



Research Article

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Pharmacognostic and phytochemical studies of leaves of *Crataegus pontica* C. Koch

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ABSTRACT

Crataegus pontica (Rosaceae family) is a small tree with caducous foliage which is widely distributed throughout west and central area of Iran. Ethnomedically, The leaves of *Crataegus pontica* have been used in Iranian traditional medicine for treatment of various disease conditions without standardization. The present study deals with the pharmacognostic and phytochemical studies of leaves of the plant. The preliminary phytochemical studies indicate the presence of carbohydrates, flavonoids, cyanogenetics, phenolic compounds, saponins, phytosterols, tannins, fats and fixed oils. The important diagnostic features of the powder include anomocytic Stomata, cuticular striations, unicellular uniseriate trichome, and calcium oxalate crystals. The T.S of leaf shows the presence of epidermis, parenchymatous cells, collateral vascular bundle and unicellular trichomes.

Keywords: *Crataegus pontica*, pharmacognostic, phytochemical, Hawthorn, Bioflavonoid

INTRODUCTION

The genus *Crataegus* belongs to the family Rosaceae comprises of a complex group of trees and shrubs, native to Northern temperate zones, mostly between latitudes 30° and 50° N. Hawthorn refers to the plant *Crataegus* and is widely distributed throughout the Northern temperate region of the world with approximately 280 species [1]. Most are large spiny shrubs or small trees. The deep green leaves are alternate, simple or lobed some toothed. The white to pink flowers have 5 sepals and/or petals, depending on the species and are carried in corymbs or are solitary. Nutlets with a fleshy edible covering follow. The fruit can be black, yellow or bluish green but the majority is red [2, 3]. Almost all the parts of these plants are reported to possess various pharmacological actions including anti-inflammatory, gastro-protective, antimicrobial, cardiogenic, hypotensive and hepatoprotective effects [1, 4]. In Iranian traditional medicine, some *Crataegus* species have been used as decoction of leaves and flowers for various purposes such as cardiovascular diseases, sedative and antianxiety agent [5]. Hawthorn fruit is stated to possess cardiogenic, coronary vasodilator and hypotensive properties. Traditionally, it has been used for cardiac failure, myocardial weakness, paroxysmal tachycardia, hypertension, arteriosclerosis and Buerger's disease [1, 4, 6, and 7]. The leaves, flowers and berries of hawthorn contain a variety of bioflavonoid-like complexes that appear to be primarily responsible for the cardiac actions of the plant [4, 8, and 9].

This genus consists of 17 species in Iran. *Crataegus pontica* C.Koch (Persian name: zalzalak) is one of this genus member which widely distributed throughout west, northwest and central area of Iran [3, 10]. It is a small tree, 10-6m high, with caducous foliage; crown dense, rounded or dome-shaped; stem: slender, robust; bark: brown-greyish [3, 11]. In Iranian folk medicine, aerial parts of the plant and especially its edible fruits have been used for treatment of cardiovascular diseases, diabetes and anxiety disorders. The leaves and flowers have been also used as infusions for various purposes such as sedative, stomachic and carminative agent [5, 12, and 13].

Considering the importance of this plant, the objective of this work was to determine the macroscopical, microscopical and phytochemical properties of *C. pontica* leaves in order to better know its principal components for potential applications.

EXPERIMENTAL SECTION

Collection and authentication of plant material

Fresh leaves of *C. pontica* were collected in the month of August, 2013, from the local areas of Tarom, Zanjan, Iran. Herbariums and voucher sample were prepared and deposited in Department of Pharmacognosy, Faculty of Pharmacy, Zanjan University of medical sciences. (Voucher no.1058). The harvested plants were dried in shadow at room temperature, ground into powdered form and stored in airtight containers.

Pharmacognostical studies

The plant was studied for morphological characters including Size, shape, color, odour, taste, and extra features. Microscopical study was performed for both entire (free hand transverse sections) and powdered material using chloral hydrate as clearing agent and Safranin and Methylen blue as staining agents. The Characters observed were photographed under Carl Zeiss microscope connected to digital Sony Camera [12, 14, 15 and 16]

Fluorescence analysis

The fluorescence character of the plant powder was studied under UV light (254 nm and 366 nm) and after treatment with different reagents like Hydrochloric acid, Sodium hydroxide, Nitric acid, Sulphuric acid, Ferric chloride, Methanol [12,14 and15]

Physical evaluation

Plant leaves powder was evaluated for various physical parameters like solvent extractive value, (Hexan, methanol and Water soluble), Loss on drying, Ash values (Total, Acid-insoluble and Water soluble ash) [12, 18]

Phytochemical studies

50g powder was extracted with 300ml of methanol at 50°C and the extract was filtered by Whatman no.1 filter paper. The filtrate was concentrated in a rotary evaporator at 40°C. The crude extract was stored at 4°C in airtight bottles until further use.

The methanol crude extract of the plant was dissolved in water and partitioned subsequently with equal volumes of petroleum ether and ethyl acetate. All the extracts were filtered and evaporated to dryness under reduced pressure at 40 °C using a rotary evaporator. Phytochemical screening for various extracts was performed according to the standard procedures. [12, 14, 16, 18 and19]

RESULTS AND DISCUSSION

Pharmacognostic study

Macroscopy

Leaves are 3-6cm long, 2-6cm wide, cuneate at base, ovate or obovate-flabellate, deeply lobed, lobes oblong-linear; with acute teeth, venation palmate, Greenish grey to pale green, glabrous on upper surface, pubescent to rarely glabrous on lower; petiole short, finely hairy (Fig.1).

Microscopy

Transverse section (TS) of *C. pontica* leaf

Leaf shows dorsiventral structure; the epidermis covered by thin cuticle present on both the surfaces; a single layer of palisade parenchyma underneath the upper epidermis occupying more than half the portion of the mesophyll tissue; spongy parenchyma 2-3 layers; Mid-rib consists of upper epidermis, single layer of parenchymatous hypodermis and collateral vascular bundle surrounded by 3-6 layers of collenchymatous cells; calcium oxalate rosette crystals present in mesophyll and collenchymatous cells; unicellular trichomes are present on both surface (Fig. 2).



Fig. 1: leaves of *C. pontica*

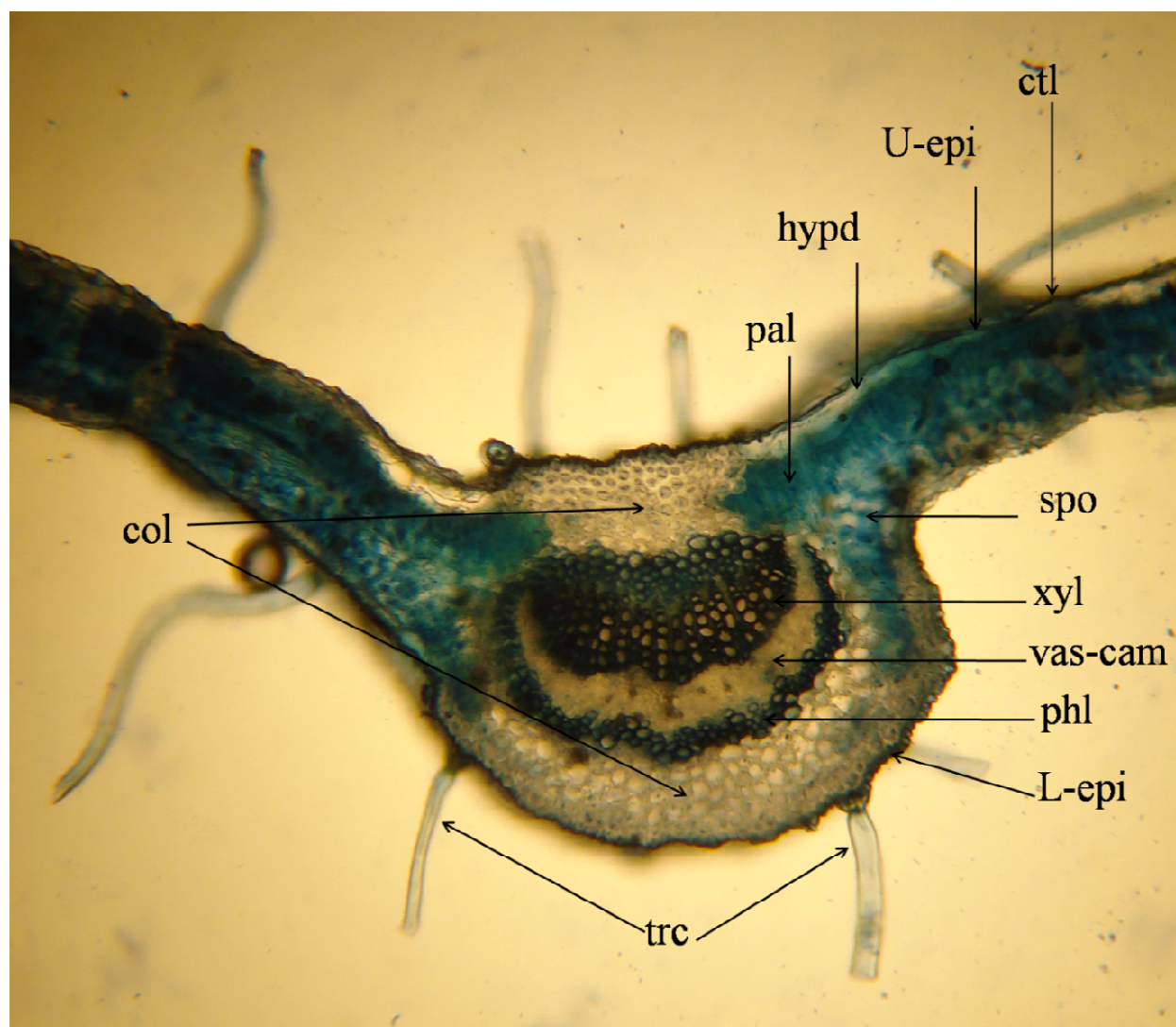


Fig. 2: TS of *C. pontica* leaf

ctl- Cuticula; *U-epi*- upper epidermis; *hypd*- Hypoderme; *pal*- Palisade parenchyma; *col*- Collenchymatous cells; *spo*- Spongy parenchyma; *xyl*- xylem; *phl*-phloem; *vas-cam*-vascular cambium ; *L-epi*- Lower epidermis; *trc*- trichome

Powder study of the *C. pontica* leaf powder

Powder of the herb is fine, greyish-green, slightly bitter and having indistinct odor. Under microscopic observation it shows presence of the epidermis (cells with thin, slightly sinuous walls) (Fig.3); anomocytic Stomata(Fig.4); cuticular striations, cicatrix(Fig.5); unicellular uniseriate trichome (Fig.6); prism crystals of calcium oxalate (Fig.7); spirally and reticulately thickened vessels, Pollen grains (Fig.8) and phloem tissue (Fig.9).

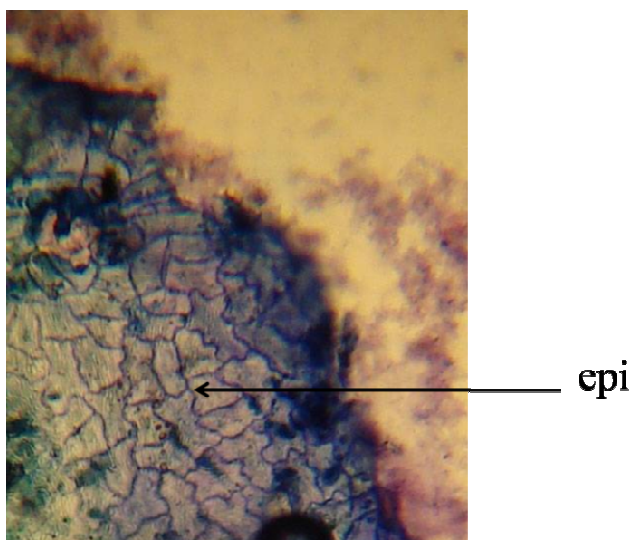


Fig.3: epi-The epidermis

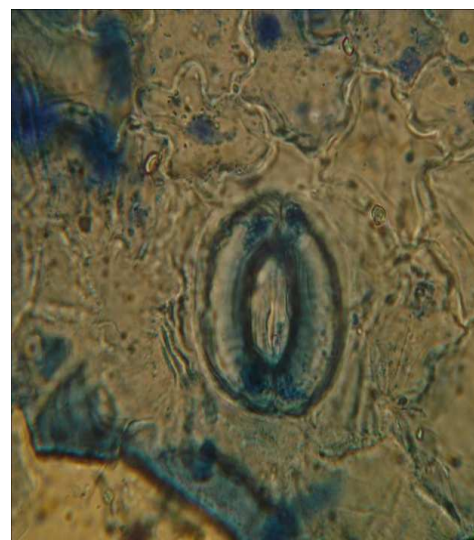


Fig.4: Anomocytic Stomata

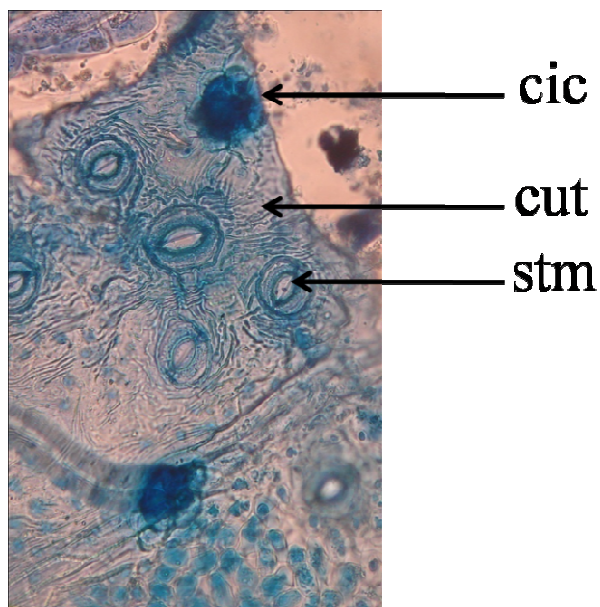


Fig.5: cic-Cicatrix; cut-Cuticular striations; stm-Stomata

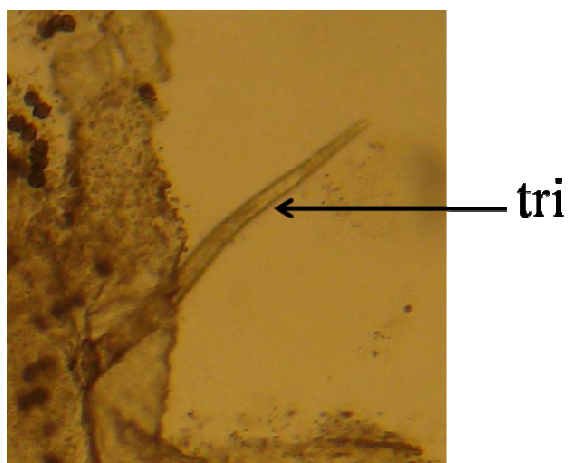


Fig.6: tri-Unicellular uniseriate trichome

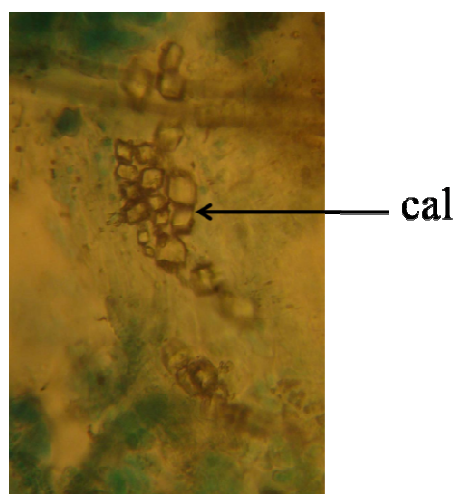


Fig.7: cal-Prism crystals of calcium oxalate

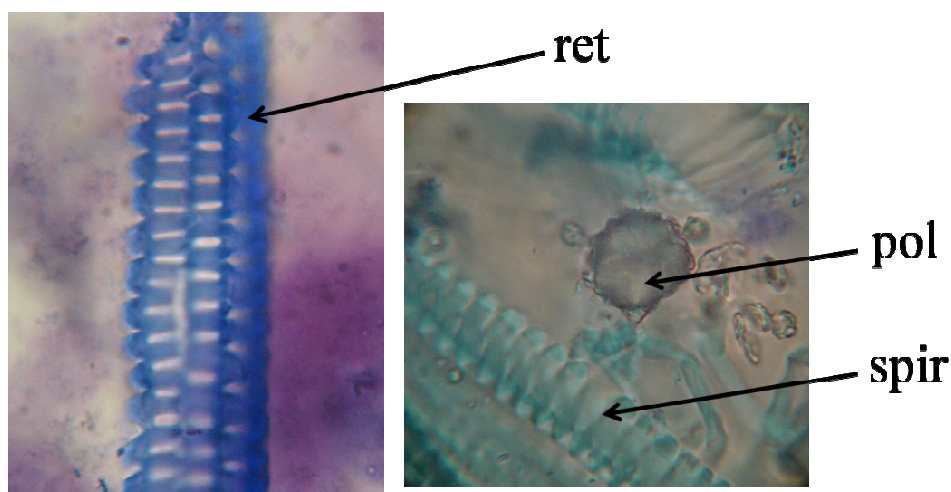


Fig.8: spir-spirally vessels; ret-reticulately vessels; pol- Pollen grains

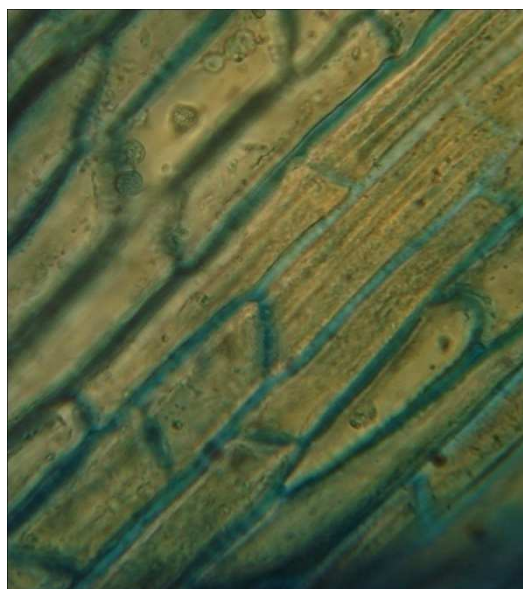


Fig.9: phloem tissue

Physico-chemical evaluations

Data of Physico-chemical parameters visually ash, extractive values and Loss on drying are given in Table 1. Water-soluble ash value was found to be more than acid insoluble ash value. The plant showed higher Methanolic soluble components than water soluble components. Qualitative phytochemical examination revealed the presence of carbohydrates, Phenolic compounds, Flavanoids and Cyanogenetics in Methanolic and Ethyl acetate Extract where as Petroleum ether extract exhibited the presence of Steroids, Fixed Oils and Fats. Saponins are present in the Methanolic extract (Table 2). The fluorescence analysis of *C. pontica* leaf under day light and UV light is recorded in Table 3.

Table1. Physical evaluation of *C. pontica* leaves

S. No.	Particulars	Result (% w/w) Mean (n = 4) \pm S.D.
1	Total ash	10.20 \pm 0.482
2	Acid insoluble ash	0.74 \pm 0.232
3	Water soluble ash	1.35 \pm 0.514
4	Methanolic soluble extractive value	31.12 \pm 0.632
5	Water soluble extractive value	17.32 \pm 0.321
6	Hexane soluble extractive value	2.78 \pm 0.426
7	Loss on drying at 105°C	12.21 \pm 0.344

Table 2. Phytochemical screening of *C. pontica* leaves

Phytoconstituents	Name of test	Methanolic Extract	ethyl acetate Extract	petroleum ether Extract
Carbohydrates	Molisch test	+	+	-
	Fehling's test	+	-	-
	Iodine test	-	-	-
	Gums test	-	-	-
Phenolic compounds	Ferric Chloride test	+	+	-
	Lead acetate test	+	-	-
	Gelatin test	+	-	-
Alkaloids	Wagner's test	-	-	-
	Dragendorff's test	-	-	-
	Mayer's test	-	-	-
Anthraquinone	Borntrager's test	-	-	-
	Modified Borntrager's test	-	-	-
Cyanogenetics	picrate test	+	+	-
Flavanoids	Shinoda test	+	+	-
Saponins	Foam test	+	-	-
Fixed Oils and Fats	Spot Test	-	-	+
Steroids	Liebermann Burchard Test	-	-	+

(-)=Absent (+)=Present

Table3. Fluorescence analysis of powdered leaf of *C. pontica*

S. No.	Reagent	Ordinary light	Short wave (254nm)	Long wave (365nm)
1	Powder as such	Greenish grey	Green	Greenish Blue
2	Powder+1N H ₂ SO ₄	Green	Brown	Brown
3	Powder+1N HNO ₃	Yellowish brown	Greenish brown	Brown
4	Powder+1N HCl	Pale green	Green	Green
5	Powder+1N NaOH	Citrine green	red	Brick red
6	Powder+FeCl ₃ 5%	Greenish Blue	Greenish black	Black
7	Powder+ Ammonia	Citrine green	Greenish black	Brown

Ethnomedically, The leaves of *C. pontica* have been used in Iranian traditional medicine for treatment of various disease conditions without standardization. Pharmacognostical evaluation of parameters like macroscopic appearance, organoleptic characters, microscopic characteristics, and presence or absence of important classes of phytoconstituents is necessary for standardization of herbals [16].

The pharmacognostic standards for leaves of *C. pontica* are carried out for the first time in this study. These macroscopical, microscopical and Physiochemical characters of the leaf can serve as diagnostic parameters in confirming the quality and purity of plant and its identification in crude form [20, 21].

Microscopical studies indicated the presence of anomocytic stomata and unicellular uniseriate trichomes in powder of the herb. Presence of cicatrix, prism crystals of calcium oxalate, spirally and reticulately thickened vessels are the characteristics of the plant. Ash values and extractive values can be used as reliable aid for detecting adulteration. The total ash value of drug indicates contamination with foreign matter such as metallic salts or silica and other impurities present along with drug. The amount of acid-insoluble ash reveals contamination with earthy and siliceous material and the water soluble ash is used to detect the presence of materials exhausted by water. [16, 12] Extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents. The water soluble extractive values indicate the presence of sugar, acids and inorganic compounds and the alcohol soluble extractive values indicate the presence of polar constituents like phenols, alkaloids, steroids, glycosides and flavonoids. Nonpolar constituents like Fixed Oils and Fats are soluble in Hexane extract [18, 20]. The fluorescence character of the plant powders helps in qualitative evaluation which can be used as a reference data for the identification of adulterations [16].

In conclusion, these parameters which are being reported in this study could be useful in the development of pharmacopoeial standards for the *Crataegus pontica* C.Koch in the future studies.

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