results proved our postulates and the explanation suggested the high antioxidant activity of the extract. In the antibacterial section, black nightshade was studied by culturing three gram-positive and three gram-negative bacteria in MHA medium containing the plant extract and experimenting with sterilized disks and wells, and by measuring the diameters of zones of growth inhibition (that were reported in mm). The largest zone of growth inhibition diameter (23mm) was that of the gram-positive aureus bacteria and the chloroform extract. DPPH test has the highest test power with the R^2_{adj} value of 99.97%. Many studies and artificial methods have been introduced for measuring antioxidant capacity. These methods are influenced by various conditions such as the antioxidant solubility ratio between the aqueous and organic phases, temperature, light intensity, antioxidative conditions, the end-point reaction (in special methods) (Yildirim, Mavi *et al*., 2001), and the extent of oxidation, and yield of different results. Therefore, a single method should not be used to measure antioxidant capacity. Esmaeili *et al*. (Esmaeili and Rohani, 2012) studied the desirable antimicrobial activity of *Tanacetumpinnatum*, the main component of which is camphor, and used three methods to examine the antioxidant activity of this research. The difference between their research and this study is that, besides using Minitab 16 and the RSM method, five methods were employed in this research (which, in itself, is a reason for the high antioxidant activity of black nightshade).

It has been shown by studies that increased levels of flavonoids in diets can reduce the severity of some diseases (Hertog, Feskens *et al*., 1993). The total phenolic compounds and flavonoids could explain the antioxidant activity in black nightshade extracts; moreover, optimization of the data carried out in this study led to the conclusion that the extract had its high antioxidant power.

Intermediate elements such as iron can form free radicals with peroxides based on Fenton reactions, leading to the probable development of cardiovascular diseases in people. Similar results were obtained in this research in the section on determining antioxidant quantities using the reductive activity. The difference between the methods used in this research and other methods related to antioxidants was the optimization of the data using Minitab 16 and the RSM method in which the R^2_{adj} values of test power were significant in all of these tests.

In the section on data analysis using the mentioned software, it was found that the quantity of the extract and the type of the solvent, and the interaction between these two were significant at α=5%, which is a proof of the high antioxidant activity of the studied plant. In the section of diagrams, the diagram of main effects showed that absorption increased with a relatively steep slope. 800mg/L extract had the maximum absorption among the various doses as shown in the diagram of interaction. The diagram of fitted values against residuals did not reveal any special trend suggesting any inadequacy of the model. Moreover, the diagram of residuals against fitted values did not demonstrate any special trend suggestive of model inadequacy either. Furthermore, the diagram of residuals against fitted values showed a constant variance, which indicates the adequacy of experimental data. Finally, optimization of the data using the mentioned software, besides confirming our statements, proved the high antioxidant power of this plant. Results showed that the RSM method could be well fitted into the experimental data obtained from the extract and the optimal point suitably conformed to laboratory data.

CONCLUSION

The aim of this research was to identify compounds of three extracts and investigate antioxidant and antibacterial activity of methanol, hexane, chloroform and water extracts. *S. nigrum* found in Iran were studied using the RSM method. Alkane and alkane acid derivatives were the major components of the various extracts. Antioxidant activity showed high data efficiency and significant differences were found between the extracts of the solvents in this respect ($P<0.05$, $R^2_{adj}=99.97\%$). As for antibacterial activity, gram-positive bacteria for chloroform extract were the largest zone of growth inhibition diameter.

REFERENCES

Adams RP (2012). Identification of essential oils by ion trap mass spectroscopy. Academic Press.

Aligiannis N *et al* (2001). Composition and antimicrobial activity of the essential oils of two Origanum species. *Journal of Agricultural and Food Chemistry,* **49**(9): 4168-4170.

Esmaeili A and H Amiri (2011). The *in vitro* antioxidant and antibacterial activities of *Tanacetum pinnatum* boiss. grown in Iran. *Bulgarian Chem. Com,* **43**: 267-271.

Esmaeili A and M Khakpoor (2012). Biological activities and chemical composition of solvent extracts of *Stoechospermum marginatum* (C. Agardh). *Acta Biochim. Pol.*, **59**: 581-585.

Esmaeili A *et al*. (2013). Antioxidant activity and isolation of luteoline from *Centaurea behen* L. Grown in Iran. *Journal of Chemistry*, **1**: 1-6.

Esmaeili A and S Rohani (2012). The *in vitro* antioxidative properties and essential oil composition of *Melissa officinal* is L. *Journal of Essential Oil Bearing Plants,* **15**(6): 868-875.

Fallah Huseini H *et al*. (2005). The efficacy of Liv-52 on liver cirrhotic patients: A randomized, double-blind, placebo-controlled first approach. *Phytomedicine,* **12**(9): 619-624.

Ghasemi K, Y Ghasemi and MA Ebrahimzadeh (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak. J. Pharm. Sci.* **22**(3): 277-281.

Hertog, MG *et al*. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. *The Lancet* **342**(8878): 1007-1011.

Hsieh CC, HL Fang and WC Lina (2008). Inhibitory effect of *Solanum nigrum* on thioacetamide-induced liver fibrosis in mice. *Journal of Ethnopharmacology,* **119**(1): 117-121.

Ielpo M *et al* (2000). Immunopharmacological properties of flavonoids. *Fitoterapia.*, **71**: S101-S109.

Jainu M and CS Devi (2004). Antioxidant effect of methanolic extract of *Solanum nigrum* berries on aspirin induced gastric mucosal injury. *Indian Journal of Clinical Biochemistry,* **19**(1): 57-61.

Jainu M and CSS Devi (2006). Antiulcerogenic and ulcer healing effects of *Solanum nigrum* L. on experimental ulcer models: Possible mechanism for the inhibition of acid formation. *Journal of Ethnopharmacology,* **104**(1): 156-163.

JI YB *et al* (2004). Study on active composition of *Solanum nigrum* L. (SNL). *Journal of Harbin University of Commerce (Sciences Edition)* p.6.

Kris-Etherton PM *et al* (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine,* **113**(9): 71-88.

Kumaran A (2006). Antioxidant and free radical scavenging activity of an aqueous extract of*Coleus aromaticus*. *Food Chemistry,* **97**(1): 109-114.

Matteo V and E Esposito (2003). Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. *Current Drug Targets-CNS & Neurological Disorders,* **2**(2): 95-107.

Obeng-Ofori D and F Freeman (2004). Efficacy of products derived from *Ricinus communis* (L.) and *Solanum nigrum* (L.) against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) in stored maize. *Ghana Journal of Agricultural Science*, **34**(1): 39-47.

Osawa T and M Namiki (1985). Natural antioxidants isolated from eucalyptus leaf waxes. *Journal of Agricultural and Food Chemistry,* **33**(5): 777-780.

Perez GR *et al* (1998). Neuropharmacological activity of *Solanum nigrum* fruit. *Journal of Ethnopharmacology,* **62**(1): 43-48.

Podlech D (1996). Karl Heinz Rechinger und die Flora Iranica. *Annalen des Naturhistorischen Museums in Wien. Serie. B. für. Botanik. und Zoologie.*, **1**: 57-65.

Son YO *et al* (2003). Ripe fruits of *Solanum nigrum* L. inhibits cell growth and induces apoptosis in MCF-7 cells. *Food and Chemical Toxicology,* **41**(10): 1421-1428.

UD-DIN AM *et al* (2009). Chemotaxonomic significance of flavonoids in the Solanum nigrum complex. *Journal of the Chilean Chemical Society,* **54**(4): 486-490.

Yildirim A, A Mavi and AA Kara (2001). Determination of antioxidant and antimicrobial activities of rumex crispus L. extracts. *Journal of Agricultural and Food Chemistry,* **49**(8): 4083-4089.

Yu L (2001). Free radical scavenging properties of conjugated linoleic acids. *Journal of Agricultural and Food Chemistry,* **49**(7): 3452-3456.