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Original article

Pharmacognostic and phytochemical investigation of *Heracleum persicum* Desf. ex Fischer

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Abstract

Background and objectives: *Heracleum persicum* aerial parts have been used in Iranian folk medicine for their therapeutic activities and as a spice and flavoring agent in Iranian foods and pickles. The present research was conducted to evaluate the pharmacognostic profile of *H. persicum* which will be useful in standardization for quality, purity, and sample identification. **Methods**: For quality control, phytochemical and physicochemical parameters such as macroscopic and microscopic evaluations, loss on drying, total ash value, acid insoluble ash, fluorescence standards of the drug and other tests were carried out using the flowers, fruits, stems and leaves of the plant. **Results**: Light microscopic studies showed various characteristic features including, collateral vascular bundle, oil ducts (vittae) and uniseriate, unicellular trichomes in leaf; collateral and closed vascular bundles, vittae and kidney shaped strands of collenchymatous tissue in stem; parenchymatous cells, commissural and solitary vittae in fruit; and tapetum tissue, pollen grains and fragments of stamens and styles in flower. Phytochemical screening of the aerial plant parts revealed the presence of flavonoids, tannins and steroids. **Conclusion**: The pharmacognostic characters observed in this study will be helpful in correct identification and characterization of *H.persicum*. Preliminary phytochemical studies may be helpful in further isolation and purification of lead compounds.

Keywords: *Heracleum persicum*, pharmacognostic, phytochemical, standardization

Introduction

The genus *Heracleum* L. (Apiaceae) comprises 65-120 species, with the Caucasus and the Sino-Himalayan Mountain Region being its two major centers of diversity [1]. Based on the Flora Iranica, this genus is represented by 10 species in Iran, 4 of which are endemic [2].

Heracleum persicum Desf. ex Fischer commonly known as Persian hogweed or "golpar" in Persian, is an aromatic medicinal plant native to Iran, Turkey and Iraq [2,3]. The plant is widely distributed in Iran, particularly over the northern parts of the country which has humid climate [4-6]. Its fruits and young stems are commonly used in Iranian food and pickles as a spice and flavoring agent. The fruits have also been used in Iranian folk medicine as a carminative, digestive, analgesic and antiepileptic drug [7,8].

Pharmacological screening has shown various activities for this species including anticonvulsant

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[9], antioxidant [10], anti-inflammatory [11] and Immunostimulatory effects [12].
Available literatures revealed that, there is no extensive pharmacognostic study about the aerial parts of the plant till date. Considering the importance of this plant, the present work has attempted to bring out the pharmacognostic features of *H. persicum*, which will assist in standardization for quality, purity and sample identification.

Experimental

Plant material

The aerial parts of *H. persicum* were collected from Tarom, Zanjan, Iran, in May 2014. Voucher specimen (ZUMS-4003) was deposited at the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran. Fresh plant materials (fifteen samples) were used for the microscopic study, while some part of the plant was shade dried and subjected for 60 Mesh [13] powdering for further studies.

Macroscopy

Various organoleptic and macroscopic characters of the plant material such as colour, odour, size, shape and taste were recorded [14,15].

Microscopy

Cross sections of fresh samples were made by hand, cleared with chloralhydrate solution, stained with phloroglucinol-hydrochloric acid (1:1) and toludine blue and mounted in glycerin medium after staining [16,17]. The sections were examined using ZEISS KF2 light microscope using 100×, 400×, and 1000× magnifications and with Sony DSC-W35 camera [18].

Powder microscopic examination

Shade dried, coarsely powdered herbal drug was examined for stomata, covering trichomes, sclerenchyma, collenchyma, epidermal cells, vascular strand and other following standard procedures [15,19].

Phytochemical investigation

For phytochemical screening, 250 g of each powdered plant samples were extracted successively with analytical grade petroleum ether (60-80°), ethyl acetate and methanol using Soxhlet apparatus. All the extracts were concentrated under reduced pressure in IKA-RV05 rotavapor. Qualitative screenings for the presence of various phytochemical compounds such as alkaloids, anthraquinones, saponins, flavonoids, sterols and cyanogenetics was performed according to the standard methods [13,15,18].

Physicochemical investigations

Plant (stem, leaf, flower and fruit) powders were used for determination of various physicochemical parameters such as ash values, extractive values, loss on drying, etc., according to the WHO guidelines [20].

Fluorescence analysis

Fluorescence study of plant powder was performed under ultraviolet (UV) light (254 nm and 365 nm) and after treatment with different reagents as per reported procedure [15,22]. The changes in appearance and color were observed and recorded.

Results and Discussion

According to WHO, the macroscopic and microscopic evaluation is a reliable, cost effective and time saving method to establish the right identification of the herbal material [20,21].
Macroscopically, the stems were shortly pubescent, angular, hollow, grooved, and red-brown from base. The leaves were alternate, base sheath-like, deeply divided with sharp points, pinnately lobed with 2-3 pairs of lateral leaf segments and less deeply serrates. Upper leaves were progressively smaller, with the upper leaf surface glabrous and the underside pubescent.
The fruits of *H. persicum* had 2 homomorphic mericarps that were compressed and covered by uniseriate, unicellular trichomes. Fruits were polachenarium, obovoid to ellipsoid, flattish, five

dorsal and three ventral intermediate filiform ridges, ventral side with 2 vittae up to half of the mericarp and slightly club-shaped. The vittae on the dorsal side of mericarps (n=4) were longer and narrower than those on the ventral side. The Surface was dull, yellowish light-brown and vittae were blackish-brown (figure 1).

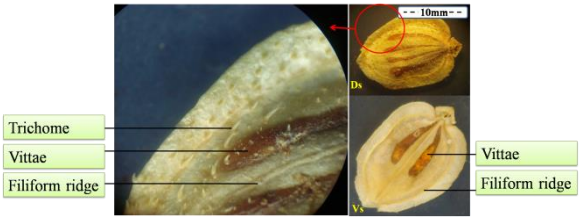

Figure 1. *H. persicum* fruit

The plant showed flowers with 5 petals, 5 stamens, pistil of 2 fused carpels and small vestigial sepals. The flowers were white-pale lime green, arranged in convex umbels with 30 to 50 rays, the side umbels were rather small compared with the main umbel (figure 2). The whole plant smelled of aniseed with acrimonious taste.

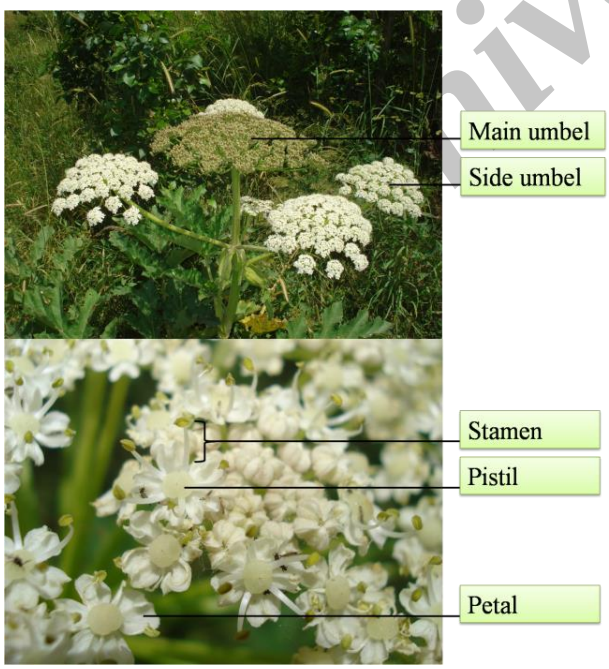

Figure 2. *H. persicum* flowers

Transverse section of the stem was somewhat circular with undulate outline. The epidermis consisted of single layered, thick walled squarish cells with a thin layer of cuticle followed by 3-5-layered cortical cells. The ground tissue contained wide thin walled compact angular parenchyma cells. The angles of the stem were occupied by kidney shaped strands of collenchymatous tissue. Some oil ducts of secretory tissue were located immediately below these collenchymatous strands and the others were scattered throughout the stem with no particular arrangement. The vascular tissue was arranged circularly in two series of concentric vascular strands, consisting of cylinders of xylem produced towards the inside and cylinders of phloem outwards, which was enclosed by a thin band of sclerenchyma elements Uniseriate, unicellular trichomes were present on the upper epidermis (figure 3).

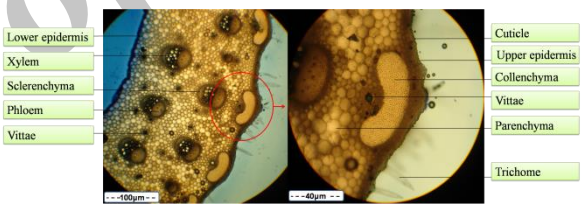

Figure 3. Transverse section of stem of *H. persicum*

The leaf had thick, adaxially and abaxially projecting midrib and thin dorsiventral lamina. The adaxial part of the midrib was prominent and pyramid like and the abaxial part looked like a heptagon. The epidermal layer of thin midrib consisted of square to rounded, thick walled cells with thick cuticle. The inner part of the adaxial cone and angles of abaxial part included a cluster of angular collenchyma thick walled cells. Beneath the collenchymatous tissue, oil ducts (vittae) were typically seen at the corners of abaxial part. The ground tissue around the vascular structure was spongy parenchyma. The cells were wide thin walled, angular and compact. In the midrib, the vascular tissue was found in a series of biconvex vascular strands, consisting of a cluster of wide circular thin

walled xylem elements produced towards the inside and a thick band of phloem elements outwards. Uniseriate, unicellular trichomes were mostly present on the underside (figure 4).

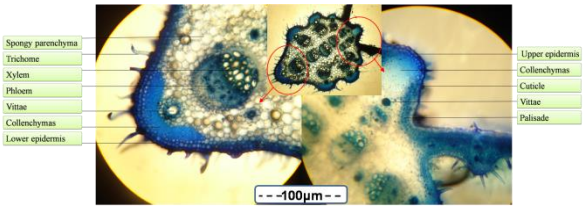

Figure 4. Transverse section of leaf through midrib of leaf of *H. persicum*

In transverse section of fruit, the commissural area between the two mericarps composed of parenchymatous cells and a central carpophore. There were two commissural vittae as well as four solitary vallecular vittae in each mericarp. Unicellular trichomes were present on dorsal side. Transverse section of mericarp structure is given in figure 5.

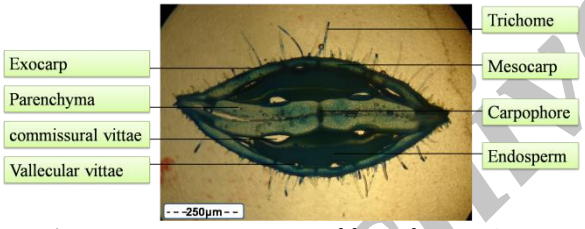

Figure 5. Transverse section of fruit of *H. persicum*

Powder microscopy of the stem showed the presence of lignified elongated fibers and spiral and pitted xylem vessels. The epidermis composed of longitudinally elongated cells with scattered anisocytic stomata and unicellular uniserriate covering trichomes (figure 6).

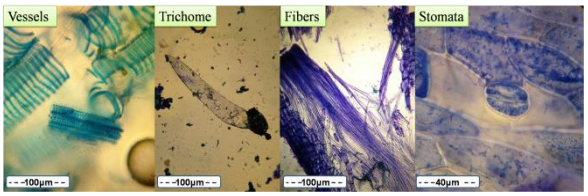

Figure 6. Microscopic characteristics of stem powder of *H. persicum*

The powder drug microscopy of leaves showed the following diagnostic characters: The upper epidermis composed of rather irregularly shaped cells with slightly thickened walls (figure 7A). The lower epidermis composed of cells with thinner, sinuous walls. The anisocytic stomata and unicellular uniserriate covering trichomes were present on both the lower and upper epidermis (figure7b). The underlying palisade cells were fairly large and loosely. Spiral xylem vessels usually occur in small groups (figure 7C).

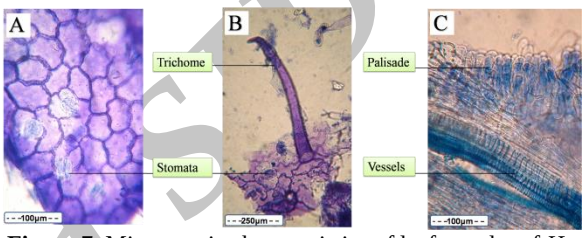

Figure 7. Microscopic characteristics of leaf powder of *H. persicum*

The powdered flower of *H. persicum* under microscopic investigation showed the presence of upper and lower epidermis of the corolla. The lower epidermis mainly composed of thick-walled polygonal cells, striated cuticle and anisocytic stomata. The upper epidermis composed of thin walled cells which were usually markedly sinuous in outline. The cells of the lower and upper epidermis were papillose. Small fragments of stamens and styles were frequently seen in the powder. The epidermis of filament fragments composed of elongated rectangular cells. The presence of pollen grains and tapetum tissue were as distinguished diagnostic features of the powder. The anomocytic stomata and unicellular uniserriate covering trichomes are observed on the style epidermis (figure 8).

The yellowish-green colored powder showed the groups of sclereids from the sclerenchymatous layers of the mesocarp which were usually longitudinally elongated cells with moderately thickened walls. The covering trichomes, which were nearly always found detached from the epicarp were conical, slightly curved and

unicellular. The fairly numerous orange-brown fragments of the vittae were a diagnostic feature of the powder (figure 9).

The types and content of phytochemical constituents of the plant can be concluded from physiochemical reactions which gives an indication of the pharmacologically active metabolites present in the plant [24].

Qualitative analysis of stem, leaves, flowers and fruits of *H. persicum* showed the presence of various phytochemicals. Flavonoid contents were detected in ethyl acetate and methanol extracts of the stem, leaves, flowers and fruits.

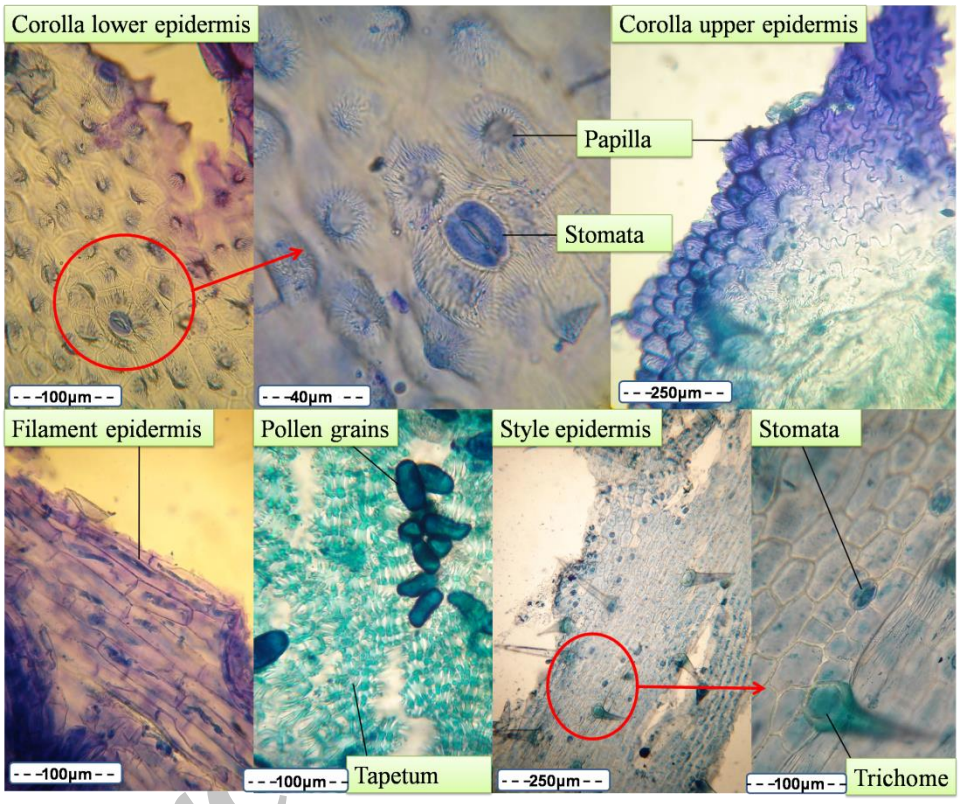

Figure 8. Microscopic characteristics of powdered flower of *H. persicum*

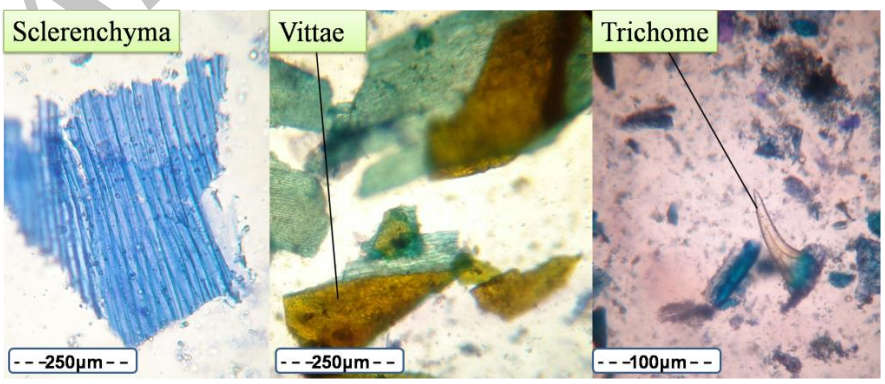

Figure 9. Microscopic characteristics of powdered fruit of *H. persicum*

Analysis also showed the presence of fixed oils, fats and steroids in the ethyl acetate and petroleum ether extracts. Tannins were detected in methanol extract of all four samples. Carbohydrates, alkaloids, anthraquinones, cyanogenetics and saponins were absent in samples (table 1). The Physicochemical parameters like ash value, fluorescence analysis and extractive values can be a reliable aid to detect adulteration. The results of fluorescence analysis are given in table 2.

Table 1. Phytochemical screening of *H. persicum* extracts

Phytoconstituents	Name of test	Methanol extract				Ethyl acetate extract				Petroleum ether extract			
		Fl	Fr	L	S	Fl	Fr	L	S	Fl	Fr	L	S
Carbohydrates	Molisch test	-	-	-	-	-	-	-	-	-	-	-	-
	Fehling's test	-	-	-	-	-	-	-	-	-	-	-	-
	Iodine test	-	-	-	-	-	-	-	-	-	-	-	-
	Gums test	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	Ferric chloride test	+	-	-	-	-	-	-	-	-	-	-	-
	Lead acetate test	+	+	+	+	-	-	-	-	-	-	-	-
	Gelatin test	+	+	+	+	-	-	-	-	-	-	-	-
Alkaloids	Wagner's test	-	-	-	-	-	-	-	-	-	-	-	-
	Dragendorrf's test	-	-	-	-	-	-	-	-	-	-	-	-
	Mayer's test	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinone	Borntrager's test	-	-	-	-	-	-	-	-	-	-	-	-
	Modified Borntrager's test	-	-	-	-	-	-	-	-	-	-	-	-
Cyanogenetics	picrate test	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	Shinoda test	+	+	+	+	+	+	+	+	+	-	-	-
Saponins	Foam test	-	-	-	-	-	-	-	-	-	-	-	-
Fixed Oils & Fats	Spot Test	-	-	-	-	+	+	+	+	+	+	+	+
Steroids	Liebermann Burchard Test	-	-	-	-	-	+	+	+	-	+	+	+

Fl: flower, Fr: fruit, L: leaf, S: stem

Table 2. Fluorescence analysis of *H. persicum*

Reagent	Day light				Short wave (254 nm)				Long wave (365 nm)			
	Fl	Fr	L	S	Fl	Fr	L	S	Fl	Fr	L	S
Powder	Hoary	Y. green	Green	Y. green	Y. orange	Y. green	Go. Yellow	Go. Yellow	Yellow	G. Blue	Go. Yellow	P. Yellow
Powder+1N H_2SO_4	Yellow	B. green	P. Brown	G. Brown	Orange	Y. orange	D. Green	D. Green	F. Yellow	D. green	Green	G. brown
Powder+1N HNO_3	Y. green	Y. brown	G. Brown	Brown	Orange	G. brown	G. brown	D. Green	Y. green	Brown	P. Brown	Brown
Powder+1N HCl	Y. green	Green	G. Brown	Y. Brown	Y. orange	Green	F. Citrine	G. brown	Y. green	G. blue	G. brown	G. brown
Powder+1N NaOH	Yellow	Brown	Y. green	Yellow	F. Yellow	Y. brown	G. brown	P. Brown	Y. orange	Y. brown	D. green	Y. brown
Powder+$FeCl_3$ 5%	Y. green	G. blue	Y. green	D. green	Y. green	G. black	D. green	D. green	Green	Black	G. blue	D. green
Powder+ Ammonia	Y. orange	C. green	Yellow	Yellow	F. Orange	G. black	Green	Green	Y. orange	Brown	G. brown	Brown

B: blackish, C: citrine, D: dark, Fl: flower, Fr: fruit, G: greenish, GO: golden, L: leaf, P: pale, S: stem, Y: yellowish.

Table 3. Physicochemical evaluation of *H. persicum*

Particulars	(% w/w)			
	Flower	**Fruit**	**Leaves**	**Stem**
Total ash	2.21 ± 0.127	9.31 ± 0.311	9.87 ± 0.251	10.24 ± 0.165
Acid insoluble ash	0.06 ± 0.001	0.12 ± 0.041	0.17 ± 0.058	0.14 ± 0.068
Water soluble ash	0.21 ± 0.036	1.41 ± 0.421	2.32 ± 0.211	2.97 ± 0.281
Methanolic soluble EV.	29.57 ± 0.412	27.23 ± 0.524	31.89 ± 0.436	14.14 ± 0.267
Water soluble EV.	14.32 ± 0.137	11.12 ± 0.641	10.24 ± 0.381	9.45 ± 0.234
Hexane soluble EV.	16.32 ± 0.451	6.81 ± 0.391	11.76 ± 0.217	8.81 ± 0.243
Loss on drying at 105°C	6.14 ± 0.246	9.81 ± 0.317	8.93 ± 0.457	7.23 ± 0.416

±: Calculated as SEM of three readings

The moisture content of herbal drug is directly related to chance of microbial growth, chemical deterioration in sample materials and consequently with the less shelf life of crude drug [19,23,24].

Physicochemical parameters of plant (flower, fruit, leaf and stem) powders are shown in table 3. The ash value was determined in three forms viz., total ash, water soluble ash, and acid insoluble ash. The total ash and acid insoluble ash give an idea of inorganic composition and other impurities present along with drug [19,22].

As shown in table 3 the total ash values ranged from 2.21% ± 0.127 (for flower) to 10.24% ± 0.165 (for stem). The most water soluble ash value was 2.97% ± 0.281 for stem powder which indicated the presence of cellulosic substances and the high acid insoluble ash values for leaves (0.17% ± 0.058) indicated the presence of silicacious substances.

The extractive values given in table 3 are useful to evaluate the phytoconstituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent [25].

In conclusion, the present study on pharmacognostical evaluation of *H.persicum* can be useful for identification and authenticity of this important useful medicinal plant and can be also helpful for the preparation of a monograph.

Acknowledgments

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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