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Development of quality control parameters for the standardization of *Limonia acidissima* L. bark

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Abstract
Limonia acidissima Linn. belongs to family Rutaceae commonly known as wood apple or elephant apple. It is an important traditional medicinal plant. The present study provides pharmacognostic, physicochemical and phytochemical details and macroscopic characters. The successive extraction of plant bark was undertaken by using various solvents of increasing polarity and the extracts thus obtained were subjected for Phytochemical analysis. The phytochemical investigation revealed the presence of alkaloids, tannins, saponin, sterols etc. The above studies provide useful information in regard to its correct identity and evaluation, and help to differentiate from the closely related other species of *Limonia acidissima,* Linn.

Keywords: *Limonia acidissima,* bark, standardization

1. Introduction
Limonia acidissima belongs to family Rutaceae synonomicaly known as *Feronia limonia Swingle* (The wealth of India, 1956) [1], *Schinus limonia Linn* (Chopra *et al,* 1956) [2] and commonly also called as wood apple, Kapitthab, Koyha, *Kava*th and elephant apple. It is distributed throughout the India, common in the wild in dry plains. It is also grown throughout Asia tropical, Asia temperate, Southern America and northern Malaysia. The decoction of the leaves is used in the treatment of constipation, vomiting, cardio tonic and diuretic (Anjaria *et. al.* 2002) [3]. The leaves contain coumarin, triterpinoids and steroids (Patra *et al.* 1988) [4]. The fruits are used for tumors, asthma, wounds, cardiac debility and hepatitis (Saima *et al.* 2000) [5]. The leaves are reported to passess hepataprotective activity (Kamat *et al.,* 2003) [6]. The different parts of plants are reported to show wound healing, antioxidant activity (Ilango and Chitra, 2010) [7], analgestic activity (Khare and Khare, 2011) [8], antidiabetic activity (MohanaPriya, 2012) [9], antiprolifetative effect (Pradhan, 2012) [10], antioxidant activity (Sonawane and Arya, 2013) [11], antimicrobial and cytotoxic activity (Hossain, 2013) [12], phytochemical and antimicrobial activity (Panda *et al.* 2013) [13] etc.

Hence, the objectives of the study were to evaluate various pharmacognostic parameters like macroscopic characters, phytochemical and physicochemical characterization for standardization of Bark.

2. Materials and Methods
a. Plant material collection
The stem bark was collected by self in the month of July Latitude N19º,52',38.6"Longitude E075º,37'72.1" Altitude 466.5 m, from Jayakwadi, Paithan Dist-Aurangabad. Bark was pulverized in the mechanical grinder to a fine powder to carry out different pharmacognostical and phytochemical evaluation and was stored in a well closed airtight vessel for further analysis.

b. Behaviour of bark powder towards some chemical reagents.
The powder of bark was treated with different chemical reagents. The mixture of the powdered drug and chemicals were allowed to warm and cold down for two hours. Changed colour of powdered drug was noted (Table No: - 2).

c. Physico-chemical Evaluations
Physico-chemical parameters such as water soluble ash, water insoluble ash, acid insoluble ash, acid soluble ash, total ash, loss of weight on drying 105^0 was determined. Considering the diversity of chemical nature and properties of contents of drugs, different solvents benzene, petroleum ether, chloroform, methanol, water, alcohol, chloroform water of extractive values were determined as per reported methods (Mukherjee, 2002 [14], Kakate, 1994 [15] and Khandelwal, 2005 [16]) (Table No: - 3).

d. Phytochemical screening
Qualitative examination of inorganic matters and determination of heavy metals was done as per reported methods. The dried powdered bark was subjected to preliminary phytochemical screening for qualitative detection of phytoconstituents. The dried powdered bark (100g) was extracted successively hexane, petroleum ether, benzene, benzene, chloroform, acetone, methanol, water in Soxhlet Extractor by continuous hot percolation. Each time before extracting with the next solvent of higher polarity the powdered material was dried in hot air oven below 50^0C for 10 minutes. Each extract was concentrated in vacuum on a Rote Evaporator and finally dried in hot air oven. The dried extracts were dissolved in respective solvents, with it was extracted, and were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material (Harborne, 2005) [17] (Table No: 4 & 5).

3. Results and Discussion

Pharmacognosy enfolds the knowledge of history, distribution, cultivation, collection, processing for market and preservation, the study of organoleptic, physical, chemical and the uses of crude drugs. The objective of pharmacognosy is to contribute towards establishment of rational relationship between the chemical moieties of naturally occurring drugs and their biological and therapeutic effects, which ultimately helps in the standardization of the plant bark drugs.

Table 1: Organoleptic of Stem Bark of *Limonia acidissima.*

Parameters	Characteristic
Condition	Dried
Colour	Outer surface- Brownish white or brownish green coloured
	Inner surface- Yellowish white or sandal coloured
Odour	Aromatic
Taste	Slightly pungent
Texture	Rough with fracture
Fracture	short, straight, deep fractured and fibrous in inner bark
Size	Length 4-7cm Thickness 5-17mm

Table 2: Reactions of bark powder with different chemical reagents.

Sr. No.	Chemical Reagents	Observation
1	Conc. Sulphuric acid	Reddish Brown
2	Conc. Hydrochloric acid	Dark brown
3	Conc. Nitric acid	Yellow
4	Picric acid	Dark yellow
5	Glacial Acetic acid	Light brown
6	Iodine solution	Light yellow
7	Sodium hydroxide	Brown
8	Potassium hydroxide	Reddish brown
9	Ferric chloride	Green
10	Powder as such	Pale yellow
11	Methanol	Brown
12	10% NaOH	Reddish brown
13	Chloroform	Light yellow
14	Petroleum ether	Dark green
15	Distilled water	Pale green

Table 3: Physico-chemical properties of bark.

Sr. No.	Quantitative Standards	%w/w
1	Total ash	9.80
2	Acid soluble ash	6.30
3	Acid insoluble ash	2.20
4	Water soluble ash	5.70
5	Water insoluble ash	0.90
6	Loss of weight on drying 105^0C	76.50
7	Alcohol soluble extractive value	2.90
8	Water soluble extractive value	5.30

Table 4: Successive extractive values of bark.

Sr. No.	Solvent	Weight of Drug	Average Extractive Value (%w/w)
1	Methanol	10gm	1.60
2	Alcohol	10gm	8.50
3	Benzene	10gm	6.31
4	Petroleum ether	10gm	0.40
5	Chloroform	10gm	4.80
6	Acetone	10gm	2.00
7	Water	10gm	13.60

Table 5: Observation of quantitative analysis of organic of bark.

Sr. no.	Test of organic mater	Petroleum ether	Chloroform	Acetone	Methanol	water
1	Tannin	+	-	+	-	+
2	Alkaloid	+	+	-	-	+
3	Saponin	-	-	+	+	+
4	Sterols	+	-	-	-	-
5	Flavonoids	-	+	-		+

4. Discussion

The phytochemical investigation revealed the presence of tannins, alkaloids, saponin, and sterols compounds mainly in the stem bark of *Limonia acidissima*Linn. The physical evalution furnished different ash values, extractive values in different values. Water soluble ash (5.70), total ash (9.80), and acid soluble ash (6.30), acid insoluble ash (2.20) values were also determined. Thus a variety of standardization parameters viz. morphology, physic-chemical, phytochemical were studied and data was generated for the assessment of quality of plant material, and also to check the adulteration and substitution etc. which may be helpful for future reference.

After present investigation it can be concluded that the pharmacognostic study of stem bark of *Limonia acidissima* have furnished a set of qualitative and quantitative parameters that can serve as an important source of information. All these parameters which were being reported could be useful in identification of distinctive features of the drug.

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