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# Assessment of Alkaloid Content and Antibacterial Activity of *Hyoscyamus Albus* and *Hyoscyamus Muticus* Collected in Two Different Climatic Regions in Algeria

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## Abstract

Medicinal plants are considered as a very important source of secondary metabolites and therefore bioactive molecules. Our comparative study deals with two species: *Hyoscyamus albus* and *Hyoscyamus muticus* harvested in two different climate regions namely: Arris (semi-arid, wilaya of Batna) and Djanet (arid, wilaya of Illizi), respectively. The phytochemical screening revealed that aerial parts of the two species contain almost all classes of secondary metabolites such as flavonoids, quinones, anthraquinones, saponins, tannins, coumarins and alkaloids. The quantitative assessment of alkaloids in hydroalcoholic extracts of the two plants showed that the total alkaloid content varies depending upon the species and climate conditions. The alkaloid level was found to be 0.51% and 0.32% in *Hyoscyamus albus* and *Hyoscyamus muticus* respectively. Moreover, the antibacterial test vis-à-vis some pathogen bacteria such as: *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed that both alkaloid crude extracts and hydroalcoholic extracts are moderately active against the tested bacterial strains.


**Keywords:** *Hyoscyamus albus*, *Hyoscyamus muticus*, Alkaloid content, climatic factors, Antibacterial activity.

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## Introduction

Ecological factors such as soil composition, temperature, elevation, humidity, bright quality, rainfall, evaporation**,** minerals, and $CO_2$ have a straightforward effect on the increase of a plant and accretion of secondary metabolites and, hence, affect unswervingly the quality of the species for healing application, and remedial assessment. Plants have the capacity to overcome biological, physical, chemical, and ecological restrictions by regulating the improvement of secondary metabolites. As a result, investigations on the control of ecological factors on the build-up of secondary metabolites in medicinal plants become a warm topic of major concern (Hanif et al., 2018, fellah, 2018).

The genus *Hyoscyamus* belongs to the tribe Hyoscyameae Miers of Solanaceae family with 20 species all over the world (Yousaf et al., 2008) and 3 species in Algeria (quezel and santa, 1963). The species of *Hyoscyamus* were characterized by high content of alkaloids. Among environmental components, temperature is considered influential. The proportion of alkaloids increases at high temperatures; conversely, more unsaturated fatty acids are produced by the same species at lower temperatures. Linder (2000) and Bernath and Tetenyi (1979, 1981) described the effect of environmental factors on growth, development and alkaloid production of poppy including day-length, light intensity and temperature; some important variations were recorded. Amiri et al (2017) investigated the environmental impact on the assessment of total alkaloid extracted from the *Atropa belladonna* using Life Cycle Assessment. It was found that green processes should be utilized for extraction of alkaloids, and according to the impacts of chloroform utilized in the extraction process; the chemical materials with minimal damage to the environment should be replaced. Wei et al (2015) studied the effect of ecological factors on the production of active compounds in the anti-cancer plant *Sinopodophyllum hexandrum* (Royle) T.S. Ying. The correlation between the contents of the active ingredients derived from the roots and rhizomes of the plant and the ecological factors were determined step-by-step by series of computational biology methodologies. The results revealed that ecological factors had significant impacts on the contents but not on the types of the active ingredients in eight production locations. The primary ecological factors impacting the active compounds consisted of the annual average precipitation, July mean temperature, frost-free period, sunshine duration, soil pH, soil organic matter, and rapidly available potassium in the soil. The annual average precipitation was the main determinant factor and was significantly and negatively correlated with the active ingredient contents ($P < 0.001$). In contrast, organic matter was the main limiting factor and was significantly and positively correlated with the active compounds. These ecological factors resulted in 98.13% of the total geographical variation of the active ingredient contents. The climate factors involved more to the active ingredient contents than did the soil factors. It was concluded that from the view of the contents of the secondary metabolites and ecological factors of each growing location, in Jingyuan, Ningxia Province, and Yongdeng, Gansu Province, conditions were favorable to the production of podophyllotoxin and lignans, whereas in Shangri-La, Yunnan Province, and Nyingchi, Tibet, conditions were favorable to the production of quercetin and kaempferol. Urban et al (2018), undertook a study on evaluation of the content of α-solanine, α-chaconine and total glycoalkaloids (TGA) in fourteen new potato cultivars with purple and red flesh in comparison with yellow- and white-fleshed control potatoes cultivated in a friendly way in integrated agriculture. The results were obtained from three-year trials on two locations. TGA levels in tubers' flesh differed from 33.69 to 167.77 mg/kg fresh matter (FM), and the ratio of α-chaconine to α-solanine from 1.18 to 3.78. No TGA safety limit was exceeded for any cultivar. The glycoalkaloids content was not significantly affected by flesh color, howevere the cultivar genotype had a decisive impact on their content. Eight cultivars with colored flesh yielded a more preferable lower TGA content as compared with the yellow-fleshed control cv. Agria (86.3 mg/kg FM); on the contrary six cultivars showed higher TGA values. The highest average TGA content was revealed to be in the purple-fleshed Bora Valley cultivar (165 mg/kg FM), the lowest was in the red-fleshed Red Emmalie cultivar (43.6 mg/kg FM), whereas the white-fleshed cv. Russet Burbank reached 67.0 mg/kg FM. The glycol-alkaloid content was significantly influenced by location and year weather conditions. As far as our investigation is concerned we aimed mainly to study the quantity of alkaloids in the two *Hyoscyamus* species collected in two very different climate conditions as well as their antibacterial effect against some pathogenic strains.

## Materials and Methods

*Plant material and isolation*

The aerial parts of the two species *Hyoscyamus albus* and *Hyoscyamus muticus* were collected from two different regions. The first species (*Hyoscyamus albus*) was collected in the semi-arid region of Arris (Batna) and the second species (*Hyoscyamus muticus)* was collected in the arid region of Tassili (Janet, Illizi) during the flowering period (April). The two species were identified by a specialist and a voucher material was deposited at the Laboratory of Natural Resources and Management of Sensitive Environments (directed by Pr. Abdelkader Khiari) under the number of ZK 7. After drying in shade at ambient temperature during three weeks in order to preserve the maximum the integrity of the vegetable material, both plants were crushed in an electric mill and prepared for extraction.

*Hydroalcoholic extracts preparation*

Two hundred (200) grams of dried powder of each plant were extracted exhaustively with water and methanol mixture in proportion of 3:7 for 48 h. The extracts thus obtained were filtered and solvent was removed under vacuum.

*chemical Phytoscreening:*

The plants were evaluated by phytochemical qualitative reactions for usual plant secondary metabolites. The screening was done for flavonoids, tannins, anthraquinones, quinones, alkaloids, saponins, coumarins. The color intensity or the precipitate formation was utilized as analytical responses to these tests.

*Determination of Alkaloids using Harborne (1973) method:*

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed and the percentage of alkaloid is expressed mathematically as

$$\%\ of\ alkaloids = \frac{\text{weight of crude } alkaloids}{\text{weight of sample}} \times 100$$

*Antibacterial activity*

The antibacterial activity test was carried out by disk diffusion method against four human pathogenic bacteria, including gram-positive and gram-negative bacteria namely *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus* subsp. *aureus* and *Pseudomonas aeruginosa.*

- *Disk-diffusion method*

The discs with 6 mm diameter were placed on the plates with 80 mm diameters containing Mueller Hinton agar (MHA) with $1.5\times10^8$ bacterial cells. Hydroalcoholic extracts and alkaloid extracts were dissolved in dimethyl Sulfoxide (DMSO) so as to prepare a 0.5 $g.mL^{-1}$ solution. 20 µl of each sample were placed on the discs and then incubated at 37 °C for 24 h. Antimicrobial activity was assessed by measuring the zone of inhibition surrounding the discs. Oxaciline was utilized as positive control and DMSO used as negative control (Billah *et al,* 2013).

## Results and Discussion

*Climatic data of Batna*

Table 1: mean climatic Data of Batna over 11 years (2004-2014)

| Balance over 11 years | Max Temperature (°C) | Min Temperature (°C) | Mean Temperature (°C) | Precipitation (mm) |
|---|---|---|---|---|
| January | 19.3 | -2.4 | 6.5 | 22.9 |
| February | 20.5 | -3.2 | 7.0 | 24.0 |
| March | 25.8 | -3.0 | 10.2 | 31.4 |
| April | 29.2 | -0.4 | 13.6 | 38.0 |
| may | 33.3 | 3.1 | 17.6 | 46.0 |
| June | 39.03 | 6.6 | 22.9 | 20.8 |
| July | 41.4 | 11.6 | 26.9 | 5.0 |
| August | 40.9 | 12.3 | 26.4 | 18.6 |
| September | 36.5 | 8.09 | 21.8 | 30.8 |
| October | 29.4 | 2.7 | 17.5 | 15.2 |
| November | 25.5 | -1.4 | 11.1 | 28.7 |
| December | 20.5 | -3.1 | 7.1 | 29.1 |

- *Temperature:*

The temperature is a very significant climatic factor which reacts directly, in interaction with the vegetation and the phenomenon of the evapo-transpiration. The evolution and the permanent change of the temperature affect the surface water and thus its quality. The temperature depends on the altitude, the distance from the sea, and the topographic position (Toubal Boumaza; 1986).

Based on the data of the table above, we note that the hot season is well marked. The maximum temperature was recorded during July to be 41.4°C and February is the coldest month with a minimal temperature of -3.2°C.

- *Precipitation:*

The term Precipitation indicates any type of water falling from the sky, in liquid or solid form. It includes rain, snow, and hail. Rainfall is defined as being the primordial factor which determines the type of climate.

According to the climatic data, annual precipitation is 290.7 mm where May is the rainiest month with 46 mm; and July is the driest month with an average precipitation of 4mm.

- *Ombrothermal diagram of Bagnouls and Gaussen:*

The ombrothermal diagram of Bagnouls and Gaussen is a graphical method which allows to define the dry and wet periods of the year, where are plotted on the abscissa the months, and on the ordinate the averages (P) expressed in millimeters and the temperatures (T) in degrees Celsius, with p = 2T. A period can be considered dry if p≤2T, that is to say if the total precipitation is less than or equal to twice the average temperature. When the umbel curve passes under thermal curve, it is precisely in this situation where P <2T.

The analysis of the diagram (Fig.1) shows that the dry period is approximately 5 months extending from June until October, while the wet period is about 7 months, extending from November until May.

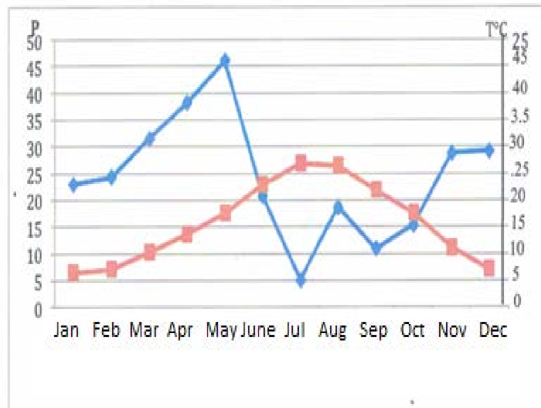


**Fig. 1:** Ombrothermal diagram of Bagnouls and Gaussen (Batna region)

*Climatic data of Djanet*

Table 2: mean climatic Data of Djanet (2004-2014).

| Balance over 11 years | Max Temperature (°C) | min Temperature (°C) | Average temperature (°C) | Precipitation (mm) |
|---|---|---|---|---|
| January | 26.5 | -1.3 | 12.04 | 0.5 |
| February | 29.7 | -1.3 | 15.5 | 50 |
| March | 34.6 | 0.2 | 20.1 | 1.5 |
| April | 35.4 | 10.9 | 25.4 | - |
| may | 40.5 | 15.5 | 29.2 | 0.5 |
| June | 42.6 | 18.2 | 31.6 | 3.3 |
| July | 41.6 | 20.6 | 31.2 | 1.09 |
| August | 41.6 | 20.1 | 31.01 | 0.2 |
| September | 39.5 | 16.5 | 27.1 | 0.4 |
| October | 36.03 | 11.6 | 24.8 | 1.09 |
| November | 31.2 | 7.5 | 18.7 | - |
| December | 27.5 | -0.4 | 13.7 | 0.3 |

- *Temperature:*

Based on the data in the above table, it is noted that the hot season is well marked. The maximum temperature is recorded during June where it reaches 42.3°C and January is the coldest month with a minimal temperature of -1.3°C.

- *Precipitation:*

According to the climate data, the annual precipitation is 58.88 mm where February is the rainiest month with 50mm. The ombrothermal diagram (Fig .2) shows that the dry period is about 11 months. It extends throughout the year (except February)

- *Ombrothermal diagram of Bagnouls and Gaussen:*

as shown in fig 2, the dry period may cover a whole year.

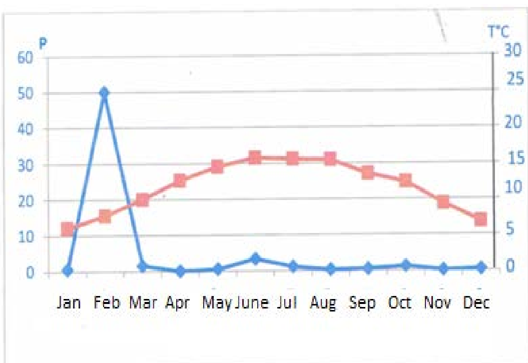


**Fig. 2:** Ombrothermal diagram of Bagnouls and Gaussen (Djanet Region)

*Phytochemical study*

- *Phytoscreening:*

All results of phytochemical analysis are showed in the Table 3

**Table 3:** results of phytochemical analysis

| Chemical class | Results |
|---|---|
| flavonoids | Appearance of a yellow color (+) |
| saponins | Formation of a persistent foam (+) |
| alkaloids | Presence of precipitation (++) |
| tannins | Appearance of a blue coloring black (+) |
| anthraquinones | Confirmed by a turn of the aqueous phase in red (+) |
| quinones | The aqueous phase transfers with the yellow, red or purple(+) |
| coumarins | observation of an intense blue fluorescence(+) |

The hydroalcoholic extracts of the aerial part of the two species showed that they are rich in: flavonoids, quinones, anthraquinones, saponins, tannins, alkaloids and coumarins,

- *Alkaloid content:*

The estimation of the alkaloid content shows that the accumulation of this bioactive substance found in *Hyoscyamus albus* harvested from the Batna region characterized by a semi-arid climate was 0.51%. On the other hand, the alkaloid content in *Hyoscyamus muticus* in Djanet arid zone is only 0.32%, which may be due to the specific characteristics of the species and the influence of climatic conditions.

Many studies dealing with the effect of climatic conditions on secondary metabolites content have been reported. For instance, Khichi et al (2018) investigated Effect of Abiotic Stresses on the Adaptation of Metabolites like Total Phenols, Flavonoids and Alkaloids in Tissue Cultured Plant of *Gloriosa Superba* and they concluded that the temperature was highly responsible for the best adaptation of secondary metabolites in *G. superba* as compared to pH and photoperiods. Kizil et al (2017) studied that effect of different nitrogen doses on some agricultural characteristics and alkaloid content of *hyoscyamus reticulatus* and *hyoscyamus niger* such as plant height, stem diameter, number of branches per plant, number of capsule per plant, capsule width, capsule length, number of seed per capsule, 1000 seed weight, seed yield per plant and total alkaloid content and ended up with the fact that nitrogen doses were important for investigated characters but not important for Hyoscyamus species. Another study carried out by Gümüşçü (2015) studied the Effect of Climate Change on Yield Components of Opium Poppy and found crop poppy is affected extremely weather conditions such as very low temperatures and precipitation. When rainfall is very high amount relatively normal weather conditions at flowering stage, the pollination and then capsule developing is negatively affected and then alkaloid accumulation in capsule, also seeds forming less than the normal. Ghanavi et al (2015) inspected the effect of environmental factors on sanguinarine and berberine levels in roots of *Chelidonium majus* by HPLC- PDA/MS method and established that there is a direct correlation between longitude and altitude with the amount of 25 alkaloids in plant, which means the more the levels of these variables, the more the amount of mentioned alkaloids. 26 In an opposite manner, levels of the alkaloids reversely correlated with latitude coordinates and temperature, in a 27 way that decreasing these variables resulted in raising the amount of the alkaloids.

*Antibacterial activity*

The antibacterial activity of the extract (crude methanolic extract, alkaloid extract) of the aerial part of the two species *Hyoscyamus muticus* and *Hyoscyamus albus* is tested against four bacterial strains via the agar diffusion method. The average of the inhibition diameter was calculated from three replicates. The results that have been obtained indicate that the zone of inhibition is different from one extract to another, from one strain to another. The aromatogram is the result of the study of the sensitivity of a micro-organism to various extracts. It provides information on the bacteriostatic activities of the extracts. The sensitivity or resistance of the bacterium is appreciated by measuring around the disc containing the antibiotic it is the diameter of the zone of inhibition of its growth. The diameter varies with the concentration (or extract) and diffusion power of the antibiotics employed (Andersson and Hughes, 2011).

Table 4: results of Antibacterial activity (diameter of inhibition zone)

| Bacterial strain | oxacilline | hydroalcoholic extract of *H. albus* | Alkaloid Extract of *H. albus* | Hydroalcoholic extract of *H. muticus* | Alkaloid Extract of *H. muticus* |
|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | 13±1.42 | - | 7±1.44 | - | 9±0.7 |
| *Staphylococcus aureus* | 15±0.7 | 10±0.5 | 7±2.12 | 9±1.82 | 8±0.84 |
| *Staphylococcus* subsp. *aureus* | 9±1.32 | - | 8±2.42 | - | 7±2.1 |
| *Escherichia coli* | 13±0.86 | 12±0.7 | 7±1.24 | 12±0.4 | 7±2.42 |

All the tested bacterial strains appeared sensitive to the alkaloid extracts of the two species where the best diameter of the inhibition zone (9+0.7mm) is attributed to the alkaloid extract of *Hyoscyamus muticus* against *Pseudomonas aeruginosa.*

The strains *Staphylococcus* subsp*. aureus* and *Pseudomonas aeruginosa* have a potential of resistance against the antibacterial action of the crude extracts of the two species, on the other hand the strains *Staphylococcus aureus* and E coli are sensitive to the same extracts. The antibiotic proves higher activity on the gram positive bacteria than on the gram negative bacteria

## Conclusion

Phytochemical screening revealed that the aerial parts of both *Hyoscyamus muticus* and *Hyoscyamus albus* plants contain almost all classes of secondary metabolites, including flavonoids, quinones, anthraquinones, saponosides, tannins, alkaloids and coumarins. The quantitative and qualitative study of the extracts of two plants shows that the content of total alkaloids varies from one species to another and from one region to another and from one climate to another. *Hyoscyamus albus* has a higher alkaloid content (0.51%) than that found in *Hyoscyamus muticus* (0.32%). During this study we performed an antibacterial test against some bacterial strains, the results showed that both alkaloid extracts are active. Knowing that Algeria has a huge biodiversity and each plant is characterized by a fairly large reservoir of secondary metabolites with particular therapeutic and pharmacological characteristics that need to be exploited further.

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