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Production of Bioactive Alkaloids from Turkish Geophytes

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Abstract: The variation in climate in different regions of Turkey has resulted in a very rich flora and a long history of many beautiful bulbous plants. Some of them have been cultivated in European and American gardens as an ornamental plant for centuries. A number of them have also some importance for their biological activities. The bulbs of *Galanthus*, *Narcissus* and *Leucojum* have an interest because of their content of galanthamine in order to be use in the treatment of poliomyelitis. In addition, there is a great accumulation of traditional medicine in Turkey. Therefore, medicinal plants have a great potential for producing new drugs of great benefit to human. Phytochemical and pharmacological studies have led to the development of several important compounds as medicinal agents. Turkey exports geophytes for horticultural purposes mainly collected from the wild. The amount of material exported from Turkey has increased each year.

The loss of biological diversity due to human activities has become one of the major problem. It was also directed at the potential permanent loss of unique chemicals which may have enormous importance to people. The sustainable utilization of medicinal plant diversity perform a complex. Saving, studying and using are the three basic elements of the conservation of bioresources. The major task of the parties concerned is to cultivate the awareness among the general public on the importance of conserving the biological diversity and develop it sustainably. For this purpose, the bulbs of exported geophytes have been examined for their bioactive compounds. In this study, the characterization of these compounds of Amaryllidaceae plants along with some regulations to conserve the native flora will be reviewed.

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INTRODUCTION

Several flowering plant families contain species which have underground storage organs such as bulbs, corms and tubers; these plants are known as Geophytes. Most of the bulbous plants in Turkey are known for their ornamental properties depends on their flowers, however a number of them also have important biological activities. Amaryllidaceae is one of the large family in terms of bulbous plants such as *Pancratium*, *Narcissus*, *Galanthus* and *Leucojum* (ref.1). Recently, the bulbs of these plants have increased an interest due to their content of Galanthamine, an alkaloid found to be of use in the treatment of poliomyelitis. On the other hand, Turkey exports Geophytes for gardening purposes. The amount of material exported from Turkey has increased every year (ref.2). The loss of biological diversity due to human activities has become one of the major problem. It was also directed at the potential permanent loss of unique chemicals which may have enormous importance to people. The sustainable utilization of medicinal plant diversity perform a complex. Saving, studying and using are the three basic elements of the conservation of bioresources. The major task of the parties concerned is to cultivate the awareness among the general public on the importance of conserving the biological diversity and develope it sustainably. For this purpose, Turkish government has developed legislative measures for the conservation and sustainable utilization of Geophytes. In this context we aim to search, identify and develop new biologically active constituents of the exported material. To date, some 170 Amaryllidaceae alkaloids have been described, but the structures of half of these have not been established (ref.3). Amaryllidaceae alkaloids exhibit *inter alia* antitumour, antiviral and anticholinergic activities. Some of them have been used in the treatment of myastenia gravis, myopathy and diseases of the nervous system (ref.4). In the aformentioned context, we systematically study uninvestigated Amaryllidaceae plants for the alkaloids, isolated from the bulbs plants of exported geophytes. In this study, the characterization of these compounds from Amaryllidaceae plants along with some biological activities and some regulations to conserve the native flora will be reviewed.

MATERIALS AND METHODS

Plant material

The bulbs of *Galanthus elwesii* Hooker fil., *G. ikariae* Baker *Leucojum aestivum* L. and *Narcissus tazetta* ssp. *tazetta* L. were provided by the export firm known MARLA located near Yalova-Turkey.

Extraction, Isolation and Purification of Alkaloids

The preparation of extracts and fractions along with the purification of alkaloids were obtained using the published methods by one of us previously (ref.5).

General

The melting points were taken on a Buchi 535 melting point apparatus. a polatronic D Polarimeter was used for measuring the optical rotations. The IR spectra were recorded on a Jasco IRA-1 infrared spectrophotometer. The ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Brucker AM 400 NMR spectrometer at 400 MHz. The mass spectra were recorded on a Varian MAT 312 double-focussing spectrometer connected to a PDP 11/34 computer system.

RESULTS

The family of Amaryllidaceae is represented by 6 genera in Turkey and this constitutes a large family of very ornamental bulbous plants (ref.6). We systematically studied uninvestigated Amaryllidaceae plants growing in Turkey, e.g. Pancratium maritimum L. previously investigated by us. Extensive chromatography of the extracts afforded alkaloids belonging to the skeletally different groups of the Amaryllidaceae alkaloids have reported (ref.7-11). Lycorine has been found as one of the major alkaloid from Amaryllidaceae plants. Therefore, it content has been determined by HPLC. For this purpose, HPLC analysis was performed on column packed with C-18 bonded silika gel operated in reversed phase mode, using chloroform-methanol (9:1) as mobile phase. Lycorine was determined at 290 nm using photodiod-array detector (ref.12). Lycorine amounts determined from the bulbs of these plants were given as follows;

Galanthus elwesii	0.011 %
G. ikariae	0.043 %
Leucojum aestivum	0.078 %
Narcissus tazetta ssp. tazetta	0.089 %

From the air-dried and powdered bulbs, the alkaloids were extracted by routine acid-base work-up procedure. Isolation and purification studies have afforded (-)-lycorine (ref.5) along with six other major Amaryllidaceae alkaloids obtained for the first time from these plants.

Alkaloids of *Galanthus elwesii*

From the spectral data, (-)-buphanisine, (+)-9-o-demethylhomolycorine, (+)-haemantamine, (-)-galanthamine, (+)-3-epihydroxybulbispermine and (+)-tazettine have been isolated from the bulbs of *Galanthus elwesii.* (ref.7,13-15).

Alkaloids of *Galanthus ikariae*

From the spectral data, (+)-9-o-demethylhomolycorine, (-)-galanthamine, (+)-3-epihydroxybulbispermine and (+)-tazettine have been isolated from the bulbs of Galanthus ikariae (ref.7,13,14).

Alkaloids of *Leucojum aestivum*

From the spectral data, (-)-galanthamine and (+)-3-epihydroxybulbispermine have been isolated from the bulbs of *Leucojum aestivum* (ref.13).

Alkaloids of *Narcissus tazetta* ssp. *tazetta*

From the spectral data, (-)-buphanisine, (+)-9-o-demethylhomolycorine, (+)-haemantamine, (-)-galanthamine, (+)-3-epihydroxybulbispermine and (+)-tazettine have been isolated from the bulbs of *Narcissus tazetta* ssp.*tazetta* (ref.7,13-15).

Antibacterial activity

Antibacterial activity studies were performed by the agar well diffusion method using Mueller Hinton Agar medium(16). The ethanolic extracts of *Narcissus tazetta* ssp. *tazetta* and *Leucojum*

aestivum at 200 mg/100 mL concentrations have shown antibacterial activity against *Staphylococcus aureus, Pseudomonas pseudomolli, Vibrio cholerae, Enterobacter cloacea, Corynebacterium hoffmanni, C. diphteriae and Salmonella typhi* using ampicillin (30 mg/100 mL) and Tobramicin (10 mg/100 mL) as standard antibiotic.

Antifungal activity

Antifungal activity of the ethanolic extracts of these plants was measured by the tube dilution method (ref.17). A solution of the each extracts in DMSO was added to molten Sabouraud dextrose Agar (SDA) to prepare slants. The slants were inoculated with the fungi and incubated at 29°C for seven days. Inhibition of the growth was observed, and MIC values were determined against a standard antifungal compound (griseofulvin) on the 8 days. Four of the plant extracts have shown significant activity against *Nigrospora oryzae, Microsporum canis, Pleuralus ostreatus, Curvularia lunata* and *Trichophyton longifusus.* Besides, the extract of *Narcissus tazetta* ssp. *tazetta* has shown significant activity against *Drechsleza rostrata, Aspergillus niger, candida albicans, Pleuralus ostreatus* and *Alefcheria boydii.*

Antiplatelet activity

The antiplatelet effect of these extracts against human platelet aggregation induced by arachidonic acid, collagen and platelet activated factor have been examined. All plant extracts as well as lycorine showed complete inhibition on platalet aggregation caused by AA,collagen and PAF. In addition, the extracts of *Narcissus tazetta* ssp. *tazetta* and *Leucojum aestivum* along with lycorine showed inhibition on the contraction of rat aorta caused by high potasium and norepinephrine. This predicts that ethonolic extract of this plant may contain calcium-channel blocking agents.

Antimalarial activity

Galanthamine and lycorine exhibit cytotoxic activity *in vitro* against fibroblastic murine non-tumour cells. It has been noticed that some compound possessing cytotoxic activity will also possess antimalarial activity. For this purpose, the activity of Amaryllidaceae alkaloids and the extracts of Amaryllidaceae plants was studied *in vitro* against a chloroquine resistant (K_1) strain of *Plasmodium falciparum*. using mefloquine and chloroquine as standard. The IC_{50} values were calculated. The extract of *Narcissus tazetta* ssp. *tazetta* and *Leucojum aestivum,* lycorine, tazettine and galanthamine were found to exhibit antimalarial activity as predicted.

However, galanthamine has less potency than others as well as standards.

Insecticidal activity

Insecticidal activity of the extracts of *Galanthus elwesii, Leucojum aestivum* and *Narcissus tazetta* ssp. *tazetta* were recorded as 90 % or greater mortality within six days against Milkweed bug (ref.18).

Conservation of Geophytes in Turkey

Biodiversity has to be thought of in a number of ways such as evolutionary tension between insects and plants and contribution to human society. Therefore, biodiversity has to be indexed, used and reserved. Natural products are played an important role in world pharmaceutical

market. The loss of biological diversity due to human activities has become one of the major problem. It was also directed at the potential permanent loss of unique chemicals which may have enormous importance to people. The sustainable utilization of medicinal plant diversity perform a complex. The major task of the parties concerned is to cultivate the awareness among the general public on the importance of conserving the biological diversity and develope it sustainably. Turkey exports bulbs for horticultural purposes mainly gathered from the wild collection. The amount of material exported from Turkey has increased continuously each year. Since the majority of these bulbs are taken from the wild, the Turkish government has recently limited the export to some extent in order to conserve the native flora. The cultivation of these bulbs has also started in Turkey. For this purpose, Turkish government has developed legislative measures for the conservation and sustainable utilization of Geophytes. The most important of the developments are:

- the strengthening of collection and export regulations to bring them in line with the *"Convention on the International Trade Endangered Species of Wild Fauna and Flora"* (CITES) terminology and
- improvements in the propagation of these bulbs.

In addition, the exportation of bulbs either form wild collected or artificially propagated sources has been significantly limited.

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