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Pharmacognostic and phytochemical evaluation of Bijapur (*Citrus medica* Linn.) fruit

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Abstract
Context: Although Bijora, botanically known as a *Citrus medica* Linn. is an important plant in Ayurveda, little is known about its pharmacognostic evaluation.
Objectives: The present study evaluated pharmacognostic and physicochemical standards.
Methods: Macroscopy, microscopy, phytochemical screening, Thin layer chromatography were carried out
Results: Macroscopy reveals the organoleptic properties of the fruit and microscopic study shows the presence of oil glands and vessels responsible for essential oil production in rind. Water soluble ash value is more than acid insoluble ash indicates that the acid-insoluble siliceous matter was less than that of water soluble ash. Thin layer chromatography of methanolic extract has been carried out and result was mentioned as R_f value. The preliminary phytochemical investigations indicate the presence of carbohydrates, amino acids, flavonoids, tannins and phenolic derivatives, steroids etc.
Conclusions: Pharmacognostic and phytochemical parameters of *Citrus medica* fruit can be considered as distinctive enough to identify the authenticity of fruit.

Keywords: Bijora; *Citrus medica*, pharmacognosy, phytochemical analysis

Introduction
Pharmacognostic study gives the scientific information about the purity and quality of the plant drugs (Dhanabal *et al*., 2005) [4]. The detailed pharmacognostic evaluation gives important information regarding the macroscopic, microscopic and physical characteristics of the plant drugs (Sharma, [date unknown]). *Citrus medica* Linn., commonly known as a Citron in English and Bijora in Gujarati is member of rutaceae family (Kirtikar and Basu, 2007) [10]. It is a slow-growing shrub reaching up to 15 ft (4.5 m) in height with rigid branches, twigs and spines in the leaf axils. The flower buds are white or purplish and large. The peel is yellow, usually uneven, rough and very thick. The pulp is greenish or pale-yellow divided into as many as 14 or 15 segments, hard, not very juicy, acidic or sweet and contains many seeds (National tropical, [date unknown]). Various parts of Bijora are extensively used in Indian conventional system of medicine. Leaves are useful as sedative (Bhavamishra, 2004) [3]. In prehistoric literature citron was mentioned as an antidote of every type of poison (Beatriz and Luis, 2005) [2]. Both the leaves and juice of the citron are used by the community of South-Eastern Nigeria for febrile sickness (Ajaiyeoba *et al*., 2003) [1]. Many pharmacological studies have been carried out to establish the traditional claim of *Citrus medica* L. in an attempt to validate its use as a multi-purpose medicinal agent. *Citrus medica* leaves possesses estrogenic and anthelmintic activities; fruit has insulin secretagogue, analgesic, antiulcer and anticancer activities; peel possesses many activities including hypoglycaemic, hypolipidemic, antimicrobial, anticholinesterase, hypocholesterolemic, and anthelmintic; seed has antidiabetic, hypolipidemic, anticoagulant, hypocholesterolemic and estrogenic activities (Panara *et al*., 2012) [14].

The organoleptic and microscopic explanation of a medicinal plant is the first step towards identification and determination of purity. No scientific parameters are available to ensure quality of plant material and to identify the proper plant material. Therefore the present study has been undertaken to ascertain the various pharmacognostic and phytochemical investigations, which could serve as a measure of confirmation and quality control for marketable samples of the plant drug. Proper recognition and standardization of the crude drug is an important prerequisite to ensure reproducible quality of herbal drug which will contribute to its safety and efficacy. Simple pharmacognostic studies used in standardization of plant material consist of its macroscopical, microscopical, biochemical, phytochemical and histochemical characteristics.

Materials and Methods

Collection, authentication and drying of the plant material

Fruits of *Citrus medica* L. were purchased from local market of Rajkot, Gujarat. Fruits were identified and authenticated by Dr. (Mrs) Sunita Garg, Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources, Delhi, India and voucher specimen deposited in herbarium of the institute. Fruits of *Citrus medica* were properly cleaned, cut in small pieces and dried in shade to remove excess of moisture. The dried material was then subjected to fine powder and passes the powder through sieve no. 50#.

Pharmacognostical evaluation

Macroscopic Characters

The organoleptic characters of fruits such as shape, size, odour, colour, surface, taste etc. were studied for morphological evaluation (Dutta, 2007 [5]; Handbook of Ayurvedic Pharmacopoeia, 2001).

Microscopic Characters

Free hand transverse sections of fresh fruit were taken and washed with chloral hydrate solution. Sections were first observed in distilled water then stained with phloroglucinol and concentrated HCl (Handbook of Ayurvedic Pharmacopoeia, 2001). Powder microscopy of shade-dried fruits powder was also performed. Photomicrographs were taken by Almicro binocular microscope attached with DCE-2 digital camera.

Histochemical Analysis

Histochemical analysis for oil, lignin, starch, tannin and crystals also carried out (Khandelwal, 2008a) [8].

Physico-chemical Evaluation

Physico-chemical parameters like foreign matter, loss on drying, total ash, alcohol soluble extractive, water-soluble extractive values and foaming index were determined as per procedures prescribed in WHO guidelines on quality control methods for medicinal plants materials (WHO, 2011) [18].

Phytochemical Screening

Preliminary phytochemical studies of dried fruits powder were carried out according to the standard procedures described by Horborne (2007a) [6]. All the extracts were dried and weighed. The presence of various phytoconstituents viz. steroids and terpenoids (liebermann burchard test), alkaloids (dragendroffs test), tannins and phenolics (ferric chloride test), flavonoids (shinoda test), amino acids (ninhydrin test), sugars (fehling solution test), carbohydrate (molish test) etc. was detected by usual method prescribed in standard texts (Kokate, 2001; Khandelwal, 2008b; Harborne, 2007b) [11, 9, 7]

Thin Layer Chromatography (TLC)

Methanolic extract of *C. medica* fruits was prepared by using soxhlet extraction method. The extract was filtered and concentrated on rotary evaporator. Thin layer chromatography fingerprinting was achieved by using Silica Gel G as a stationary phase. The concentrated extract was spotted using a capillary tube on a normal phase plate previously activated at 110 °C for 2 h. The plate was developed using mobile phase of Benzene: Acetone (9:1). Isolated spots were visualized under ultra violet light at 254 nm and 366 nm. Further, the plate was sprayed with 10% vanillin sulphuric acid. The retardation factor (R_f) was determined using following formula (Wagner *et al*. 1996) [17].

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Results

A Brief Taxonomic Description of the Plant

Citrus medica Linn. is shrub, about 3.6 m high with tiny, thick and spiny branches, cultivated sparingly throughout the warm-moist regions of the country (Handbook of Ayurvedic Pharmacopoeia, 2001). Its leaflets are 3-6 inch long, ovate-lanceolate with short, wingless or nearly wingless petioles; flowers are small, 5-10 in a raceme; petals are generally more or less pink; fruit is globose ovoid or oblong often mamillate at the apex, with a leathery peel, green when unripe and turns to yellow when ripe. This plant is found apparently wild in Khasia Hills, Garo hills, Chittagong, Upper Yunzalin valley, Kumaon, Pachamarhi, Sikkim, Western Ghats and Saputara range in Central India (Kirtikar and Basu, 2007) [10].

Macroscopic Features (Fig 1)

Weight	2 kg
Shape	oblong to ellipsoid
Colour	green when unripe and turns to lemon yellow when ripe
Diameter	11.85 cm
Length	22.34 cm
Rind Thickness	2.25 cm
Apex Shape	mammiform
Pulp	scanty, sub acid
Base Shape	truncate
Surface	warty, leathery
Axis	hollow
Odour	strong aromatic
Taste	acidic to slightly sweet
Rind Colour	white
Pulp Colour	pale yellow

Organoleptic character of powder

Fine fibrous powder of *Citrus medica* possesses acidic to slightly sweet taste with lemon odor, brown color. (Table 1)

Microscopic Characters of the Fruit

Transverse section of *Citrus medica* fruit shows the following tissue arrangement (Fig 2).

Pericarp: It shows single layer of epidermis covered with thick cuticle, occasionally paracytic stomata present (Fig 3)

Hypodermis: Below the pericarp 2-3 rows of small sized compactly arranged parenchymatous cells of the hypodermis found

Mesocarp: it contain large and porous cells with big oval to spherical lysigenous oil cavities (Fig 4)

Vascular bundle: present in mesocarp (Fig 5)

Endocarp: cells of the endocarp layer are thin walled and elongated

Few simple starch grains and small prismatic crystals of calcium oxalate are scattered throughout the parenchymatous tissue of the section.

Powder Microscopy

The powder microscopy of *Citrus medica* showed xylem vessels, fibers, epidermal cells with calcium oxalate crystals, paracytic stomata and starch grains. Isolated xylem elements were seen united in a bundle. The bundle consists of fibers, vessels and parenchyma cells. Fragment of pitted and spiral vessels were seen scattered in the powder (Figure 6).

Histochemical Analysis
The histochemical analysis of fruit powder confirms the presence of lignin, tannin, oil globules and crystals (Table 2).

Physico-Chemical Evaluation
Various physico-chemical parameters ash values likes (total ash, sulphated ash, acid insoluble ash and water soluble ash), alcohol soluble extractive, water soluble extractive and foaming index of fruit powder were determined and the data are depicted in Table 3.

Qualitative Phytochemical Screening
The percentage of successive soxhlet extractives was calculated and results are shown in Table 4. Preliminary phytochemical studies showed the presence of steroids, flavonoids, terpenoids, phenolic compounds, alkaloids and carbohydrates (Table 5).

Thin Layer Chromatography Profile of Methanolic Extract of *Citrus medica* Fruit
Six different compounds were separated when sprayed with 10% vanillin sulfuric acid (Figure 7, Table 6).

Discussion
In ethnomedicinal practices the traditional healers use *Citrus medica* as 'Bijora' in treatment of various ailments and diseases of human beings and animals. In the present study, the phytochemical standards and pharmacognostic parameters for the *Citrus medica* fruits have shown for the first time. Morphological and microsopical studies of the fruit will enable the recognition of the crude drug and is one of the important parameter in modern monograph. Various analytic characters were identified in the fruit like epidermis, hypodermis, oil cell, mesocarp, endocarp, vascular bundle, starch grains and calcium oxalate crystals. Lysigenous cavities (oil cavities) are internal secretory structure which is identical character of the most of the citrus plant. Secretory cavities are spaces that result from dissolution of cells partly disintegrated cells occur along the margin of the cavity. Higher water soluble extractive value than alcohol show that fruit of *Citrus medica* possesses more water soluble constituents. These characters might be a very significant tool to authenticate the drug for future reference. The information obtained from physicochemical evaluation will be helpful in finding out the genuity of the crude drug. In that respect, various physicochemical parameters like moisture content, ash values, loss on drying and extractive values were also determined and these can be used as reliable aid for detecting adulteration in the crude drugs. Also, for recognition of allied drugs as well as adulterants, such parameters are helpful. Plants show their therapeutic activity through their secondary metabolites, those are actually the biologically active constituents of the plant. Therefore, during standardization of the plant drug, the quality and quantity of such phytoconstituents must be evaluated. In the present investigation, preliminary screening was carried out by conducting qualitative chemical tests and the results of tests showed the presence of terpenoids, steroids, flavonoids, alkaloids, glycosides and carbohydrates as phytoconstituents. Thin layer chromatography analysis was performed with the methanolic fruit extract that exhibited the presence of six components in the extract. It is a potent and most economical tool for correct identification of the plant material, especially in respect of its chemical constituents. Thin layer chromatography fingerprint has become significant quality control tool for herbal samples in natural origin medicines. Fingerprint analysis has been established by world health organization as a methodology for the quality control of herbal samples (Tistaert *et al*., 2011, Handbook of General Guidelines, 2000) [16, 19]. Physicochemical constants and phytochemical screening can serve as an important basis of information and afford appropriate standards to establish the quality of this plant material in future research or applications.

Table 1: Organoleptic characters of powder of *Citrus medica* fruits

Parameters	Observations
Texture	Fine fibrous powder
Color	Brown
Taste	Acidic to slightly sweet
Odor	Lemon flavor

Table 2: Histochemical analysis of powder of *Citrus medica* fruit

Reagent	Constituents	Color change	Result
Iodine	Starch	Blue	+
Ferric chloride solution	Tannin	Bluish black	+
Sudan iii	Oil	Red	+
Phloroglucinol +HCl	Lignin	Pink	+
Phloroglucinol +HCl	Calcium oxalate crystal	Effervescence	+

Table 3: Physicochemical parameters of powder of *Citrus medica* fruit

S. No.	Physicochemical Parameter	Powder of *Citrus medica* fruit
1.	Foreign matter	Nil
2.	Loss on drying	16 % w/w
4.	Total Ash value	5 % w/w
5.	Water soluble ash	2.5 % w/w
6.	Acid insoluble ash	0.15 % w/w
7.	Sulphated ash	11 % w/w
8.	Cold water soluble extractive	20.66 % w/w
9.	Hot water soluble extractive	47 % w/w
10.	Ethanol soluble extractive	26 % w/w
11.	Foaming index	<100
12.	Swelling index	1 ml

Table 4: Successive extractive values of *C. medica* fruit

S. No.	Solvent	Colour in day light	Consistency	Average value of extractive %w/w
1	Petroleum ether	Cream	sticky	2.0%
2	Benzene	Pale brown	sticky	1.4%
3	Chloroform	Cream	sticky	0.4%
4	Ethyl acetate	Pale brown	sticky	0.7%
5	Methanol	Dark brown	sticky	23.1%
6	Chloroform: Water	Dark brown	sticky	17.7%

Table 5: Qualitative phytochemical screening of successive extracts of fruits of *C. medica*

S. No.	Test	P. Ether (60-80 °C)	Benzene	Chloroform	Ethyl acetate	Methanol	Water
1	Alkaloids	-	-	-	-	+	+
2	Carbohydrates	-	-	-	+	+	+
3	Steroids & terpenoids	+	+	+	-	+	+
4	Fixed oils and fats	+	+	+	-	+	+
5	Glycosides	-	-	-	-	+	+
6	Phenolic comp. & tannins	-	-	-	+	+	+
7	Proteins & amino acids	-	-	-	-	+	+
8	Flavonoids	-	-	-	+	+	+

Table 6: TLC profile of methanolic extract of fruits of *C. medica*

Spot No.	R_f	Colour
1	0.03	Light blue
2	0.11	Purple
3	0.26	Light green
4	0.33	Light Pink
5	0.39	Purple
6	0.66	Light Pink

Fig 1: citrus medica fruit

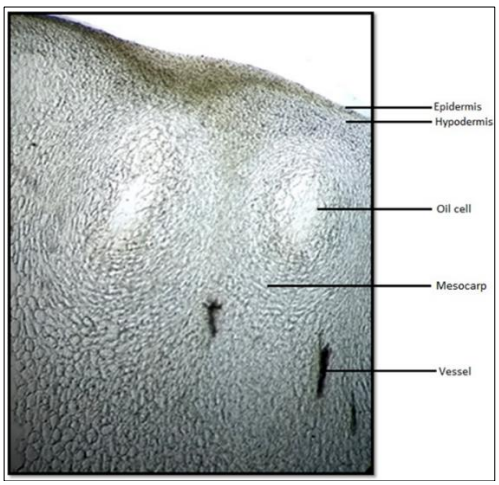

Fig 2: transverse section of citrus medica fruit

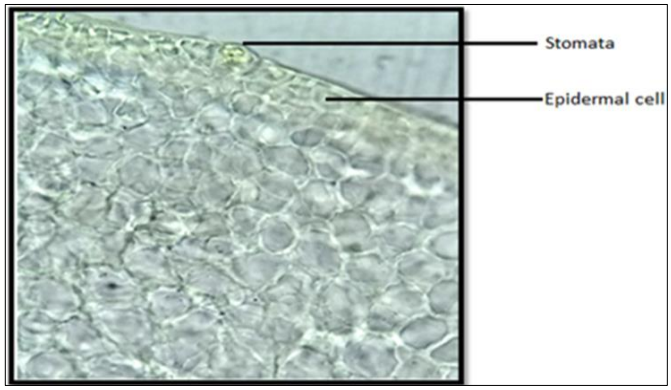

Fig 3: epidermis in TS of citrus medica fruit

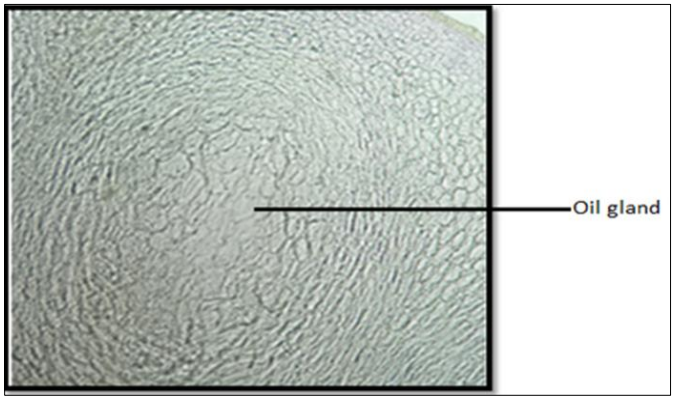

Fig 4: oil gland in TS citrus medica fruit

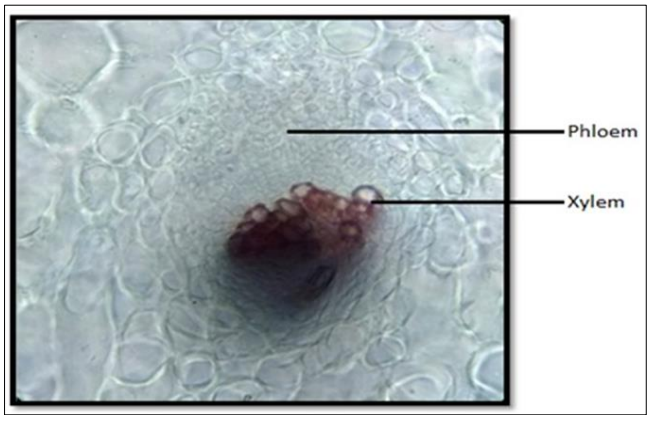

Fig 5: Vascular bundle in TS of citrus medica fruit

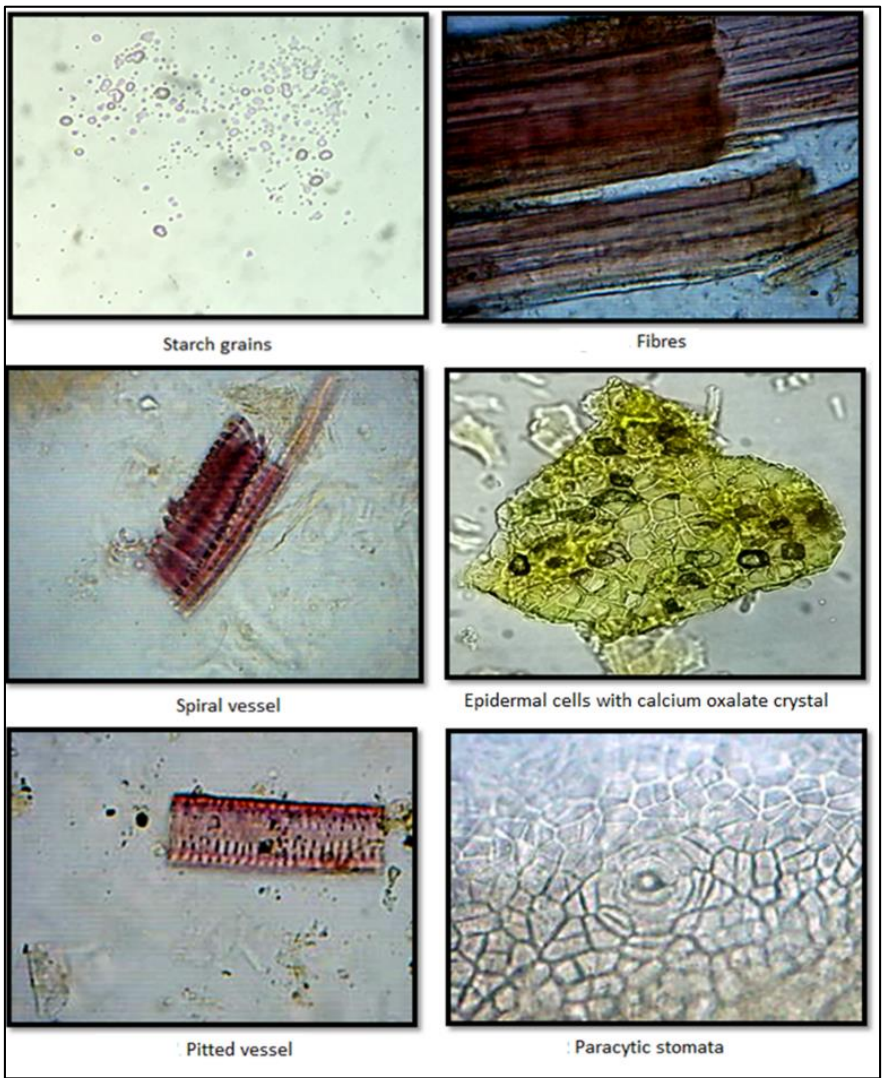

Fig 6: powder microscopy of citrus medica fruit

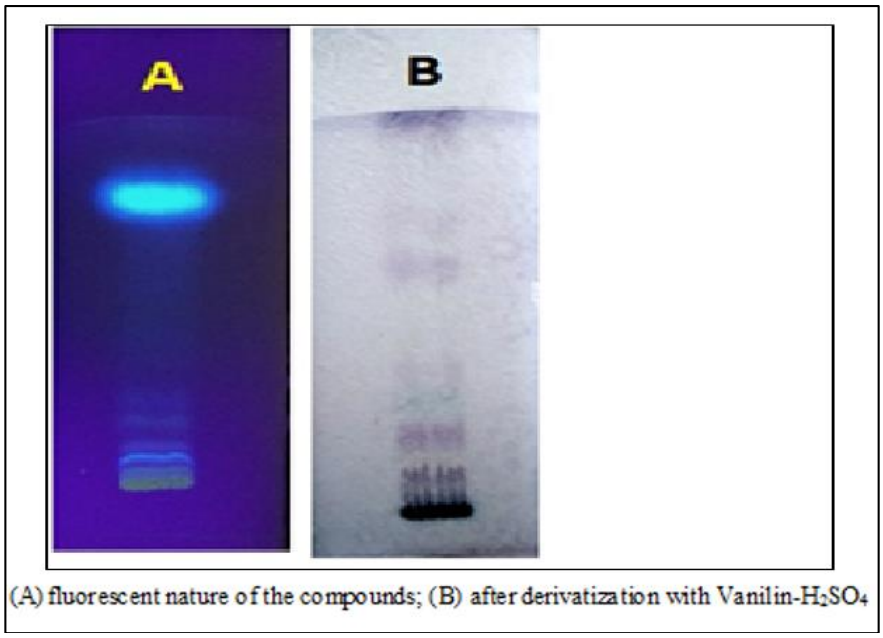

Fig 7: TLC of methanolic extract of fruits of *C. medice*

Conclusion

Establishing standards is an essential part of recognizing the quality, purity and correct identity of a crude drug. These standards must be established before entry of any drug in the pharmacopoeia. The most of the information on the purity, identity and quality of the plant material can be obtained from its morphology, microscopy, phytochemical and physiochemical parameters. Pharmacognostical studies of the *Citrus medica* fruit provided a set of quantitative and qualitative standards that can serve as an essential source of

information to find out the identity and to determine the purity and quality of the plant materials for future studies. These studies can also help the manufacturers for identification and selection of the raw material for drug production. These parameters also will serve as standard data for quality control studies of pharmaceutical preparations which include *C. medica* fruit. In conclusion, these parameters can be considered as distinctive enough to decide and identify the authenticity of this drug in herbal industry. As there is no record on phytochemical and Pharmacognostical study of *C. medica* fruit, the present research work was undertaken to construct some Pharmacognostical and phytochemical standards.

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Conflict of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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