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# Extraction and comparison of alkaloids in different organs during different phonological periods of *Nitraria schoberi*

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**ABSTRACT**

*Nitraria schoberi (Zygophyllaceae) is native to the desert regions of Mighan in Arak of Iran. Also because many alkaloids have many biological properties. Among the properties that can contract effect on blood vessels called action and relief providers, and resistance to drought, salinity and contamination of the plant, the results indicate that is resistant to drought and salinity tolerance to pollution $CuSo_4$ at a concentration of 600 mg/m3. In study Nitraria schoberi of family Nitrariaceae and Spindale's order, the total amount of alkaloids in different organs during different phonological were studied. The aim of different organs (leaf, root and branch) and (leaf, roots, stems, flowers) during the flowering stage and (leaf, roots, stems, crown of branch, fruits) during the fruiting stage, at various times, were collected from the desert region of Mighan Arak of Iran. After extraction, using three methods: Soxhlet, ultrasonic and reflux, the total amount of alkaloids in different organs using spectroscopic and gravimetric methods were extracted. The results showed that the different of mean total alkaloids in organs of the plant (leaf, stems, roots, flowers, crown branch, fruits) in all courses is significant ($p<0.01$) and leaf of plant, are more alkaloids than other organs. Among the different phonological period fruiting period, much more alkaloids in all organs, other than the period indicated. The alkaloid production in the fruiting period to reach its highest. The alkaloid obtained from the gravimetric method is higher than the spectroscopic method, because, some alkaloids no complex formation with BCG, therefore, spectroscopic methods showed lower levels of alkaloids.*

**Keywords:** *Nitre billardierei*, Alkaloids, methods of Soxhlet, ultrasonic , reflux

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## INTRODUCTION

The utilization of plants as therapeutic medicines is an ancient tradition, far older than the contemporary sciences of medicine, pharmacology, or chemistry; it is a tradition that can claim both historical and prehistoric precedent, having its origins in an era when medicine, magic, religion, and pharmacology were facets of a single empirical discipline. In many indigenous cultures, plantbased, traditional medicine has remained essentially unchanged from the form practiced by our ancestors 5000 or more years ago [1]. 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-resistance and salt resistance make it an ideal plant with remarkable ecological values [2,3]. The leaves, fruits and seeds of some species are often used in folklore medicine as an antispasmodic, antineuropathic, and anti-arrhythmic agent [4, 5]. The fruits are in particular recommended for the treatment of hypertension, abnormal menstruation and indigestion [3]. Despite their wide medicinal use, scientific data concerning the photo chemical composition of Nitraria species are scarce. Isolated from aerial parts of the plants, alkaloids of the Nitraria genus are classically classified into three major groups tripiperidine alkaloids (e.g., schoberine), indole alkaloids (e.g., nitrarine), and the group of spiro alkaloids. The latter can be divided in two sub-groups: simple spiro alkaloids (e.g., sibirine) and complex spiro alkaloids (e.g., nitraramine 1, 1-epinitraramine

2). A variety of alkaloids, like Nitramin 1, Nitrarine 2, Nazlinin 3 and Nitraramine 4, have been isolated from different species of the Nitraria family1. An interesting feature of these alkaloids is the fact that they are isolated as race mates, even in cases where the molecule contains five or six stereocenters2. This has been observed before for alkaloids like Akuammicine3, Yuehchukene 4 and Lucidene 5. Prior studies have shown the presence of several classes of secondary metabolites including sterols, fatty acids, alkaloids and flavonoids derivatives [6,7, 8, 9]. This species which is widely distributed all over Asia, Turkey, Iran and the Middle East, is a weekly understood plant for its chemical composition. Therefore, different parts of this plant are worth to be undertaken into a photochemical 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 [10]. On the other hand, the previous studies determined that the fruits of Nitraria are a source of phenolic compounds [11]. Plant phenolic compounds possess strong antioxidant activity and may help to protect cells against the oxidative damage caused by free-radicals [12]. 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 [13]. 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. schooner have yet been published. The present study was carried out to provide pharmacological evidences to support the use of fruits from *N. schooner* as antioxidant agent.

## MATERIALS AND METHODS

Plants collected from natural habitats Nitraria schoberi of 15 kilometers from the city of Arak (Iran) in three steps. Stage of before flowering on 3thApril, 2012 and stage of flowering in 15thApril and fruiting stage on 16th September 2012, was performed in region of Mighan desert of Arak. Plant samples were consistent with the Flora Iranica were identified by experts at Arak Agricultural Research Center.

**1-Determination of the total alkaloids using gravimetric methods:**
Different parts of the plant collected separately during different phonological in vitro were dried and milled. And a rate of 50 grams of powdered leaf was poured into the flask and NH4OH (25%) were soaked. Then 150 ml of methanol were added and using three methods: Soxhlet, reflux (Reflux) and ultrasonic extraction was performed.

**1-1-Soxhlet method:**
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 colorless. The extracts were concen traded to ~1 ml under reduced pressure on a rotary evaporator. The extracts were stored in sealed vials at 4°C until biological analysis. The metabolic 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).

**1-2-Reflux method:**
50 g of plant powder was poured into a flask with 100 ml of NH4OH (25%) were soaked. Then 150 ml of methanol were added and connected to the fridge. The temperature was set at 80 ° C, and reflux extraction method was used, also the extract obtained was weighed.

**1-3-Ultrasonic methods: Ultrasound -assisted extraction (ultrasonic)**
50 g of plant powder was poured into a flask with 100 ml of NH4OH (25%) were soaked. Then 150 ml of methanol were added and connected to the ultrasonic. The solvent was then filtered and the extract obtained was weighed.

**2- Determination of total alkaloids using spectroscopic methods:**
**2-1- Preparing a solution Bromocresol Green (BCG) with concentrations 0/0001 M:**
69.8 mg BCG with 3 ml solution of NaOH (2 normal) and 5 ml of distilled water were dissolved and with distilled water, was brought to a volume of 1 liter.

**2-2- Preparation of phosphate buffer at pH 7.4:**
79.6 g $Na_2HPO_4$ dissolved in one liter of distilled water (2 mM ) and the citric acid 0.2 M , to pH = 4.7.

**2-3- Calibration curves for atropine:**
For the calibration curve, the concentration of 5 was used. 100ppm solution was initially prepared from atropine (1 mg in 10 ml of distilled water) and then dividing by 5 to funnel removed and, respectively, 0.5 , 1, 1.5 , 2 and 5/2 ml atropine was added and each 5 ml of phosphate buffer (PH,4.7) and BCG solution was added 5 mL and was vigorously stirred. 5 ml of chloroform was added to each of them. After the flash, was isolated by chloroform phase.

5 to 10-mL flask isolated by chloroform phase was removed, and the way they move and was brought to volume with chloroform. Absorption at a wavelength of 470 nm of each flask was measured, read before a witness was prepared to absorb. After reading the absorption observed uptake little balloons and calibration graphs were plotted.

**2-4- Extracting the alkaloids:**

50 g of dried plant powder was poured into the flask, and was added to the 150 ml n-hexane. Then, using soxhlet extraction was performed for 2 h. After filtration, the residue was dissolved in 150 ml methanol and Soxhlet extraction procedure was performed again for 12 hours. After evaporation of the resulting solution was filtered off and dissolved in 150 ml of distilled water Normalized using HCl 1 to 2.5 was completed. 1 mL of solution was transferred to a separating funnel and 10 ml of chloroform were added Stir after MEOH phase was discarded. The aqueous phase normalized by NaOH 1 to pH = 9.5.

And 5 ml of the BCG, and 5 ml of phosphate buffer was added and was extracted with 4 ml of chloroform and the volume reached 10 ml flask chloroform phase, and absorption in the UV wavelength 470 nm was read on the device. Plant uptake was repeated three times for each sample was the average of the three replications. Data were analyzed with the use of SPSS software.

## RESULTS AND DISCUSSION

Data obtained by using SPSS software were analyzed with one-way ANOVA test. First, normality tests were performed (Kolmogorov Smirnov test) and Q-Q Plot graphs showed that the distribution is not normal. Since the conversion of the data , their distribution was not normal. The non -parametric equivalent of a one-way Anova Kruskal-Wallis test was used for data analysis. The results showed that the mean difference between the 99% and 95% level is significant. The results showed that all organs are *N. schoberi* contain alkaloids. *N. schoberi* leaf the gravimetric method with a value of 1.086 % highest alkaloid and stalk of rapeseed with 0.087 of the alkaloid showed the lowest the findings Fan lianlian (2002) concluded that leaf and fruit species *N.sibirica*, Rich in mineral elements and their values were higher in leaf, is consistent. Spectroscopic methods for *N. schoberi* leaves 1.58 % highest alkaloid and stalk *N. schoberi* with 0.0812 cent alkaloid showed the lowest. Spectroscopic method for the determination of total alkaloids in need of standard substance (atropine) and a dye (BCG), respectively. Property selected pigment was given a colored complex with the alkaloid and Absorption at a wavelength of 470 nm and showed well. So in this way, alkaloids with BCG complex formation and absorption was read, the calibration curve was determined using, the results are presented. The alkaloid obtained from the gravimetric method is higher than the spectroscopic method, this could be due to the fact that some of the alkaloid with BCG does not complex formation and therefore the lower part shows the amount of spectroscopic methods. In the study of Khajeddini et al, (2012), 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 [14]. Except for myristic acid, other fatty acids have been reported in the other species of Nitraria [10]. 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 [15, 16]. In accordance with the previous studies on the other species of genus, gamma sitosterol (4.27%) and cam-pestrol (2.43%) were the main identified phytosterols in the fruits of N. Schoberi [10]. 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 [12]. The occurrence of polyphenols in the fruits of Nitraria is consistent with previous chemical investigations that have been shown the presence of flavones and anthocyanin derivatives in the fruit [17,18]. The high value of phenolic content indicates that the plant has high antioxidant activity. The result showed that the production of alkaloids in the fruit reaches its peak that may result in development of fruits (due to the developmental stage of the plant) is the activity of enzymes responsible for the production of alkaloids. Moreover, when the plants are mature relatively more nitrogen into the structure of alkaloids. Greater proportion of younger plants because their amino acid, apply for the initial metabolism, while more mature plants can better support the secondary metabolism , and the maximum amount of alkaloids in leaves of *N. schoberi* are three phonological periods and minimum value of the *N. schoberi* plant there. In the gravimetric method, the total alkaloid originally extracted using three methods: Soxhlet, ultrasonic and reflux. In the course soxhlet higher alkaloid obtained. With regard to the quantitative separation of *N. schoberi* for the first time in this study took place, further research on the identification of alkaloids in the plant and compared with the results of this research can be fulfilled.

**Table 1 - Alkaloid content of plant leaves using different**

| Extraction method | Alkaloid (mg) |
|---|---|
| Soxhlet method | 627 mg |
| Reflux method | 601 mg |
| Ultrasonic methods | 612 mg |

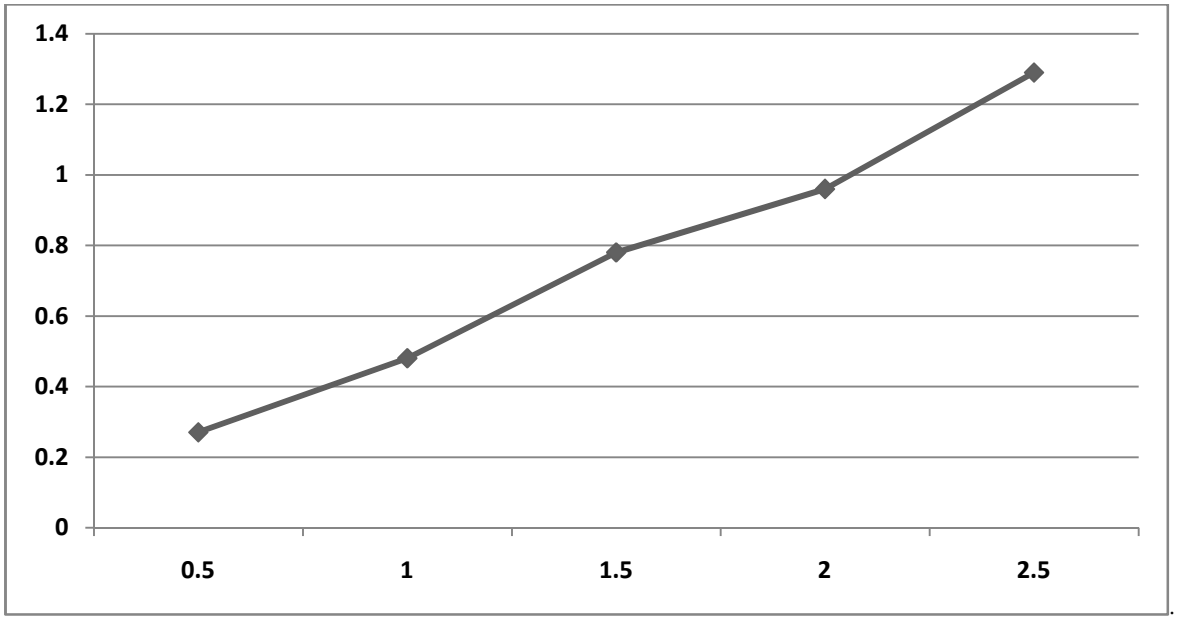


**Fig. 1 - Calibration curve based on the concentration of atropine**

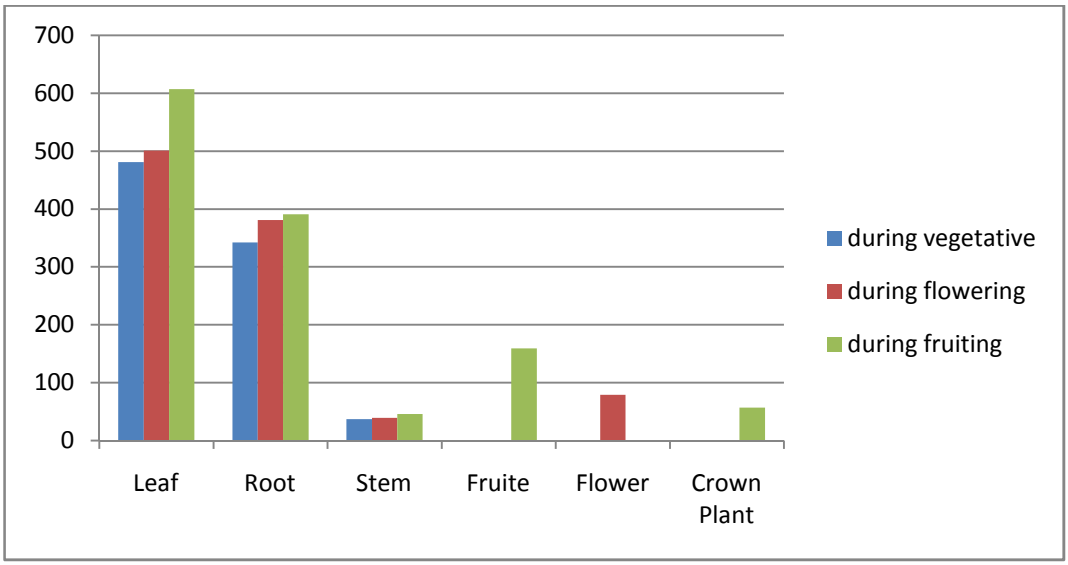


**Fig. 2 - Comparison of alkaloids in different organs during different phonological gravimetric method**

**Table 2 - The Alkaloids gravimetric method during vegetative**

| Organs used in plant | Leaf | Root | Stem |
|---|---|---|---|
| The material terms of milligrams | 492 | 352 | 39 |
| % | 0.984 | 0.704 | 0.078 |

**Table 3 - The Alkaloids gravimetric method during the flowering period**

| Organs used in plant | Leaf | flower | Root | Stem |
|---|---|---|---|---|
| The material terms of milligrams | 512 | 82 | 387 | 42 |
| % | 1.024 | 0.164 | 0.774 | 0.084 |

**Table 4 - The Alkaloids gravimetric method during the fruiting period**

| Organs used in plant | Leaf | flower | Root | Stem | Plant Crown |
|---|---|---|---|---|---|
| The material terms of milligrams | 627 | 168 | 407 | 50 | 63 |
| % | 1.25 | 0.37 | 0.81 | 0.1 | 0.13 |

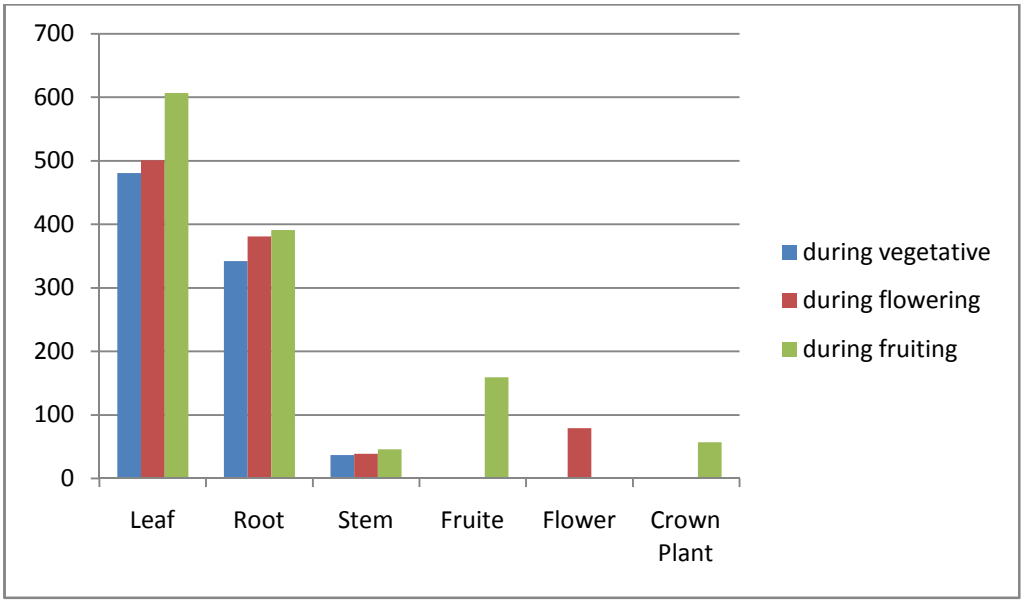


**Fig. 3 - Comparison of alkaloids in different organs during different phonological periods in spectroscopic methods**

**Table 5 - Observed absorption spectroscopy method during vegetative**

| Organs used in plant | Leaf | Root | Stem |
|---|---|---|---|
| Absorption observed | 21.18 | 15.06 | 1.26 |

**Table 6 - The Alkaloids gravimetric method during the flowering period**

| Organs used in plant | Leaf | flower | Root | Stem |
|---|---|---|---|---|
| Absorption observed | 22.07 | 3.48 | 16.78 | 1.71 |

**Table 7 - The Alkaloids gravimetric method during the fruiting period**

| Organs used in plant | Leaf | Root | Stem | Plant Crown | fruit |
|---|---|---|---|---|---|
| Absorption observed | 26.73 | 17.2 | 2.02 | 2.52 | 7.01 |

**Table 8 - The amount and percentage of total alkaloids spectroscopic methods during vegetative**

| Organs used in plant | Leaf | Root | Stem |
|---|---|---|---|
| The material terms of milligrams | 481 | 342 | 37 |
| % | 0.96 | 0.68 | 0.074 |

**Table 9 - The amount and percentage of total alkaloids spectroscopic methods during flowering**

| Organs used in plant | Leaf | flower | Root | Stem |
|---|---|---|---|---|
| The material terms of milligrams | 501 | 79 | 381 | 39 |
| % | 1 | 0.16 | 0.76 | 0.08 |

**Table 10 - The amount and percentage of total alkaloids spectroscopic methods during fruiting**

| Organs used in plant | Leaf | flower | Root | Stem | Plant Crown |
|---|---|---|---|---|---|
| The material terms of milligrams | 607 | 159 | 391 | 46 | 57 |
| % | 1.21 | 0.32 | 0.78 | 0.09 | 0.11 |

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