Determination of Atropine, Hyoscine and Rutin Content of Henbane Seeds from Different Regions in Iran

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ABSTRACT

Seeds of plants belonging to Solanaceae family like *Hyoscyamus niger* are well known for their alkaloidal secondary metabolites, but there also exists some non-alkaloidal compounds like flavonoids, which are less explored. The purpose of the present study was to determine the content of tropane alkaloids including hyoscine and atropine as well as rutin content of black henbane seeds, which were collected from three different regions of Iran (Kermanshah, Kandovan and Karaj, 2200, 1900 and 1300 meter above sea level, respectively). Determination of alkaloids and rutin content were performed by the GC/MS and HPLC methods, respectively. All seeds from the three mentioned collection sites contained hyoscine, atropine and rutin in different amounts. The highest and the lowest hyoscine content (0.77 and 0.057 g.g dw) were obtained at 1300 and 1900 m altitude, respectively. Also, seed atropine content was showed the same trend to altitude as hyoscine content. Hyoscine was the predominant tropane alkaloid in seeds of black henbane, while this sample contains a low amount of rutin. The seed rutin content was increased with the increase of altitude, i.e., the high value of rutin content (25.76 g.g dw) was observed at 2200 m altitude above sea level.

Key words: *Hyoscyamus niger*, Tropane Alkaloid, Flavonoid, Hyoscine, Atropine, Rutin.

Introduction

Human life depends on plants. Plants represent a nearly unlimited source of phytochemicals, metabolites of primary and secondary metabolism. The secondary metabolites are of major interest because of their different functions and their impressive biological activities [9]. Therefore, secondary metabolism contributes to the economical importance of plants. Secondary metabolites with diverse chemical structures are usually synthesized in some plant tissues at certain developmental stages. The rates of metabolite formation are often greatly influenced by internal hormone balances and external stimuli [3].

Alkaloids are a diverse group of low-molecular-weight, nitrogen-containing compounds (Fig 1) found in about 20% of plant species. Solanaceous plants are regarded as rich sources of alkaloids, namely the pharmaceutical by interesting tropane derivatives. Tropane alkaloids, especially atropine andhyoscine, are widely used in medicine for their mydriatic, antispasmodic, anticholinergic, analgesic and sedative properties [11]. The synthetic production of these alkaloids is more expensive than their extraction from plant materials and they are, therefore, currently industrially extracted from various Solanaceous plants belonging to the genera *Atropa*, *Duboisia*, *Datura* and *Hyoscyamus*. Different species of *Hyoscyamus* are rich sources of tropane alkaloids. Tropane alkaloids are synthesized in roots and then transported to the aerial parts of the plant [7]. Biosynthesis of alkaloids, although controlled genetically, could be affected by different environmental factors, such as light, high temperature, stress and nutrients [2]. While, the poisonous henbane is well known for its alkaloidal content, the occurrence of non-alkaloidal metabolic constituents is not well explored. The anticholinergic plants species also produce non-alkaloidal secondary metabolites such as saponins, glycosides and falavonoids. Phytochemical reports were showed the occurrence of flavonoids like rutin in the seeds of *H. niger* plants. The antioxidant activity of rutin, quercetin and flavone C-glucosides was determined by different chemical assays and has been recently reported [12,13]. The main objective of the present study was to quantify tropane alkaloids...
(atropine and hyoscine) and flavonoids (rutin) in the seeds of *H. niger* plants from three different altitudes in Iran.

![Chemical structure of atropine, hyoscine and rutin.](image)

**Fig. 1:** Chemical structure of atropine, hyoscine and rutin.

**Materials and Methods**

2.1. Seed materials:

The henbane seeds were collected from three different sites in Iran, Kermanshah, Kandovan and Karaj, which are located at 2200, 1900 and 1300 meter altitude as above sea level (Table 1 and Fig 2). The seeds were harvested in July at plant seeding stage, and then were dried at ambient temperature in the shade for following biochemical analysis.

**Table 1:** The *h. niger* seed collection sites and their altitudes (meter above sea level).

<table>
<thead>
<tr>
<th>Seeds Collection Site</th>
<th>Altitude (m)</th>
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<tbody>
<tr>
<td>Kermanshah</td>
<td>2200</td>
</tr>
<tr>
<td>Kandovan</td>
<td>1900</td>
</tr>
<tr>
<td>Karaj</td>
<td>1300</td>
</tr>
</tbody>
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![Map of three studied hyoscyamus niger seed collection sites in Iran (Kermanshah, Kandovan and Karaj, which is shown with green, yellow and pink colour, respectively).](image)

**Fig. 2:** Map of three studied *hyoscyamus niger* seed collection sites in Iran (Kermanshah, Kandovan and Karaj, which is shown with green, yellow and pink colour, respectively).
2.2. Alkaloids extraction and analysis:

Seed samples were ground into fine powder and sieved with laboratory mesh (size 30, mesh opening 545 μm). A subsample of 2 gram from each sample was added to appropriate volume of CHCl₃: MeOH: NH₄OH 25%, (15:5: 1), sonicated for 20 min and then kept at water bath (40 °C) for one hour. Subsequent sample preparation and alkaloids extraction were based essentially on the method described by Kamada [6]. Alkaloids extracted were identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis. Gas chromatography analysis was performed using a GC system equipped with a flame ionization detector (FID) and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220 and 290 °C, respectively. The column temperature was initially kept at 50 °C for 5 min, then gradually increased to 300 °C at a rate of 3 °C/min and maintained for 3 min. The flow rate of gas helium was 0.8 mL/min. Then 1 μL of extract was directly injected into the gas chromatograph. Each extraction was repeated three times and the compound percentages are the means of the three replicates. GC-MS analysis was carried out on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, USA) fitted with a fused silica HP-5MS capillary column (30m×0.25mm×0.25μm). Oven temperature was programmed from 50 °C to 285 °C at 3 °C/min, and helium was used as carrier gas (0.8 mL/min). Mass spectra were obtained in an Agilent 5973 system operating in electron impact mode (EIMS) at 70 eV, coupled to a GC system. The identification of alkaloids was based on the comparison of their GC retention time and mass spectra (MS) data with their standards substances (HYO. HCl and SCO. HBr, Merck).

2.3. Rutin Assay:

An amount of one gram of ground seed was extracted with 10 ml of solution (methanol-acetic acid-water; 100:2:100) for 1 hour on a shaker at laboratory temperature. Then 2 ml of the extract were centrifuged for 10 min at 2000 rot/min. Thereafter, solution was filtered through a micro filter with a regenerated cellulose membranes of the pore size 0.22. The filtrate was applied for HPLC. UV detector was carried out at 355 nm. Rutin was eluted at 6.72 min and the peak area was compared to the standard.

Results and Discussion

The results indicated that the content of tropane alkaloids (hyoscine and atropine) and rutin were different in seeds of *Hyoscyamus niger* plants, which were collected from various ecological sites. The results have been summarized in figures 4 and 5. As can be observed, the highest and the lowest hyoscine content (0.77 and 0.057 g.g dw) were obtained at the 1300 and 1900 m altitude, respectively. On the other hand, maximum hyoscine content of seeds was observed at the lowest altitude. Also, seed atropine content was showed the same trend as hyoscine to altitude changes. The highest atropine content (0.143 g.g dw) was observed at 1300 m altitude in the Karaj site. Obviously, maximum total alkaloid (hyoscine + atropine) content (0.913 g.g dw) was also calculated in low elevation, Karaj.

Rutin content of seeds from different locations also showed markedly fluctuations with altitude (Fig 5). The seed rutin content was increased with the increase of altitude, i.e., the high value of rutin content (25.76 g.g dw) was observed at 2200 m altitude above sea level. However, in this case the difference between 1900 m and 2200 m, Kandovan and Kermanshah, were not prominent as compared to that of Karaj.

Fig. 3: Biosynthetic pathway for tropane alkaloids.
This study showed that the production of secondary metabolites, tropane alkaloids and rutin, were affected differently by climatic conditions like altitude. According to the results of current study, hyoscine was the predominant tropane alkaloid in the seeds of *H. niger* plants from Karaj, which contain the lowest elevation (1300 m).

As regards hyoscine heat labile and temperature decrease by increasing altitude also temperature decrease leads to hyoscine increase [8]. In our current study also high level of hyoscine content was gained at lower altitude, which may be due to temperature constraint in high altitude area.

The data also showed that the highest amount of hyoscine to atropine ratio (5.38) was obtained in seeds of Karaj region. Although, plant heredity controls the biosynthesis of alkaloids, some environmental (e.g., biological and abiological factors as elicitors) could enhance or inhibit tropane alkaloids production [4,1,5]. The biosynthesis of tropane alkaloids (Fig 3) is often tissue specific, occurs in most plants in their roots, and the produced alkaloids are exported from roots to other organs [10]. Atropine and hyoscine, derived from phenylalanine, ornithine and arginine, are synthesized almost exclusively in the plant roots. Hyoscine is synthesized from atropine via 6b-hydroxyhyoscyamine. The hyoscyamine 6b-hydroxylase catalyzes the two-step epoxidation of atropine to hyoscine. However, not much information is available on the effect of climatic conditions like altitude on the content of seed alkaloids and flavonoids content in *Hyoscyamus niger* plants.
References


