The GC-MS analyses of the n-hexane extract of *Nitraria schoberi* L., its total phenolics and in vitro antioxidant activity

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The present study was carried out for analysis of n-hexane extract of *Nitraria schoberi* fruits and also for evaluation of the total phenolics and antioxidant activity of the fruit extracts. The n-hexane extract was obtained by Soxhlet apparatus and analysed by gas chromatography-mass spectrometry (GC-MS). Total phenolic content (TPC) was determined using the Folin-Ciocalteau reagent and the antioxidant capacity of the n-hexane and methanolic extracts as well as essential oils, was evaluated with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test. Seventeen constituents, representing 88.89% of the oil, were identified, belonging to different groups of chemical compounds namely fatty acids, sterols, esters and hydrocarbons. Linoleic, palmitic, oleic and myristic acids were identified as the dominant fatty acid components, and gamma sitosterol and campesterol were the major elucidated sterols. The results indicated that methanolic extract had higher antioxidant activity than n-hexane extract and essential oils. TPC was relatively high and determined as 5.72 µg gallic acid equivalent (GAE)/mg of dry weight (DW). This study revealed that *N. schoberi* is an attractive source of fatty acid components, especially the essential ones, as well as that of effective natural antioxidants.

**Key words**: Antioxidant activity, gas chromatography-mass spectrometry (GC-MS), *Nitraria schoberi* L., total phenolic content.

**INTRODUCTION**

The genus *Nitraria* (Zygophyllaceae), comprising ca. 15 species, is widely distributed in the Middle East and Central Asia as well as in the Northwest region of China. Its special physiological characteristics of drought-resistant and salt-resistance make it an ideal plant with remarkable ecological values (Zhao et al., 2002; Li et al., 2006). The leaves, fruits and seeds of some species are often used in folklore medicine as an antispasmodic, antineuropathic, and anti-arrhythmic agent (Xing, 1991; Liu, 1988). The fruits are in particular recommended for the treatment of hypertension, abnormal menstruation and indigestion (Li et al., 2006). Despite their wide medicinal use, scientific data concerning the phytochemical composition of *Nitraria* species are scarce. Prior studies have shown the presence of several classes of secondary metabolites including sterols, fatty acids, alkaloids and flavonoids derivatives (Tulyaganov and Allaberdiev, 2001; Tulyaganov and Allaberdiev, 2003; Hadi et al., 2011; Suo and Wang, 2010).

*Nitraria schoberi* L. is a promising member of the genus *Nitraria*, from which three bioactive fractions were isolated, two with serotonin-like activity and one with vascular smooth muscle relaxing activity (Üstünés, 1988). This species which is widely distributed all over Asia, Turkey and the Middle East, is a weakly understood plant for its chemical composition. Therefore, different parts of this plant are worth to be undertaken into a phytochemical analysis. The chemical composition of *n-
hexane extract of *N. schoberi* fruits was included in the present study. Considering the high abundance of fatty acid stores that regulate a variety of physiological and biological functions in fruit oil of *Nitraria*, investigating their composition is of vital industrial importance (Wang et al., 2007).

On the other hand, the previous studies determined that the fruits of *Nitraria* are a source of phenolic compounds (Senejoux et al., 2011). Plant phenolic compounds possess strong antioxidant activity and may help to protect cells against the oxidative damage caused by free-radicals (Kahkonen et al., 1999). Free-radicals and other reactive oxygen species (ROS) were reported to be a causative agent of various diseases such as arthritis, asthma, dementia, mongolism, carcinoma and Parkinson’s disease (Perry et al., 200). In recent years, there is an increasing interest in finding antioxidant phytochemicals that can inhibit the propagation of free-radical reactions and protect the human body from diseases (Kinsella et al, 1993). One of the best approaches for discovering new antioxidants is the screening of plant extracts.

Despite the reported high phenolics content in the fruit of *Nitraria* and its attribution to antioxidant activity, no study of total phenolics and/or antioxidant activities of the *N. schoberi* have yet been published. The present study was carried out to provide pharmacological evidences to support the use of fruits from *N. schoberi* as antioxidant agent. The objective of this investigation were to (1) identify the chemical composition of n-hexane extract from fruits of the *N. schoberi*, (2) to analysis the total phenolic content (TPC) of fruit extract and (3) to assay the antioxidant activity of its essential oil, n-hexane and methanolic extracts in order to find out the proper solvent for the extraction of the antioxidant compounds.

**MATERIALS AND METHODS**

**Plant materials**

The fruits of *N. schoberi* L. were collected from the Payam Mountains at the East Azerbaijan province of Iran at July, 2011 by the authors. Plant sample was identified and the voucher specimen has been deposited in the Herbarium of Tabriz University of Medical Science. The samples were dried for 10 days at room temperature and then were powdered.

**Isolation of the essential oil**

The essential oil of dried fruits of *N. schoberi* was obtained via the hydro-distillation by using a Clevenger type apparatus for 4 h. The oil was used for the evaluation of antioxidant activity.

**Preparation of the hexane and methanolic extracts**

The extraction was performed using a Soxhlet apparatus in the normal way at the boiling point of the solvent used. The powdered sample (50 g) was extracted with 500 ml of solvent on a water bath until the solvent became colourless. The extracts were concentrated to ~1 ml under reduced pressure on a rotary evaporator. The extracts were stored in sealed vials at 4°C until biological analysis. The methanolic extract was used for antioxidant activity and the hexane extract was used for antioxidant activities as well as for the analysis of its chemical composition by gas chromatography-mass spectrometry (GC-MS).

**GC-MS procedure**

Recognition of compounds was carried out by a Shimadzu GC-MS-QP 5050A gas chromatograph (Shimadzu Corporation, Kyoto, Japan). The column used for the analysis was a 60 m × 0.25 mm i.d. DB1 capillary column coated with a film of dimethylpolysiloxane (J&W Scientific, Folsom, CA, USA). An aliquot of 1 µl of the n-hexane extract was injected into the GC-MS system in the split mode (split ratio 1:42). Helium was used as the carrier gas with a flow rate of 1 ml/min. The column temperature was maintained at 100°C for 2 min. Then, it was programmed to 300°C at a rate of 5°C/min and the final temperature was held for 38 min. Injector temperature and detector temperature were optimised at 270 and 310°C, respectively. The MS operating parameters were as follows: ionization energy, 70 eV; ion source temperature, 280°C; quadrupole, 100°C; solvent delay, 3.0 min; scan speed, 2000 u/s; scan range, 30 to 600 u, and EV voltage, 3000 volt. The components were identified on the basis of matching of their retention indices and mass spectra with the Wiley 229, Nist 107 and Nist 21 libraries.

**Determination of TPC**

TPC was estimated using the Folin-Ciocalteu colorimetric method described previously (Meda et al., 2005) with a little modification. Briefly, 5 g of powdered samples were individually dissolved in 50 ml of aceton-water (in 4 to 6 ratio). After 30 min, 1 ml of these solutions were mixed with 0.2 ml of Folin–Ciocalteu reagent, and 1 ml of 2% sodium carbonate (Na₂CO₃). After incubation at 40°C for 30 min, the absorbance of the reaction mixtures were measured at 760 nm by using a spectrophotometer (Shimadzu, Kyoto, Japan). Quantification was done on the basis of the standard curve of gallic acid. Results were expressed as µg gallic acid equivalent (GAE)/mg of dry weight (DW).

**1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity**

The DPPH quenching ability of plant essential oil, methanolic and n-hexane extracts was measured according to Hanato et al. (1988). Five (5) ml of the extract at different concentrations was added to 5 ml of a DPPH methanolic or chloroform solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The antiradical activity was expressed as half maximal inhibitory concentration (IC₅₀) (mg/ml), the antiradical concentration required to cause a 50% inhibition. A lower IC₅₀ value corresponds to a higher antioxidant activity of plant extracts. The ability to scavenge the DPPH radical was calculated using the following equation:

\[
\text{DPPH scavenging effect} \% = \left( \frac{A_0 - A_t}{A_0} \right) \times 100
\]

Where \(A_0\) is the absorbance of the control at 30 min, and \(A_t\) is the absorbance of the sample at 30 min.
Table 1. Chemical composition (%) of n-hexane extract from the fruits of *N. schoberi*.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>K.I.</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Myristic acid</td>
<td>1720</td>
<td>0.11</td>
</tr>
<tr>
<td>2</td>
<td>Methyl palmitate</td>
<td>1908</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>Farnesyl acetone</td>
<td>1921</td>
<td>0.21</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl 9-hexadecenoate</td>
<td>1955</td>
<td>0.78</td>
</tr>
<tr>
<td>5</td>
<td>Palmitic acid</td>
<td>1983</td>
<td>5.7</td>
</tr>
<tr>
<td>6</td>
<td>Ethyl palmitate</td>
<td>1984</td>
<td>0.52</td>
</tr>
<tr>
<td>7</td>
<td>3-methyl heneicosane</td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>8</td>
<td>Eicosane</td>
<td>2000</td>
<td>2.47</td>
</tr>
<tr>
<td>9</td>
<td>Linoleic acid</td>
<td>2152</td>
<td>62.86</td>
</tr>
<tr>
<td>10</td>
<td>2-chloro ethyl linoleate</td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td>11</td>
<td>Isopropyl linoleate</td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>12</td>
<td>Oleic acid</td>
<td>2161</td>
<td>0.19</td>
</tr>
<tr>
<td>13</td>
<td>Pentacosane</td>
<td>2500</td>
<td>4.72</td>
</tr>
<tr>
<td>14</td>
<td>Campesterol</td>
<td>3305</td>
<td>2.43</td>
</tr>
<tr>
<td>15</td>
<td>Gamma. sitosterol</td>
<td>3408</td>
<td>4.27</td>
</tr>
<tr>
<td>16</td>
<td>Stigmasteran-5,24-dien-3-ol(3 beta, 24E)</td>
<td></td>
<td>1.16</td>
</tr>
<tr>
<td>17</td>
<td>Stigmast-4-en-9-one</td>
<td></td>
<td>2.28</td>
</tr>
<tr>
<td>18</td>
<td>Fatty acids</td>
<td></td>
<td>68.86</td>
</tr>
<tr>
<td>19</td>
<td>Sterols</td>
<td></td>
<td>10.14</td>
</tr>
<tr>
<td>20</td>
<td>Hydrocarbons</td>
<td></td>
<td>7.7</td>
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<tr>
<td>21</td>
<td>Esters</td>
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<td>1.8</td>
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<tr>
<td></td>
<td>Other compounds</td>
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<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>88.89</td>
</tr>
</tbody>
</table>

Statistical analysis

The biological tests performed as a completely randomized design with three replications. Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan multiple range test at P <0.05 significant level.

RESULTS AND DISCUSSION

GC-MS analysis

The compounds were identified by mass spectra, database, Kovats index retention, and the literature. The identified compounds and relative quantitative distribution are shown in Table 1. A total of 17 components were identified, representing 88.89% of the extract. Fatty acids (68.86%) were determined as the first major groups of constituent in the extract. The sterols (10.14%) were the second group, followed by hydrocarbons (7.7%).

In the present study, linoleic (62.88%) and oleic acids (0.19%) were identified as the main unsaturated fatty acids (UFAs) and palmitic (5.7%) and myristic acids (0.11%) was found as the major saturated fatty acid. Except for myristic acid, other fatty acids have been reported in the other species of *Nitraria* (Wang et al., 2007). The amounts of UFAs were higher than saturated ones. In recent years, unsaturated and polyunsaturated fatty acids are the object of increasing interest due to their health promoting activity related to the observed reduction of cardiovascular diseases associated with their ingestion (Connor, 2000; Mensink et al., 2003). Therefore, the fatty acids of the fruits of *N. schoberi* may have health promoting effects.

In accordance with the previous studies on the other species of genus, gamma sitosterol (4.27%) and campestrol (2.43%) were the main identified phytosterols in the fruits of *N. schoberi* (Suo and Wang, 2010). Stigmasterol and sitosterol, two 24 methyl sterols, are major constituents of the sterol profiles of plant species. Beside their vital role in the structure and dynamics of membranes, they are also involved in the embryonic growth of plants (Schrick et al., 2002; Schaller, 2004).

In addition to the major mentioned functional groups, hydrocarbons, esters, 1 chlorinated compound (2. chloro ethyl linoleat) and 1 keton (farnesyl aceton) were also identified. Organohalogen compounds which are rarely reported in plants (Platikanova et al., 2005; Djozan et al., 2008) possess some defensive functions as antifeedants and protect various plants against insects and herbivores (Engvild, 1986). Among the recognised compounds, farnesyl acetone is outstanding as some of its derivatives have anticonvulsant properties at non-sedative doses in
Table 2. DPPH scavenging ability of different extracts of *N. Schoberi*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Methanolic extract</th>
<th>N-hexan extract</th>
<th>Essential oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH radical scavenging activity [IC$_{50}$ (mg/ml)]</td>
<td>0.26 ± 0.001$^c$</td>
<td>1.75 ± 0.005$^a$</td>
<td>1.37 ± 0.003$^b$</td>
</tr>
</tbody>
</table>

$^*$Data were expressed as mean ± SD. Values with different superscript letters are statistically different (P<0.05).

Figure 1. Comparison of DPPH scavenging ability [IC$_{50}$ (mg/ml)] in different extracts of *N. Schoberi*.

mice (Spence et al., 1979).

**Total phenolics content**

Based on the measured absorbance value of the plant extract reacting with Folin–Ciocalteu reagent, and in comparison with absorbance values of gallic acid solutions in the standard curve, the amount of total phenolics in the extract was estimated at 5.72 μg GAE/mg DW. This value is comparable to the values reported in the literature for other *Nitraria* species. The previous studies determined that the fruits of *Nitraria sibirica* Pall. are a source of phenolic compounds, with an amount being 23.6 μg GAE/mg DW (Senejoux et al., 2011). The occurrence of polyphenols in the fruits of *Nitraria* is consistent with previous chemical investigations that have been shown the presence of flavonol and anthocyanin derivatives in the fruit (Halim et al., 1995; Khoo et al., 2010; Zheng et al., 2011). The high value of phenolic content indicates that the plant has high antioxidant activity.

**Antioxidant activity**

Radical-scavenging activity of the methanol and n-hexane extracts as well as essential oils from fruits of *N. schoberi* was evaluated using DPPH radical assay. The DPPH scavenging activities expressed as IC$_{50}$ values (the concentrations that led to 50% inhibition) are shown in Table 2. According to this data, methanolic extract was the most efficient free radical scavenger by the lowest IC$_{50}$ value of 0.26 mg/ml (Figure 1). The high DPPH scavenging activity of methanolic extract may arise from the phenolic compounds which accumulated mainly in polar extracts.

**Conclusion**

The n-hexane extract constituents of *N. schoberi* fruits were studied for the first time. In addition, the results presented in this study are the first information on the antioxidant activities and total phenolics of the essential oil as well as n-hexane and methanol extracts of the
plant. According to our results, the main constituents of n-hexane extracts were fatty acids and sterols. Regarding the high amount of UFAs, it seems that *N. schoberi* fruits may be a good dietary source for UFAs. Among the three analyzed extracts, the highest antioxidant activity was observed in the methanolic extract. The study indicated that the fruit extract is rich in phenolics antioxidants.

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**REFERENCES**


