



Pharmacognostic and Preliminary Phytochemical Investigation on *Doronicum scorpioides* Roots

Azadeh Hamedia^{a,*}, Samaneh Rivaz^{a,b}, Abdolali Mohagheghzadeh^c

^aMeymand Medicinal Plant Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

^bStudent Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran.

^cDepartment of Traditional Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

In many herbal markets medicinal plants are sold in dried forms which make it difficult to authenticate them and recognize the adulterations. In the present study the preliminary pharmacognostic parameters including microscopical studies, extractive values and thin layer chromatography analysis of roots of *Doronicum scorpioides* (Asteraceae) were evaluated. Three fractions of the plant (hexane, dichloromethane and ethanolic) were applied to the silica gel plates and run in nonpolar, semi polar and polar mobile phases. Chromatographic spots were visualized using different spray reagents. The preliminary screening of the *D. scorpioides* roots revealed the constituents of the different fractions of the roots including alkaloids, flavonoids, triterpenes saponins, coumarins and essential oils which can be a useful guide for further phytochemical and pharmacognostic research. This is the first report on phytochemical constituents of *D. scorpioides* roots. Also the pharmacognostic investigation of the *D. scorpioides* root can be a reliable method for authentication of the medicinal part of the plant and to recognize any adulterations.

Keywords: *Doronicum scorpioides*; Microscopic studies; Pharmacognostic investigation; Quality Control.

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1. Introduction

Since quality control and standardization of medicinal plants are important parts of health care in many countries specially developing societies, it is becoming a critical issue to ensure about efficacy and safety of these

herbal medicines. On the other hand, the growing beneficial market of medicinal plants and herbal medicine in one side and our lack of knowledge about these herbs ended up these markets to lots of intentional and unintentional adulterations [1, 2]. Many of these medicinal plants are collected by folks from nature and the medicinal parts are sold in dried forms which make it more difficult to recognize these adulterations [3, 4].

*Corresponding author: Dr. Azadeh Hamedia, Department of Pharmacognosy, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran, 71345-1586
Tel: (+98) 711-2424128 Ext. 246; Fax: (+98) 711-2424126
E-Mail: hamediaz@sums.ac.ir

Considering these issues different pharmacognostic studies to develop herbal pharmacopoeias have an important role in standardization and quality control of medicinal plants [5, 6].

In this study, the preliminary pharmacognostic parameters including microscopical studies of the powder, extractive values and preliminary phytochemical analysis on roots of *Doronicum scorpioides* (Asteraceae) were evaluated, and its ethno-pharmacological applications was reviewed.

Doronicum scorpioides root (Figure 1) is known as “Daroonaje-aghrabi” in Persian folk and traditional medicine because of the similarity of the roots morphology to the scorpion tail. The roots are cream-yellow white with a gray bark and an aromatic bitter flavor. According to Persian traditional literature, it can reduce phlegm and black bile and has been used in different tonic remedies for liver, spleen, heart and nerves. Also, it was mentioned that the roots can be useful as an antidote for insects' bites and venom [7, 8].

2. Material and methods

2.1. Plant material

D. scorpioides roots were purchased from Shiraz herbal market. The plant material was recognized and authenticated by Miss S. Khademyan, Taxonomist, Department of Traditional Pharmacy, Faculty of Pharmacy, Shiraz University of Medical Sciences, and the voucher specimen was preserved with the code PM181 in the department for further references. The plant material was powdered, passed through sieve number 100 and kept in dark closed container.

2.2. Microscopical studies

One drop of chloral hydrate solution was added to a small amount of roots powder on a slide and mixed well. The cover slip was added and heated on a flame. For detection of mucilage and starch water was applied instead

of chloral hydrate and the slides were not heated [9, 10].

2.3. Evaluation of extractive values

One hundred grams of the roots powder was extracted with hexane applying Soxhlet apparatus for 6 h (hexane fraction). The residuum was dried and macerated with dichloromethane, followed by ethanol in a dark and closed glass container, two days for each. Hexane, dichloromethane and ethanolic fractions were concentrated with a rotary evaporator and dried in a speed vacuum apparatus at 40 °C. The dried fractions were weighed out and kept at teflon capped tubes in -20 °C.

The ash values were determined according to the officinal methods described in the WHO guidelines on quality control methods for medicinal plant material [1].

2.4. Thin layer chromatography

Solutions of 5 mg/ml of different fractions were prepared and 10 µl of them were applied to the silica gel plated 60 F 254 (10×20 cm) from Merck With 5 µl graduated micropipettes from CAMAG. The plates were run in nonpolar (toluene: acetone, 80:20), semi polar (toluene: chloroform: acetone, 40:25:35) and polar (n-butanol: glacial acetic acid: water, 50:10:40) mobile phases. Chromatographic spots were visualized first using ultraviolet



Figure 1. Morphology of *Doronicum scorpioides* roots.

Table 1. Ash and extractive values of *Doronicum scorpioides* roots.

Total ash value (%w/w)	Acid insoluble ash (%w/w)	Water soluble ash (%w/w)	Petroleum ether soluble extractive (%w/w)	Dichloromethane soluble extractive (%w/w)	Ethanol soluble extractive (%w/w)
5.45± 0.01	1.91± 0.01	3.21± 0.01	4.66	4.98	2.96

lamps emitting at 254 and 365 nm and then using different spray reagents. For detection of essential oils and fatty acids phosphomolibdic acid reagents (vis.), for alkaloids Dragendorff reagents were applied. Also 5% potassium hydroxide for coumarins (UV₃₆₅ nm) and anthraquinones (visible and UV₃₆₅ nm), orcinol for glycosides, NP (ethanolamine diphenylborate)/PEG for flavonoids (UV₃₆₅), Libermann_burchard for steroids and triterpenes, 3% FeCl₃ for tannins and other phenolic compounds (vis.), vanillin-sulphuric acid and anisaldehyde-sulphuric acid as general reagents were sprayed on TLC plates [1].

For determination of relative R_f value in each chromatography procedure a standard natural product or drug was spotted on the TLC plate with the same method as above and with respect to the chosen spraying reagents.

Scopolamine for alkaloids, thymol for essential oils, diosgenin for steroids, quercetin for flavonoids, pyrogallol for phenolics, and warfarin for coumarins were used as standards in order to determine the relative R_f value. All chemicals and solvents were of analytical grade purchased from Merck or Sigma Aldrich.

3. Results and discussion

The roots exodermis was uniform which consists of elongate cells that had uniform shape (Figure 2). Trichomes had star shape and it was possible to observe masses of overlapped stellate trichomes. A fiber of roots is shown in Figure 2.

Results of ash and extractive values are shown in Table 1. Different ash values and extractive values represent the inorganic residue and purity of herbal drugs [12]. The

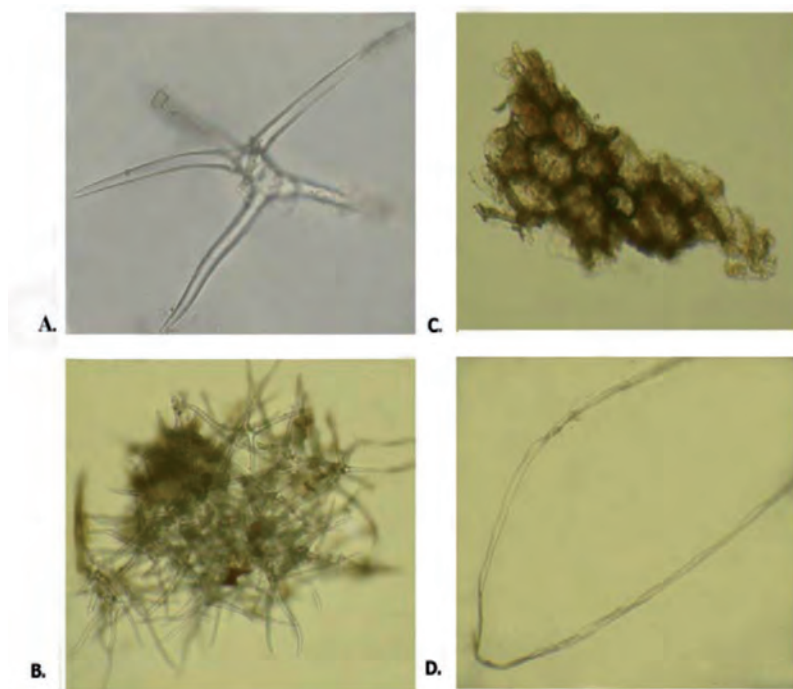


Figure 2. Microscopical analysis of *Doronicum scorpioides* roots powder. A: Star shape trichome, B: A mass of overlapped stellate trichomes, C: Roots exodermis, D: Roots fiber

Table 2. Relative RR_f values of secondary metabolites resulted from preliminary phytochemical analysis of different fractions of *Doronicum scorpioides*.

	RR_f of alkaloids to scopolamine	RR_f of Flavonoids to quercetin	RR_f of triterpenes to diosgenin	RR_f of essential oils to thymol	RR_f coumarins to warfarin
Petroleum ether fraction (run in non polar mobile phase)	2.39	0.99	2.74	1.2, 1.1, 0.92, 0.73, 0.59, 0.43	0.65 0.85
Dichloromethane Fraction (run in polar mobile phase)	-	-	1		
Ethanollic fraction (run in polar mobile phase)	-	0.97	1		
		0.82	0.45		

ash values of the powdered roots showed high percentage of water soluble ash.

The extractive values of herbal drugs can be considered as a representative of different classes of their chemical constituents. The extractive values of petroleum, dichloromethane and ethanolic fractions were determined 2.96%, 4.98% and 4.66%, respectively, which shows that high portion of secondary metabolites of *D. scorpioides* roots are semi-polar or polar.

The classes of these secondary metabolites were screened by TLC and a variety of chemical reagents.

Some chemical of the roots like essential

oils, flavonoids and coumarins could be detected under UV lamps without chemical treatments. The spray reagent used made a great knowledge of secondary metabolites of the plant roots. The petroleum fraction chromatographed in nonpolar mobile phase revealed about 7 blue spots with phosphomolibdic acid reagent which shows that the roots contain several essential oils. The relative R_f values of these spots were evaluated with comparison to thymol which was run as a standard essential oil along with the petroleum fraction (Table 2). With Libermann-Burchard reagent saponin and steroidal components in ethanol and

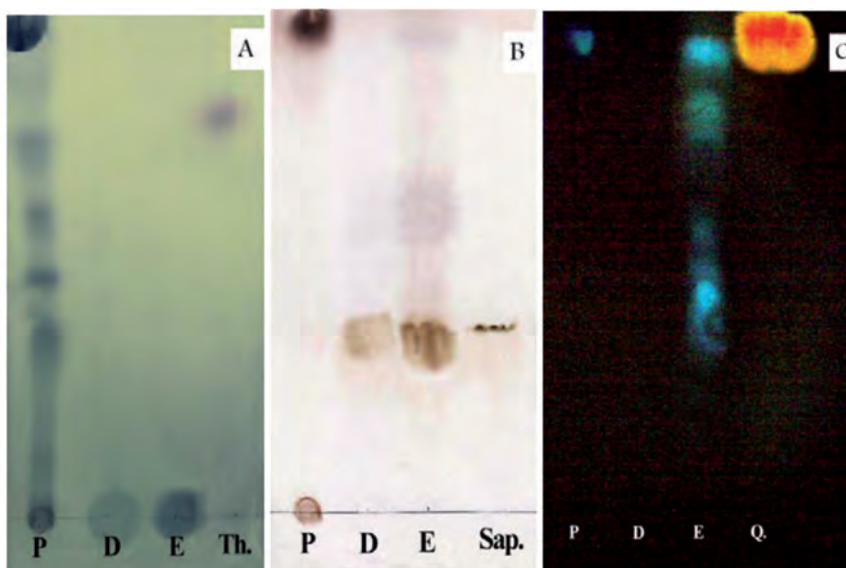


Figure 3. TLC analysis of petroleum ether (P), dichloromethane (D) and ethanolic (E) fractions of *Doronicum scorpioides* roots run in nonpolar mobile phase and treated with phosphomolibdic acid reagent (A), polar mobile phase treated with Libermann-Burchard reagent (B) and NP/PEG reagent (C). Relative R_f values were determined against thymol (Th.), diosgenin (sap.) and quercetin (Q).

dichloromethane (with less intensity) fractions of the roots which were chromatographed in polar mobile phase appeared as brown zone in visible light and green florescent under UV₃₆₅ nm with the same R_f value to R_f value of diosgenin as standard saponin. Tannins gave faded red-brown zones in ethanol fraction. According to best of our knowledge there is not any report on essential oils, saponin or steroidal constituents of *D. scorpioides* roots. Bohlmann and Abraham (1979) in a report on *D. pardalianches* aerial part and roots constituents identified thymol, tremetone and p-hydroxyacetophenone derivatives [13]. Thymol derivatives were also reported from *D. corsicum* [14] and *D. austriacum* [15]. With regard to R_f value of thymol which was run as a standard essential oil in the same TLC the roots of *D. scorpioides* doesn't contain thymol.

Alkaloids in the petroleum ether fraction which was run in polar solvent gave red-orange color with Dragendorff reagent. The relative R_f value to scopolamine was 2.39. The authors of this article couldn't find any report on isolation of any alkaloids of *D. scorpioides* roots but alkaloid otosenine which is believed to have cardiovascular properties was isolated from *D. hookeri* [16]. Petroleum ether fraction chromatographed in nonpolar solvents gave bluish-violet spots with 5% KOH and NP/PEG reagent revealed 3 blue-green fluorescent spots under UV₃₆₅ light for flavonoids in ethanolic fraction with was chromatographed in polar mobile phase. There was also a similar spot for petroleum fraction (Table 2 and Figure 3). Glycosides which were revealed by orcinol reagent are underlined in Table 2.

4. Conclusion

The pharmacognostic investigation of the *D. scorpioides* roots can be a reliable method for authentication of the medicinal part of the plant to recognize any adulteration. This

is the first report on phytochemical analysis on *D. scorpioides* roots. The preliminary screening of the *D. scorpioides* roots revealed the constituents of the different fractions of the roots including alkaloids, flavonoids, triterpene saponins, coumarins and essential oils which can be a useful guide for further phytochemical and pharmacognostic research.

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