

A preliminary phyto-pharmacognostical evaluation of *Opuntia elatior* Mill. (*Nagaphani* or *Hathalo-thore*) fruit

Research article

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Abstract

In Gujarat, *Opuntia elatior* Mill., a member of Cactaceae family known as *Nagaphani* or *Hathlo-thore* has been reported for its traditional uses as a medicinal plant. It is used as remedy in different disease conditions like anaemia, asthma etc. by tribal of Gujarat state. A detailed pharmacognostical character and pharmaceutical analysis of its fruit is not reported still yet. An attempt has been made to study the macroscopic, microscopic, physicochemical parameters and HPTLC profile of its fruit following standard procedures. Results show in Transverse section of unripe fruit, presence of outer exocarp layer with rosette crystals, followed by mesocarp and inner endocarp. Transverse section of ripen fruits, shows presence of same characters as fresh unripe fruit except the presence of betacyanins in its mesocarp layer. Powder microscopy of mature fruit shows diagnostic characters like multicellular trichomes, rosette crystals of calcium oxalate, coloring matter and starch grains. The ash value of *O. elatior* fruit is 3.59 % w/w, pH - 5.27, total solid content - 10.83 %, viscosity - 5.11 and Specific gravity is 1.042. HPTLC study showed the presence of four and two spots in short and long UV respectively, among them, 0.04 and 0.97 are the common R_f value. These observed pharmacognostical, physicochemical parameters and HPTLC profile may be useful to establish the botanical standards for identification and standardization of *O. elatior* fruit.

Key words: *Hathalo-thore*, *Nagapheni*, *Opuntia elatior* Mill, Pharmacognosy, Physicochemical

Introduction

A folklore medicinal plant, widely prevalent in the state of Gujarat, known as "*Hathlo thore*", botanically identified as *Opuntia elatior* Mill. of family Cactaceae.(1) This family is containing about 130 genera including the genus *Opuntia* and nearly 1500 species.(2) Cactaceae family is well adapted to arid and semiarid climates, where water shall be a limiting factor for cultivation.(3,4) It is found that all cacti in India did not belong to one species, but to three or four species distributed over different regions in India. *Opuntia dillenii* Haw. is found mainly in the southern parts of the India while *O. vulgaris* Mill (Syn *Opuntia monocantha* Haw.) is distributed mainly in the northern parts; *O. elatior* Mill. is being reported in western India. (5) Among them, *O. elatior* is available in Saurashtra region of Gujarat with a vernacular name '*hathlo thore*' *O. elatior*(6) can be differentiated from other by presence of 2-5 spines in each areole and the characteristic colour changes in flower at various stages (i.e from yellow at first to pink and finally bright red). *Opuntia elatior* is fleshy, succulent, perennial herbs, shrub with spines or bristles or both available in Gujarat, Rajasthan, Orissa and central India, and

distributed chiefly in semi-desert or drier regions of tropical and subtropical America and also in Africa, Mauritius, Seychelles, Sri Lanka, and Australia.(7)

Opuntia elatior Mill. possesses varied ethnobotanical claims in different diseases. In asthma baked fruits is administered internally once in a day for a week(8), pulp of one ripe fruit is used for burning sensation in the stomach(9), fruits first heated and then powdered, about 5g powder consumed at morning for 10-15 days in rheumatism(10), juice of 7-8 fruits is mixed with 50gm jaggery and taken orally by the tribal ladies for 2-3 days to develop complete sterility(11) and in whooping cough baked fruit and fruit syrup is increases the flow of bile and control spasmodic cough and expectoration.(12) This plant is used internally to relieve muscle spasms, whooping cough, cough, stomach ache & inflammation in diphtheria. Its external application is well appreciated in abscesses, boils, measles, etc.(13,14)

Recent literature review reveals that the various parts used of the plant *Opuntia elatior* Mill. has not been studied for its pharmacognostical characters till date. Hence, the present study was undertaken to establish certain botanical standards for identification and standardization of fruits of *Opuntia elatior* Mill.

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Materials and Methods

Fresh fruits of *Opuntia elatior* Mill. were used as material for this study. The study was conducted as per the guidelines of Ayurvedic Pharmacopoeia of India. (15)

Collection and authentication of sample

The plant *Hathlo thore* (*Opuntia elatior* Mill.) fruit was collected from its natural habitat, suburbs of Jamnagar, Gujarat, during November 2013 and authenticated by local taxonomist. Fruits were separated from the plant and preserved (SPECIMEN NO- PHM-6138/1/1/2014). The collected plant samples were washed under running fresh tap water to remove adherent soil and dirt.

Pharmacognostical Analysis

Organoleptic study:

Fresh and ripen samples of fruit of *Opuntia elatior* Mill. was evaluated for their organoleptic characters including taste, odour, colour, and touch. (16)

Macroscopic study:

Macroscopic characters of fruit *Opuntia elatior* Mill. was studied systematically as per the standard text book of Botany(17) as well as with the help of floras.(18,19,20)

a. Morphology

Collected fruits were observed carefully for its identical morphological characters. Morphological characters like colour, shape, size were noted down. Characters of fruit were studied as per taxonomy(21) and measurement was taken by scale through naked eyes.

b. Microscopic study

Fresh fruits were taken for detailed microscopic study. Free hand sections of fruit were taken. Microphotographs were taken by using Carl Zeiss Trinocular microscope attached with camera. Same procedure was followed for detailed powder microscopy.(22)

Pharmaceutical Analysis

Physicochemical analysis:

Fruit *Swarasa* of *Opuntia elatior* Mill. was used to carry out different parameters as mentioned in Ayurvedic Pharmacopoeia of India. pH value, Specific gravity, Total solid content, Moisture content, Ash value and Viscosity were determined following standard procedure in API.(23)

Determination of pH Value

20 ml of sample (fruit *swarasa*) was taken. pH of the sample were noted with the help of the pH paper. pH – 5.22. Volume taken= 20 ml

Weight of empty bottle (a)	17.074 g
Weight of Sample + bottle (b)	43.500 g
Weight of Sample (b-a)	43.500-17.074 = 26.43 g
Weight of bottle + Distilled water	42.428 g
Weight of Distilled water (d-a)	(42.428-17.074) g = 25.354 g

Specific gravity

Weight of sample

$$\frac{\text{Weight of Distilled water}}{\text{Weight of sample}} = \frac{25.354}{26.426} = 1.0423$$

Total solid content

Weight of dish + sample	56.700 g
Weight of dish	54.535 g
Weight of solid content	2.165 g
Volume taken of sample	20 ml
Total solid content	Wt. of solid content X 100
	$\frac{2.165 \times 100}{20}$
	= 10.83%

Specific Gravity

Moisture content

Estimation of moisture content *O.elatior* fruits was carried out five times. Fruit pulp (10 g) placed in a tarred evaporating dish and dried at 105 °C in an oven at constant weight. The moisture content and total solids were determined using following equation.(24)
 $\% \text{ Moisture content} = \frac{[(\text{initial weight} - \text{dried weight}) / \text{initial weight}] \times 100}{1} = 74.16\%$

Determination of Ash value

3g of accurately weighed fruit pulp was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450 °C until free from carbon and constant weight, cooled and weighed.
 Total ash=3.59%

Viscosity

Determination of Viscosity of OFJ
 Viscosity of OFJ was determined five times using spindle S61 of Brookfield viscometer (Model DV-II+ Pro viscometer) at 100 rpm.(25)

Chromatographic study:

HPTLC study was carried out following standard guidelines of API.

Sample for HPTLC:

Alcoholic extracts of fruit *Swarasa* of *Opuntia elatior* Mill.

MobilePhase - Toluene: Ethyl acetate: Formic acid (7.5:2:0.5 v/v)

Chromatographic conditions

1. Application mode: Camag Linomat V, 2. Development Chamber: Camag Twin trough Chamber, 3. Plates: Precoated Silica Gel GF254 Plates, 4. Chamber Saturation: 30 min, 5. Development Time: 30 min, 6. Development distance: 8 cm, 7. Scanner: Camag Scanner III, 8. Detection: Deuterium lamp, Tungstan Lamp, 9. Data System: Win cats software.

Observations and results

Pharmacognostical study

Morphology of fruit

Fruit berry oval to corn-shape with many clefts of very thin 2-5 spines in each areole all around the fruits and slight brownish in colour. Fruit has multi carpilary syncarpous with many seeds. Average number of seed is 25-30 in a fruit. Measurements of unripe fruit and mature fruit in Table 1 and average weight of fruit in Table 2.

Table 1: Morphology of fruit

Sr. No	Characters	Measurements
1	Unripe fruit	4.5 × 2.8 cm
2	Mature fruit	4.6 × 2.5 cm

Table 2: Average weight of fruit

Sr. No.	Characters	Weight
1	Unripe fruit	9.09 g
2	Mature fruit	12.52 g
3	Fruit with flower	13.19 g
4	Flower	4.10 g

Microscopy of fruit

T.S. of section of unripe fruit

Diagrammatic section of the fresh fruit shows outer exocarp, middle mesocarp, inner endocarp and treads into ground tissue.

Exocarp: One to two layers with cortical cells with rosette crystal. Cells square type.

Mesocarp: Comprises large area, made up of parenchyma cells with chlorophyll pigments later on called chlorenchyma. Some of the cells filled with starch grains, rosette crystal of calcium oxalate. Many vascular bundles distributed all over the mesocarp. Some of the specialized cells lead to mucilage cavity.

Endocarp: Endocarp made up of somewhat elongated completely arranged parenchyma cells. Some consist of starch grains and rosette crystal. Endosperm leads into the placenta to the locule. Multi capillary locule consist numerous seeds distributed all over the ground tissues. (Fig. 1a-f)

T.S. of section of matured fruit

Diagrammatic section of the matured fruit shows outer exocarp, middle mesocarp, inner endocarp and treads into ground tissue.

Exocarp: 1 to 2 layers with critical cells with rosette crystal. Cells square type.

Mesocarp: Comprises large area made up of parenchyma cells with chlorophyll pigments later on largely filled betacyanins (red-violet colour) some of

the cells field with starch grains, rosette crystal of calcium oxalate. Many vascular bundles distributed all over the mesocarp. Some of the specialized cells lead to mucilage cavity.

Endocarp: Endocarp made up of somewhat elongated completely arranged parenchyma cells. Consist some of starch grains and rosette crystal. Endosperms leads into the placenta to the lobule multi capillary lobule consists numerous seeds distributed all over the ground tissues. (Fig. 1g-l)

Powder microscopy of fruit

Powder microscopy showed that presence of multicellular trichomes, rosette crystals of calcium oxalate, coloring matter and starch grains.

Pharmaceutical Analysis

Physicochemical Analysis

Fruit *Swarasa* of *Opuntia elatior* Mill. was tested for various physico-chemical parameters such as ash value, pH, Total solid content (%), Viscosity, Moisture content. The observed results were depicted in table 3.

Table 3: Physico-chemical analysis of *Oppuntia elatior* Mill.

Sr.No	Test	Result
1	Sp.Gravity	1.0423
2	pH	5.27
3	Total solid content (%)	10.83 %
4	Viscosity	5.11 millipoise
5	Moisture content	74.16%
6	Ash value	3.59%

Data presented in table 3.4.1 shows the values generated from the physicochemical analysis of fruit *Swarasa* of *Opuntia elatior* Mill. Ash value of fruit *swarasa* is 3.59 (%w/w). Total solid content of fruit *swarasa* is 10.83 (% w/w). Moisture content of fruit *swarasa* is 74.16 (%w/w). The pH value of sample is 5.27. Viscosity of fruit *Swarasa* is 5.11 millipoise. Specific gravity of fruit *swarasa* is 1.042.

HPTLC profile:

Selective alcoholic extract is basically polar and moderately polar component here taken as a fingerprinting proof of *Opuntia elatior* Mill. After performing the HPTLC chromatogram, it can be suggested that extracts contained different compound. Under UV at 254nm, most of the bands appeared in fluorescence at different R_f values. However, under UV at 366nm, in fluorescence at different R_f . The Pharmacological activities exhibited by of the plant extracts could be attributed to the presence of secondary metabolite (Polyphenol, glycosides, steroids, quaternary ammonium alkaloid) probably in synergism with other compounds.

Table 4: HPTLC profile of alcoholic extract of *Opuntia elatior* Mill. in Toluene: Ethyl acetate: Acetic acid (7:3:1)

Data of HPTLC				
Solvent system: Toluene : Ethyl acetate:Acetic acid (7:3:1 v/v/v)				
Track	254nm (Short UV)		366nm (Long UV)	
	No of spots	Rf value	No of spots	Rf value
1	4	0.04, 0.16, 0.92, 0.97	2	0.04, 0.97

Discussion

Pharmacognosy

Opuntia elatior Mill. (cactaceae), is a fleshy, succulent, perennial herbs, shrub with spines or bristles or both. In India, three species of *Opuntia* are reported. Among them, *Opuntia elatior* Mill. is available in Saurashtra region of Gujarat with a vernacular name 'hathlo thore' *Opuntia elatior* Mill.(26) can be differentiated from other by presence of 2-5 spines in each areole and the characteristic colour changes in flower at various stages (i.e from yellow at first to pink and finally bright red). Fruit of *O. elatior* is oval to corn-shaped with many clafits of very thin 2-5 spines and slight brownish in colour. Average number of seeds is 25-30 in a fruit. Unripe fruit is 4.5 × 2.8 cm and mature fruit is 4.6 × 2.5 cm in size. Average weight of unripe fruit and mature fruit is 9.09 g and 12.52 g respectively.

Diagrammatic section of the fresh fruit shows one to two layers of outer exocarp with square shaped cortical cells having rosette crystal. Middle mesocarp comprises large area, made up of parenchyma cells with chlorophyll pigments. Inner endocarp made up of somewhat elongated completely arranged parenchyma cells. Some cells contain starch grains and rosette crystal.

Diagrammatic section of the matured fruit shows outer 1 to 2 layers of outer exocarp with critical cells and rosette crystal. Middle mesocarp comprises large area made up of parenchyma cells with chlorophyll pigments later on largely filled betacyanins. Inner endocarp is made up of parenchyma cells. Consist some of starch grains and rosette crystal. Endosperms leads into the placenta to the lobule multi capillary lobule consists numerous seeds distributed all over the ground tissues.

Powder microscopy of fruit shows diagnostic characters like multicellular trichomes, rosette crystals of calcium oxalate, coloring matter and starch grains.

The observed pharmacognostical characters of *Opuntia elatior* fruit may be useful to establish the botanical standards for identification and standardization of the plant *Opuntia elatior* Mill.

Analytical study

Ash value of fruit *Swarasa* of *Opuntia elatior* Mill. fruit was 3.59 % w/w. The ash value indicates the presence of inorganic and salt materials in the sample. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological' ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.(27) pH value of the juice is 5.27 which is low. This may be as it is comparatively rich in organic acid. pH is the main factor affecting the stability of vitamin C, thus high values of pH favoring the oxidation processes of vitamin C(28) The overall range of pH is 2 to 5 for common fruits with the most frequent figures being between 3 and 4.(29)

Total solids contents are related directly to both the sugars and fruit acids as these are the main contributors. Pectins, glycosidic materials and the salts of metals (sodium, potassium, calcium etc.), when present, will also register a small but insignificant influence on the solids figure. In the present study, total solid content of the fruit juice is 10.83%. The total solids content is significantly influenced by the combined effect of stages of maturity and ripening conditions.(30)

Viscosity of the juice is 5.11. Knowledge of the viscosity is of primary importance to the fruit juice industry. The accurate viscosity data over wide temperature and concentration regions are need for a various research and engineering applications in any branch of the food industry, such as developing food processes etc.(31) Specific gravity of the fruit juice is 1.042. Specific gravity is commonly used in industry as a simple means of obtaining information about the concentration of solutions of various materials such as brines, hydrocarbons, sugar solutions (syrups, juices, honeys, brewers wort, must etc.) and acids.(32)

The HPTLC is rapid method of drug analysis that can be used to efficiency demonstrate the characteristic constituents of a drug or new plant extract. The technique clearly illustrates differences in chemical composition of plant extracts. In the present study, alcoholic extract of *O. elatior* is studied for high performance thin layer chromatography profile at 254nm and 366nm frequency. HPTLC study of alcoholic extract of *O. elatior* showed the presence of four(4) and two(2) spots in short and long UV respectively. Among these, 0.04 and 0.97 is the common Rf value found in both the samples.

Conclusion

The observed parameters of the fruit of *Opuntia elatior* Mill, like morphology, microscopy, physicochemical parameters and HPTLC profile always constant. It may be useful to establish certain botanical standards for identification and standardization of *Opuntia elatior* Mill. for the further studies.

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Photographs

Figure 1: T.S of *Opuntia elatior* Mill. fruit

<p>Fig. 1a: Twig with flowers</p>	<p>Fig. 1b: Unripen fruits</p>	<p>Fig. 1c: Diagrammatic sketch</p>
<p>Fig. 1d: Epidermis</p>	<p>Fig. 1e: Mesocarp cells with chlorophyll pigments</p>	<p>Fig. 1f: Endocarp with seed</p>
<p>Fig. 1g: Mature fruits</p>	<p>Fig. 1h: Epidermis</p>	<p>Fig. 1i: Mesocarp</p>
<p>Fig. 1j: Rosette crystals</p>	<p>Fig. 1k: Spiral vessels</p>	<p>Fig. 1l: Mesocarp cells</p>

Figure 2: Powder microscopy of *Opuntia elatior* Mill. fruit

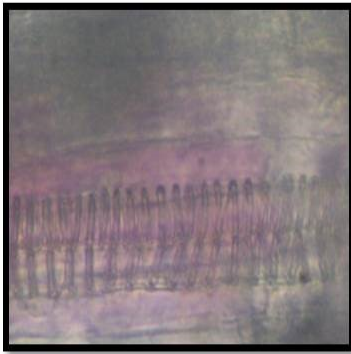


Fig. 2a: Parenchyma cells with starch grain

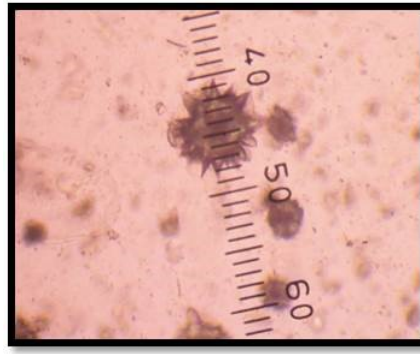


Fig. 2b: Rosette crystals

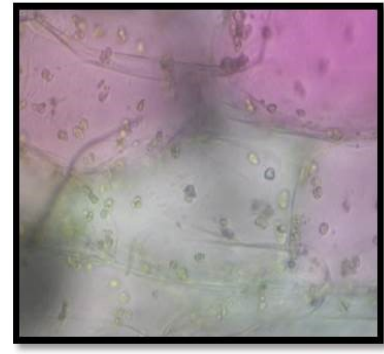


Fig. 2c: Annular and spiral vessels



Fig. 2d: Colouring matter with parenchyma cells



Fig. 2e: Multi cellular trichome

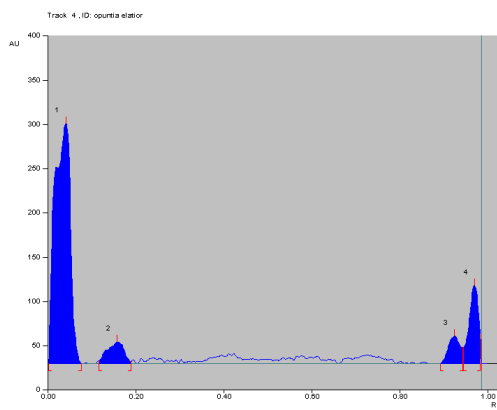


Fig. 3a: at 254nm

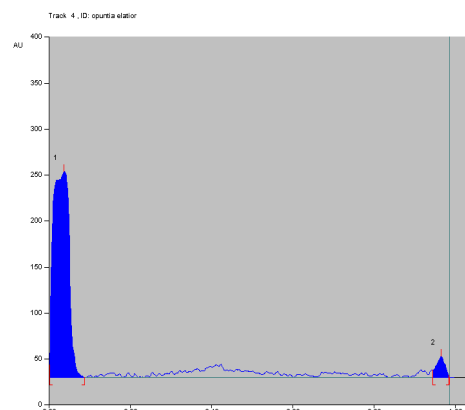


Fig. 3b: at 366 nm

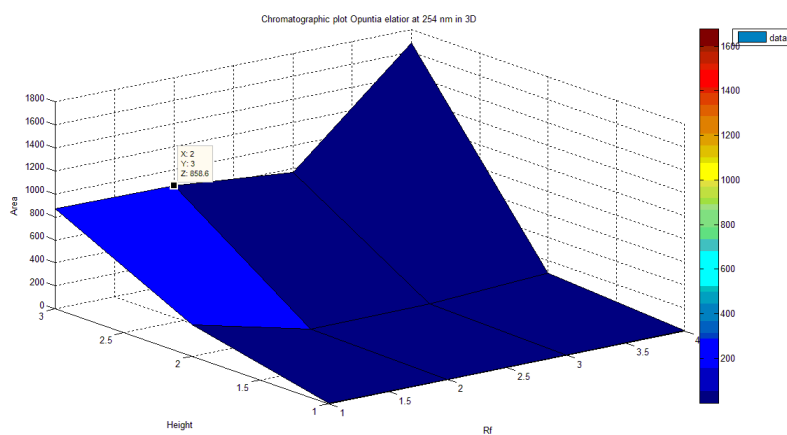


Fig. 3c: Chromatographic plot at 254 nm in 3dimension on context of evaluation report
