Pharmacognostical Evaluation of *Fagonia cretica* Linn.

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Abstract

Fagonia cretica Linn. is widely distributed in dry lands of India. It has been used in traditional system of medicine as bitter tonic and febrifuge. The whole plant of F. cretica was subjected to macro and microscopical examination followed by physicochemical study. It is small woody herb with tortuous root, highly branched stem, elliptic leaves and purplish coloured flowers. Roots are characterized by lignified cork, cortical fibres, wide phloem and xylem. Stem shows the presence of a group of lignified fibres under epidermis, pericycle (containing discontinuous groups of the fibres and stone cells) and a wide stellar region followed by small pith. Transverse section of the leaf shows palisade cells, centric mesophyll and collateral vascular bundle in midrib. Powder shows the presence of covering trichomes, epidermis, stone cells, sclerenchyma and lignified cork. Saponins, flavanoids and alkaloids were found be major components. TLC study using silica gel plate as a stationary phase and Toluene: Ethyl formate: Formic acid (5: 4: 0.1) as a mobile phase shows the presence of isorhamnetin.

Key words: *Fagonia cretica*, Isorhamnetin, TLC, Zygophyllaceae.

Introduction

Fagonia cretica Linn. (Syn.: *F. schweifurthii* Hadidi, *F. arabica* Hook. f., *F. indica* Burm. f.; Family: Zygophyllaceae.) is commonly known as Durlabha (Sans.); Dhamaasaa (Unani); Dhamaso (Guj.) and Khorason thorn (Eng.) (Rastogi and Mehrotra, 1990; Khare, 2007). It is a small spiny under-shrub, found in North West India, Punjab, Deccan and Afghanistan (Chopra *et al*., 1958; Chopra *et al*., 1956; Hooker, 1875).

The plant is highly valued in traditional medicine as a febrifuge, antiasthmatic and useful in skin diseases (Kirtikar and Basu, 1975; Nadkarni, 1954).

Flavonoids reported in plant include isorhamnetin 3-glucoside and isorhamnetin 3-rutinoside, herbacetin 8-rutinoside, kaempferol, quercetin and isorhamnetin (El-Negoumy *et al*., 1986; El-Hadidi *et al*., 1988). Oleanolic acid and ursolic acid are the triterpenoid saponins reported in the plant (Miyase *et al*., 1996; Rahman *et al*., 1982).

The present study aims at establishing quality parameters and TLC profile for isorhamnetin.

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Materials and Methods

Plant Material

Fresh, fully-grown, flowering plants of *F. cretica* Linn. were collected from Gujarat [Bhuj (A) and Jamnagar (B)] and Rajasthan [Jodhpur(C)] in the month of November, 2009. The plants collected were authenticated by taxonomist of Gujarat Ayurveda University, Jamnagar, Gujarat. Voucher specimen samples (LM 581—LM 583) were deposited at the Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad, Gujarat. The plant materials were cleaned, dried, powdered to 60 # and used for the experimental work.

Pharmacognostical Studies

Whole plants were studied for morphological characters. Microscopical study was performed for both entire (free-hand transverse sections of leaf, stem and root) and powdered material. Quantitative microscopy was carried out on leaf for determining the stomatal number, stomatal index and palisade ratio. The data was compared with the literature (Anonymous, 2006).

Moisture content (Anonymous, 2006a), ash values and extractive value were determined (Anonymous, 2002).

Phytochemical Studies

Phytochemical screening (Shah *et al.*, 2010) was performed with the use of alcoholic extract and saponin (Anonymous, 2002), flavonoid, phenolic (Kalola and Shah, 2006), alkaloid (Sreevidya and Mehrotra, 2003) and carbohydrate (Hodge and Hofreiter, 1962) contents were estimated.

TLC of Isorhamnetin

Powdered plant material of three samples (10 g) were extracted thrice with methanol separately, by sonication for 10 min and filtered. Extracts were evaporated to dryness. 5 mg of residues were dissolved in 5 ml methanol. The resulting solutions were centrifuged at 3000 rpm for 5 min and the supernatant collected were analysed for drug content by applying 3 µl of each of the solution to a plate.

A stock solution (100 µg ml^{-1}) of isorhamnetin was prepared by dissolving 1 mg in 10 ml methanol in a 10 ml volumetric flask.

Table 1 : Quantitative microscopy of *Fagonia cretica* Linn. leaf

Parameters	Samples		
	A	B	C
Stomatal number: Upper surface Lower surface	 147 ± 2 118 ± 2.4	 224 ± 4 195 ± 3	 177 ± 3 104 ± 2
Stomatal index: Upper surface Lower surface	12.52 ± 0.98 12.79 ± 0.30	17.49 ± 0.09 16.62 ± 0.49	13.19 ± 0.13 12.66 ± 0.96
Palisade ratio: Upper surface Lower surface	2.58 ± 0.14 1.83 ± 0.14	2.75 ± 0.25 2.5 ± 0.25	2.83 ± 0.28 1.83 ± 0.14

Number of readings = 3

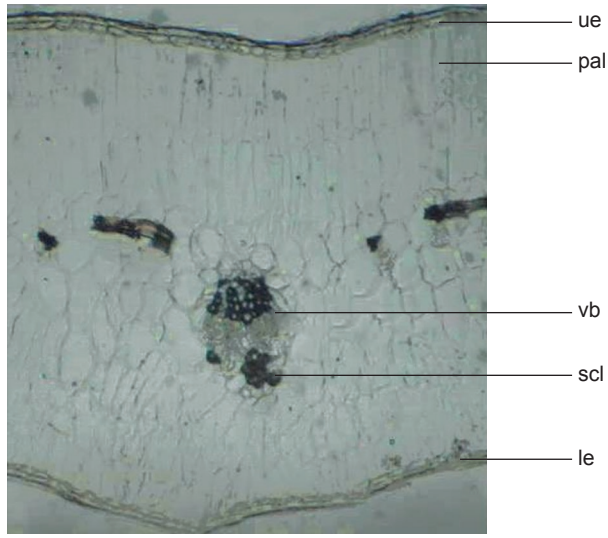
Table 2 : Physico-chemical parameters of *F. cretica* whole plant

Particulars	Samples (% w/w ± SD)		
	A	B	C
Loss on Drying	52.72	54.44	53.89
Total ash	9.86 ± 0.85	9.27 ± 0.35	7.09 ± 0.59
Water soluble ash	4.65 ± 0.28	4.61 ± 0.44	3.02 ± 0.07
Acid insoluble ash	1.14 ± 0.16	0.67 ± 0.09	0.62 ± 0.07
Water soluble extractive value	26.6 ± 0.9	26.26 ± 0.01	29.89 ± 0.53
Alcohol soluble extractive value	22.3 ± 1.51	28.0 ± 0.81	24.46 ± 0.95

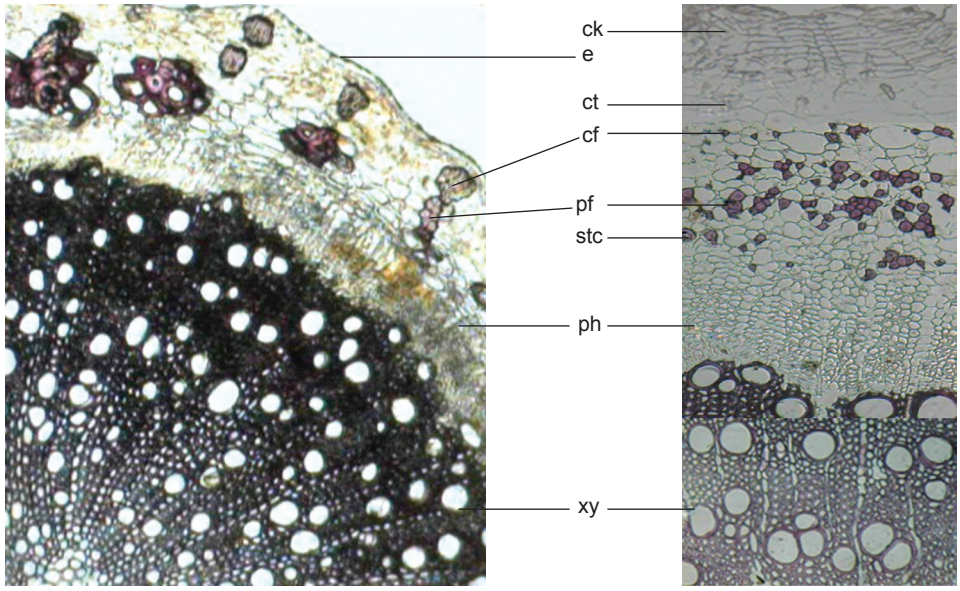
SD = standard deviation, Number of readings = 3

Table 3 : Content of phytoconstituents in *F. cretica* whole plant

Sr. No.	Phytoconstituents	Samples (% w/w)		
		A	B	C
1	Phenolic substances	0.327	0.400	0.612
2	Alkaloids	0.097	0.083	0.28
3	Flavanoids	0.950	1.411	2.263
4	Saponins: Froth number	333	250	333
5	Carbohydrates: Sugar content	3.22	3.77	4.01



A.



B. C.

Fig. 1: Microscopy of *Fagonia cretica*. A. Transverse Section of leaf; B. Transverse Section of stem; C. Transverse Section of root

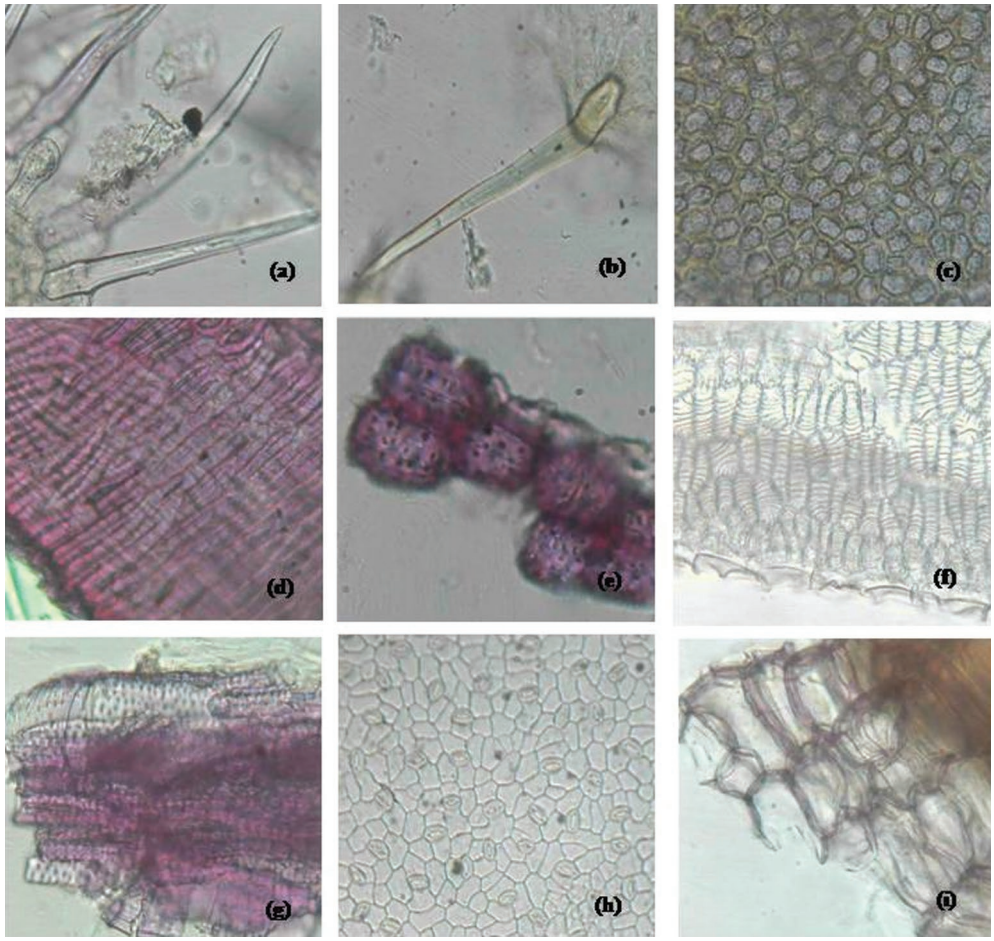


Fig. 2: Powder Microscopy of *Fagonia cretica* whole plant

a) Unicellular covering trichomes occurring in groups.
b) Isolated covering trichome with striated cuticle.
c) Fragment of testa.
d) Overlapping mesocarp and endocarp cells.
e) Stone cells.
f) Fibrous layer of anther.
g) Medullary rays and border-pitted vessels.
h) Anomocytic stomata.
i) Lignified cork.

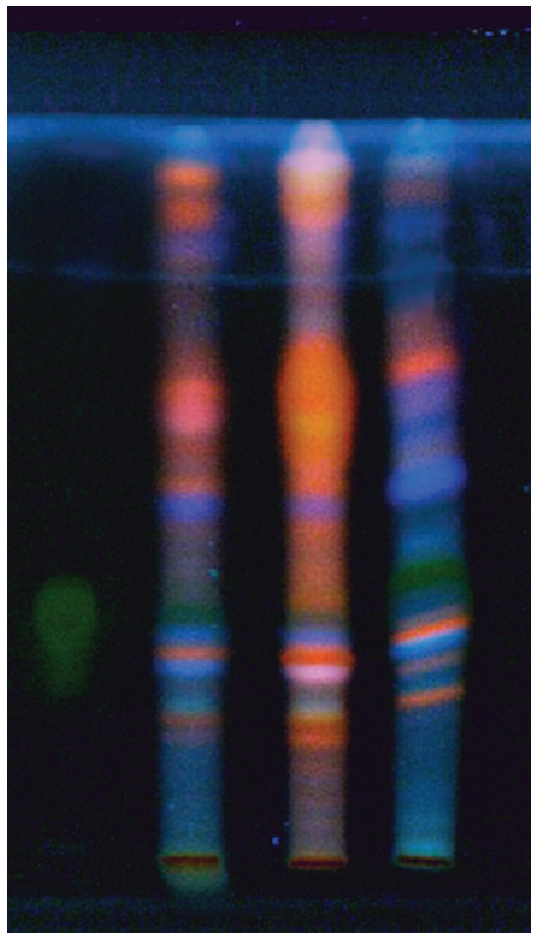


Fig. 3: Separation of isorhamnetin from *Fagonia cretica* Linn. whole plant samples (collected from Bhuj, Jamnagar and Jodhpur respectively) on TLC plate.

Results and Discussion

Morphological characters

F. cretica collected from three different places showed similar morphological characters. It is a small woody, branched and thorny under shrub, found to be growing in arid parts of northwest India. Leaf is opposite, leaflet 1.0 to 1.2 cm in length and 0.2 to 0.25 cm in width, linear to elliptic in shape, with entire margin, acute apex, glabrous surface and a small petiole; stipules spiny and arranged in whorls of 4 at node. Stem is cylindrical, glabrous, longitudinally striated with distinct nodes, internodes being 1.2–2.0 cm in length; fracture short and fibrous; yellowish brown in colour, slightly bitter and mucilaginous in taste. Root is tortuous, 0.5 to 0.8 cm in diameter, exfoliated at places, exhibiting fibrous fracture, creamish brown in colour and slightly bitter in taste. Flower is solitary, purplish-rose-coloured, petals spathulate with a marked claw, sepals imbricate,

half as long as petals; stamens 10 in number and are inserted on the disc. Fruit is schizocarp, deeply 5 partite, each one seeded cocci is compressed and pubescent with recurved peduncle, almost of the same length as that of fruit. Seeds are small, compressed, and ovate with a mucilaginous testa.

Microscopical characters

All three samples showed similar microscopical characters and can be differentiated by quantitative microscopic parameters.

The transverse section of the leaf showed a layer of upper and lower epidermis with thin walled, tabular, tangentially running cells covered with thick cuticle, an isobilateral lamina with continuously running three layered palisade tissue (pal) with sinuous cells on the upper side, and two layered on lower side with straight walled cells; a centrally located collateral vascular bundle (vb) of the midrib associated with a group of small sclereids (scl) (Figure 1).

Transverse section of the stem showed a layer of epidermis (e), narrow band of parenchymatous cortex and pericycle traversed with groups of cortical fibres (cf) and pericyclic fibres (pf), phloem (ph) and xylem (xy) encircled by small crescent shaped, centrally located obliterated pith. Phloem wide, parenchymatous, traversed with sieve tissue and uni to triseriate medullary rays; cambium distinct; xylem wide, composed of isolated and radially arranged vessels, medullary rays, parenchyma and thin walled fibres (Figure 1).

Transverse section of the root showed centrally located wide wood, occupying the major area of the section, encircled by well developed phloem (ph) traversed with groups of thick walled fibres (cf and pf) and stone cells (stc) arranged at places in discontinuous tangential bands, a very narrow parenchymatous cortex (ct) and outermost lignified cork tissue (ck). Xylem (xy) composed of vessels, fibres and pitted parenchyma, starch grains and occasional prismatic crystals of calcium oxalate traversed throughout the parenchymatous cells of the section (Figure 1).

Powder

Powder microscopy showed simple, covering trichomes with unicellular stalk scattered as such or attached to the epicarp and wall of ovary (a), cells of the former are covered with striated cuticle (b), simple trichomes uni- to bi-cellular, thick walled, lignified, with pointed apex and bulging base, of various sizes from fruit (a); fragments of testa in surface view showing polygonal thick walled cells (c); fragments of longitudinally cut thick walled, lignified

groups of sclerenchymatous cells of mesocarp often seen overlapping with the underlying cells of endocarp (d); isolated and groups of stone cells (e); fragments of fibrous layer of anther in surface view (f); radially longitudinally cut medullary rays crossing the bordered pitted vessels (g), anomocytic stomata (h) and lignified cork in surface view (i) (Figure 2).

Data of quantitative microscopy for leaves are entered in table 1.

Physicochemical and Phytochemical Evaluations

Results of physico-chemical evaluation viz. ash and extractive values are given in table 2. Qualitative phytochemical examination revealed that the plant is rich in saponin and flavanoid. The study was extended to estimate saponin, flavanoid, alkaloid, phenolics and carbohydrate.

TLC of Isorhamnetin

Both standard isorhamnetin and extracts were applied on the TLC plate and chromatographed with the mobile phase Toluene: Ethyl formate: Formic acid (5: 4: 0.1) that enabled good resolution with a sharp and symmetrical spot at R_f 0.35 for each (Figure 3).

Conclusion

The herb was woody, thorny, with petiolate leaves, glabrous stem and tortuous root. Root was characterized by lignified cork, cortical fibres in cortex, stone cells in phloem and wide zone of xylem. Stem showed hypodermis and cortex containing discontinuous groups of lignified fibres, followed by wide phloem and xylem. Characteristic diagnostic features of leaf included 2 layered palisade tissue and midrib showing a collateral vascular bundle. Powder was found to have trichomes from fruits, stone cells, overlapping cells of sclerenchyma and lignified cork. The plant showed higher water-soluble components than alcohol soluble components. Phytochemical screening revealed presence of steroid and triterpenoid, flavanoid, phenolics, saponin, alkaloid and carbohydrate. Phenolics, alkaloids, flavanoids and carbohydrates, all were found to be higher in Jodhpur sample (0.612% w/w, 0.28% w/w, 2.26% w/w and 4.01% w/w respectively) (see table 3).

TLC using precoated silica gel 60 F_{254} plate as a stationary phase, as Toluene: Ethyl formate: Formic acid (5: 4: 0.1) mobile phase revealed presence of isorhamnetin at R_f 0.35 in all samples.

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