ANATOMICAL AND PHYTOCHEMICAL STUDY OF *LILIUM LEDEBOURII* (BAKER) BOISS., A RARE ENDEMIC SPECIES IN IRAN

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ABSTRACT

Lilium ledebourii (Baker) Boiss.(Liliaceae), locally named "Susan -e Chelcheragh" is a native and rare species grown on the heights of Damash region (ca. 2100 m) in Gilan province, north part of Iran.The microscopic and anatomical features and the composition of oils of flower and corm of this unique plant were studied. The microscopic study has shown the main characteristic elements of leaf, stem, corm and flower of this plant. The composition of essential oils of flower and corm were determined by coupled GC-MS analysis. The yields of oils of flower and corm were 0.71 % and 1.65 % (v/w) respectively. The major components of flower's oil were isopulegol (55.15 %), pentacosane (18.1%), 3-methyltricosane (9.97%), tricosane (5.35%), 2-methylpentacosane (4.35%), docosane (4.28%) and linalool oxide (2.20%). The components of corm's oil were almost fatty acids. No aromatic volatile compound was found in the corm oil. Primary qualitative phytochemical tests of stem, leaf, corm and flower showed positive results for alkaloid and flavonoid (one plus) in stem and for saponin (4 plus) in corm and (2 plus) in the flower. Tests for tannin in all parts were negative. Further phytochemical and botanical studies on this unique plant is of importance.

Keywords: *Lilium ledebourii*, Anatomy, Phytochemistry, Esssential oil, Iran.

INTRODUCTION

Lilium ledebourii (Baker) Boiss (Liliaceae), locally named "Susan–e–chelcheragh", is an endangered rare species endemic to Iran. This plant is growing natively on the heights (ca 2100m) of "Damash" in the Gilan province, north part of Iran (Fig. 1) and it is under careful surveillance of regional Environmental Protection Agency (EPA).

It is a perennial plant with lanceolate yellowish scales and thick stem, 50-150 cm high. Leaves are erect, linear-lanceolate 10-14× 1-2 cm, papillar on the nerves in lower surface and ciliate margined. Flowers are white, large raceme with 2-15 flowered, pedicel up to 13 cm long which ascending, or spreading-reflexed. Bracts are lanceolate, blue at summit. Flowering period is commonly in July. Fruits are in capsule form 30-45 x 25-32 mm, erect and obovate angular almost 6-angled (1-2).

Literature search showed that no investigation has so far been carried out on the anatomy and phytochemistry of this species, which is mostly due to the specific characteristics of this plant. However, numerous reports were found on the phytochemistry of other species of *Lilium* genus which were almost entirely related to the bulbs of these species. Isolation of steroidal saponins (3-10), phenolic glycosides (6), flavonoid alkaloid (11,12) pyrroline–pyrrolidine alkaloids (13) from the bulbs and antho-cyannins from flowers of several cultivars (14) for certain species of *Lilium* genus have been reported.

Bibliography of the Iranian old medical and botanical books revealed that what are used traditionally under the general name of " Susan" do not belong exactly to the *Lilium* genus, but are rather related to the *Iris* genus (15-17).White susan (probably *L. candidum*) is described in the well known book of Avicenna, " The Canon of Medicine" (16). The bulbs, leaves and oil of the flower of this herb are considered to be useful for burns, injuries, inflammation and uterus disorders (16).

The fresh and dried bulbs of *L. candidum* have

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been recently suggested to be useful in gynecological disorders, ulcer, burns, injuries and may be used as a diuretic (18). Furthermore, the bulbs of several other *Lilium* species exhibited a wide spectrum of biological activities (19).

The aim of this study was to carry out, may be for the first time, a phytochemical and anatomical investigation on this rare plant in order to enrich scientific information of this species.

MATERIALS AND METHODS

Plant Materials: Plant samples in flowering stage were collected early in the morning of July 2001 and 2002 from "Damash" in Gilan Province, north of Iran. Flowers and corms of the samples were separated and kept in a flask in contact with ice and then were immediately transferred to laboratory for oil analysis. A voucher specimen (No. 6524) was deposited at the herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences. Classification and identification was done by one of the authors (Gh. Amin).

Instrumentation: The GC-MS analyses were performed using a Hewlett-Packard (HP-5973) instrument. The data were obtained on a 5% Methyl silicone fused silica capillary column (HP-5), 30 m x 0.25 mm i.d. (film thickness 0.25 µm) installed in a Hewlett-Packard 6890 gas chromatograph. Operation conditions were: carrier gas: He, linear velocity 32 Cm/s, column oven temp. prog. 60°C (2 min), 60-240°C at 4°C/min; sample injection port temp. 250°C; jet separator temp. 250°C; ionization voltage: 70 eV; ionization current: 60 µA; scanning speed 1s over 30-400 amu range; split injection system, 1:100.

Microscope: Leitz microscope equipped with photographic apparatus with lenses providing a range of 10X to 40X magnifications and different filters was used.

Methods

Sample preparation: Samples of leaf, stem, corm and flower were prepared by selecting each part, air-dried in shade, powdered and passed through a 70-mesh sieve and kept into light-protected tight container. Analysis of essential oils of flower and corm were performed on fresh samples collected as described above.

Anatomical study: Macroscopic and microscopic examinations were performed according to the reported procedures (20,21). A quantity of each sample was softened before preparation, made translucent by clarifying agents (20), examined under microscope and photographed. Macroscopic identity was performed by visual inspection and comparison with authentic samples.

Oil Isolation: The essential oils of fresh flower were obtained by hydrodistillation for 3 h using a modified Clevenger type apparatus. The isolated colorless oil was dried over anhydrous sodium sulfate and preserved in a sealed flask at –12 °C until the time of analyses.

Due to the high saponin content of the root the oil of the corm was not obtained by hydrodistillation, a quantity of chopped corm (150g) was treated with petroleum ether for 25 hr, and then the oil fraction was separated by distillation at reduced pressure. The final product was tested by TLC and analyzed by GC and GC-MS.

Identity Tests: Identification of major secondary metabolites (alkaloid, flavonoid, saponin, tannin) were performed on 5g air-dreid samples of stem, leaf, corm and flower according to the described methods (21,22).

RESULTS

Anatomical characterization

Macroscopic inspection: Leaf powder was rough and green with no distinctive taste and odor like dried alfalfa and was insoluble in water. Root powder was smooth and cream, with bitter taste and odor like dried alfalfa and was insoluble in water. Flower powder was yellowish white, slightly minty and herbaceous and bitter sweet taste.

Microscopic characters: The results obtained by microscopic inspection for leaf, corm, flower and pollen are presented in fig. 2-4 as follow:

Leaf revealed scalariform parenchyma cells, anomocytic types of stomata, unicellular gland trichomes and lignified spiral vessels (fig. 2 A-D). Corm presented lignified spiral vessels, scalariform and thick-walled parenchyma cells, parenchyma cells with twisted wall and starch granules (fig. 3 A-D). Flower had spiral vessels, scalariform and thin-walled parenchyma cells and large anomocytic types of stomata (fig. 4 A-D). Pollen grains found with different seizes (fig. 4 D).

Phytochemical results

The identity tests of above mentioned major metabolites were positive (+1) for alkaloid and flavonoid in stem sample, saponin were

positive for corm (4+) and Flower (2+) samples respectively. Tannin was negative in all samples. The yields of the oils from flower and corm were 0.71% and 1.60% v/w respectively. The percentage and oil constituents of flower are listed in table 1.

Table 1. Identity test of stem, leaf, corm and flower of *Lilium ledebourii*

	S	A	F	T
Stem	-	+	+	-
Leaf	-	-	-	-
Corm	++++	-	-	-
Flower	++	-	-	-

S: Saponin, A: Alkaloid, F: Flavonoid, T: Tannin

Table 2. Chemical composition and retention index (RI) of flower oil of *Lilium ledebourii* determined by GCMS

Compound	RI	Percentage
Linalool oxide*	1098	2.28
Isopulegol	1146	55.15
Docosane	2200	4.28
Tricosane	2300	5.35
3-methyltricosane	2374	9.97
Pentacosane	2500	18.01
2-methylpentacosane	2564	4.35

* Correct isomeric form not identified.

As seen in table 1, seven compounds representing ca 99.39% of the essential oil of flower were identified. Identification of components were based on comparison of their mass spectra with those published data in the literatures (23, 24), and by computer search of their 70 eV mass spectra with those stored in the library of the GC/MS data system, as well as by Kovat's retention indices. Kovat's retention indices were determined by injection of the homologous n-alkenes series (C6-C15) following the oil injection.

The most prominent components of flower's essential oil were: isopulegol (55.15%), pentacosane (18.1%), 3-methyltricosane (9.97%), tricosane (5.35%), 2-methylpentacosane (4.35%), docosane (4.28%) and linalool oxide (2.28%).

The components of corm oil were almost fatty acids including linoleic, oleic, palmitic, as well as sterols (pimaric acid). No volatile compounds were found in the oil of the corm.

DISCUSSION

Lilium ledebourii (Baker) Boiss is a rare endemic species grown on the height of Damash in north part of Iran. The phythochemical tests (Table 1) showed high quantity of saponins in corm and lower concentration in flowers. Saponins have exhibited a wide spectrum of biological activities (3), such as cholesterol lowering, interaction with steroidal hormone metabolism, digestive difficulties and others (25).

The components of the essential oil of flower showed seven compounds. No aromatic compound was identified in the essential oil of flower.

Isopulegol which consists about 55% of the essential oil of this plant has been reported as a coolant agent with a minty and herbaceous odor. The slight odor of flowers of this plant was approximately similar to the odor of isopulegol (26). It might be of value to mention that the highly purified (-)-isopulegol enantiomer, which imparts a feeling of freshness and has been used in sensate mixtures is odorless (27). Furthermore, (1R,2S,5R)-isopulegol and its carbonylated products have shown significant bactericidal activities (28). Pentacosane, the second major oil (18.01%) of flower of *L. ledebourii* is a hydrocarbon, which isolated from other plants such as Valeriana spp (29), Rosa species (30) and from honey (31).

Analysis of corm's oil did not show any aromatic or volatile essential oil, but there were some fatty acids. Identification of fatty acids was not in the scope of this investigation; hereby we just reported what was presented by GC-MS.

Microscopic characters of this plant, which is given probably for the first time, will enrich certainly the botanical information on this species.

Theses information put forth the importance of further phytochemical and botanical studies on this unique plant in spite of limited distribution of this plant.

ACKNOWLEDGEMENT

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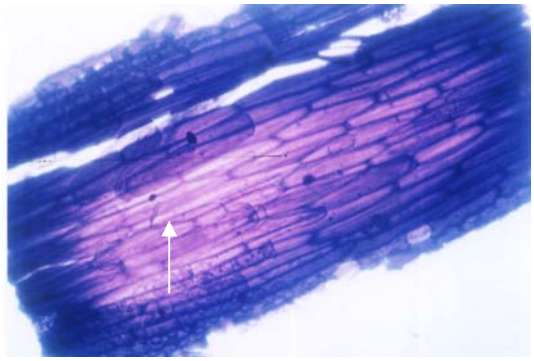
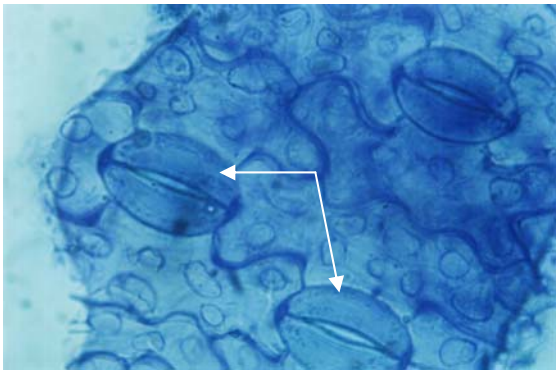
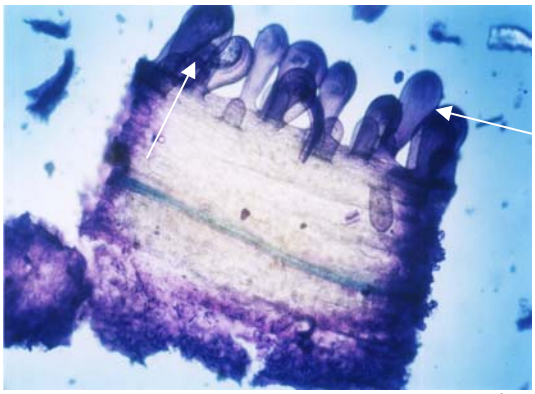
Fig. 1. Flowering aerial parts of *Lilium ledebourii* Boiss. (Susan -e Chelcheragh)



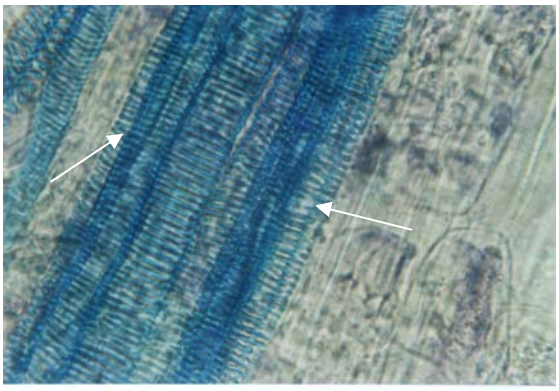
A. Scalariform parenchyma cell (X 40)



B. Anomocytic stomata (X 40)

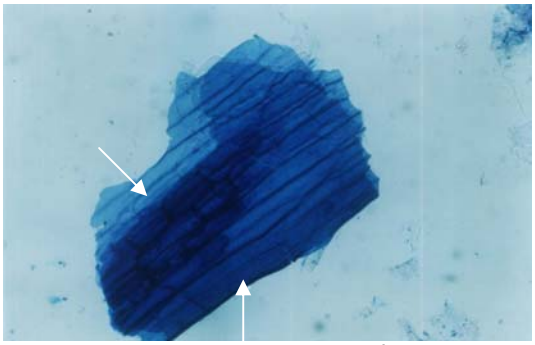


C. Epidermis unicellular glandular trichomes (X 10)

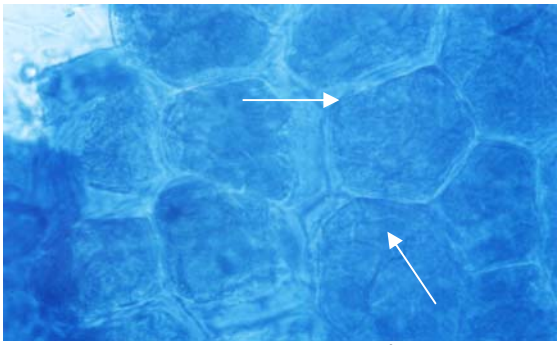


D. Lignified spiral vessels (X 40)

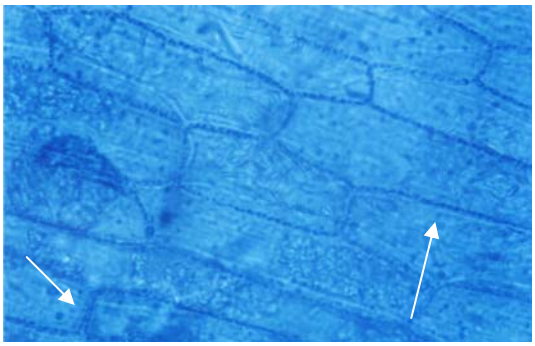
Fig. 2. Anatomical microscopic structure of *Lilium ledebourii* leaf.



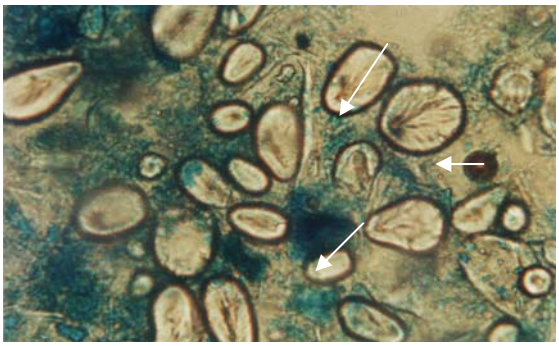
A. Scalariform parenchyma cells (X 10)



B. Thick-walled parenchyma cells (X 40)

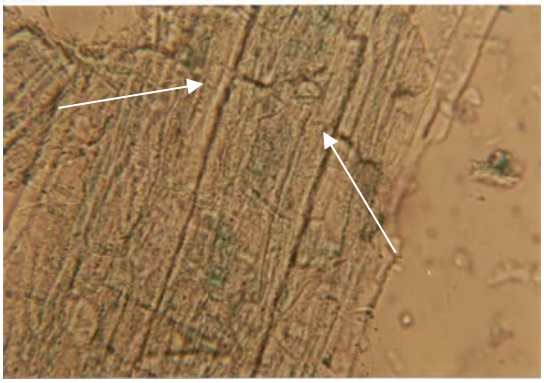


C. Parenchyma cells with twisted walls (X 40)

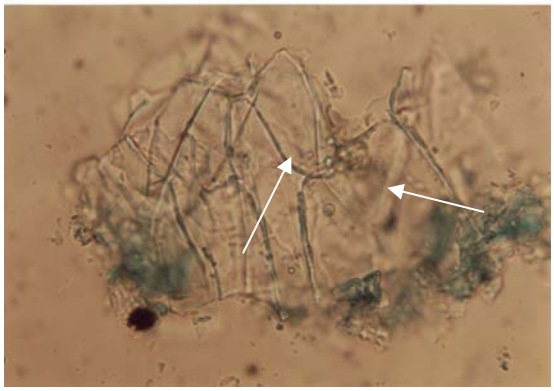


D. Starch granules (X 40)

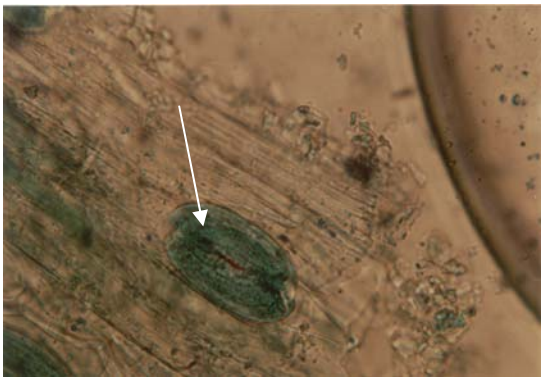
Fig. 3. Anatomical microscopic structure of *Lilium ledebourii* corm.



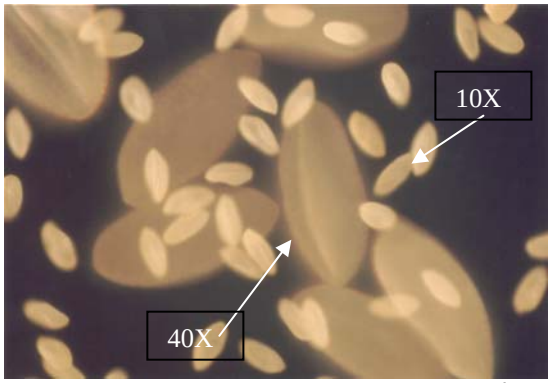
A. Scalariform parenchyma cells (X 40)



B. Thin-walled parenchyma cells (X 40)



C. Large anomocytic type of stomata (X 40)



D.. Pollen grains with various seizes, (X 10 & X 40)

Fig. 4. Anatomical microscopic structure of *Lilium ledebourii* flower and pollen.

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