



PHARMACOGNOSTICAL EVALUATION OF FRUITS OF *Embelia robusta* auct. Non roxb

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ABSTRACT

Embelia robusta auct. Non roxb. is the commonly used medicinal plant in the name of vidang. In local, it is called red vidang. But irony is, it is not the true plant of vidang. The true vidang is *Embelia ribes* Burm f. *Embelia robusta* auct. Non roxb. is other species of embelia. Both are two plants of same genus. *Embelia robusta* auct. Non roxb. is often mistaken as vidang & is being sold in market in the name of vidang. Although it has similar properties, actions & chemical constituents like embelin etc. as that of vidang. But it is not the real vidang. Many studies are done on original vidang. But a little work has been done on *Embelia robusta* auct. Non roxb.. Its description is not available even in Ayurvedic pharmacopeia of India.

Therefore preliminary pharmacognostical parameters for the fruits of *Embelia robusta* auct. Non roxb. were studied with the aim of drawing the pharmacopoeia standards for this species. These studies comprise to investigate macroscopy, microscopy, physicochemical parameters, chromatography & preliminary phytochemical screening of fruits of *Embelia robusta* auct. Non roxb.

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INTRODUCTION

Embelia robusta auct. Non roxb. is the allied species & substitute of original Vidang (*Embelia ribes*). It is a rambling shrub or small tree with pale & greenish yellow flowers & red, globose fruits belonging to family myrsinaceae. It is distributed from Ceylon to Malabar Coast through Sylhet & Assam to Singapore¹. Its fruits are antispasmodic, carminative, anthelmintic, and antibacterial. Powdered fruits are used in dysentery. Plant is used in weak pulse rate. Its stem contains embellin².

Therefore capturing its more or less similar pharmacological properties as that of original vidang, a preliminary pharmacognostical account of the fruit of *Embelia robusta* auct. Non roxb. is highly imperative which may provide critical & basic evidence towards a refined knowledge on standardization of the fruit from its original species in the way of botanical, physicochemical, phytochemical phenomena. Thus the present study was undertaken with the aim of drawing the preliminary pharmacopoeia standards for *Embelia robusta* auct. Non roxb. species.

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MATERIAL AND METHODS

Plant material

The dried fruits of *Embelia robusta* auct. Non roxb. were collected from Herbal garden of Research Institute, Indian system of Medicine, Joginder nagar, Himachal Pradesh in the month of September. The collected fruits of *Embelia robusta* auct. Non roxb. were authenticated at herbarium section, Department of Botany, Rajasthan University, Jaipur with authentication no. RUBL 211596. The shade dried powder was used for the determination of physicochemical parameters and phytochemical screening.

Materials

Chemicals used

Safaranine, iodine, ferric chloride, methanol, petroleum ether, formic acid, dragondroff's reagent, glycerine, eosin, methylene blue, ethanol, toluene, ethyl acetate, pH buffer tablet 4 & 7, hydrochloric acid, sodium hydroxide, potassium ferrocyanide, glacial acetic acid, distilled water, potassium thiocyanide, sulphuric acid, barium chloride, molybdate reagent, magnesium reagent, methanol, mollisch reagent, acetic acid, potassium chromate, potassium iodide, ammonia, ammonium chloride, ethanol, iodine solution, sodium potassium tartarate, carbon tetra chloride, sodium nitroperrusside, nitric acid, potassium bismuth iodide, lead acetate, ferric chloride, chloroform, pyridine, mayor's reagent, benzene, acetone, millon's reagent, ninhydrin



Picture 1 showing fruits of *Embelia ribes* Burm. f.



Picture 2 showing fruits of *Embelia robusta* auct. Non roxb.

Equipments and apparatus used

Digital balance, hot air oven, silica crucible, grinder, muffle furnace, dessicator, TLC plate, TLC chamber, heating mantle, soxhlet apparatus, condenser, water bath, thimble, hot plate, digital pH meter, common glass ware, rotary shaker, microscope with camera, UV chamber

Macroscopic study

Crude samples were spread on table & then examined for size, shape, colour, odour, touch & taste.

Microscopic study

For microscopical examination of fruit and seed, the specimens or outer coat of seed or fruit were taken & examined:

1. **Outer Coat**-For examining the outer coat boiled 3 or 4 seeds or fruits in caustic alkali solution in a test tube for 1-2 minutes. After boiling, placed the pieces on slide and examined them after mounting in glycerol solution.
2. **Section** -Seeds were embedded in paraffin wax blocks for section cutting. For this, a block of paraffin (0.6 × 0.5 × 1.5 cms. in size) was made and the seed was embedded in the block by making a cavity or a pit in the block with a hot teasing needle. Cut the section with a sharp razor (through the object) together with the paraffin, placed them on to the slide, removed paraffin with a needle and examined the section in *safaranine solution*³.

Physicochemical parameters

Various physicochemical parameters such as total ash, water soluble ash, acid insoluble ash, water extractable matter, alcohol extractable matter, foreign matter, moisture content were calculated as per Ayurvedic pharmacopeia of India guidelines⁴.

Chromatography

T.L.C.plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator were used as stationary phase. Toluene, Ethyl Acetate and formic acid were mixed at the ratio of 14: 5: 1 to prepare the mobile solution. Visualization was done in day light, in iodine vapours, in Dragondroff's reagent. Rf value was calculated in each medium⁵.

Preliminary phytochemical screening

The aqueous and alcoholic extracts were subjected to qualitative chemical examination for the identification of various plant constituents like carbohydrates (Molisch's test), reducing sugars (Benedict's test, Fehling solution test), monosaccharides (Barfoed's test), alkaloids (mayer's reagent test, Dragondroff's reagent test, Wagner's test, Hager's test), amino acids (Ninhydrine test, Xanthoprotic test, Millon's test, Biurate's test), Glycosides(Borntrager's test), phenolic compounds, saponin(Foam test), Steroids (Salkowaski test), tannin (Fe Cl₃, Lead acetate, potassium dichromate test). All the test were followed according to the guidelines of CCRAS⁶.



Picture no 3 showing fruits of *Embelia robusta* auct. Non roxb

OBSERVATIONS AND RESULTS

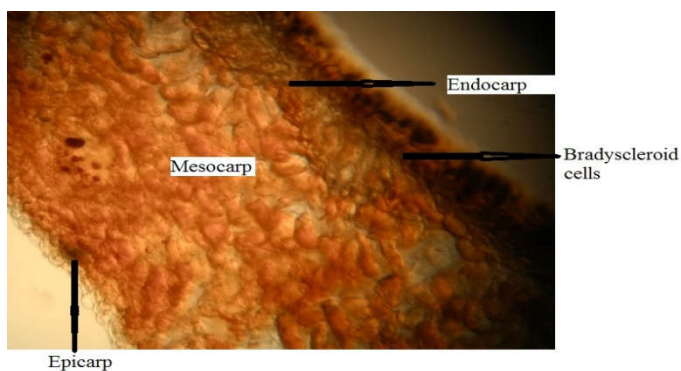
Macroscopic study



Picture no 4 showing seed of *Embelia robusta* auct.Non roxb

Fruit are reddish black in colour, globular in shape with 3-5 mm diameter. It has warty surface with a beak like projection at apex. Pericarp is brittle enclosing a single seed covered by a thin membrane. Entire seed is brown and covered with yellowish spots (*chitra tandula*). Its odour is characteristic with sweet & slight astringent taste.

Microscopic study



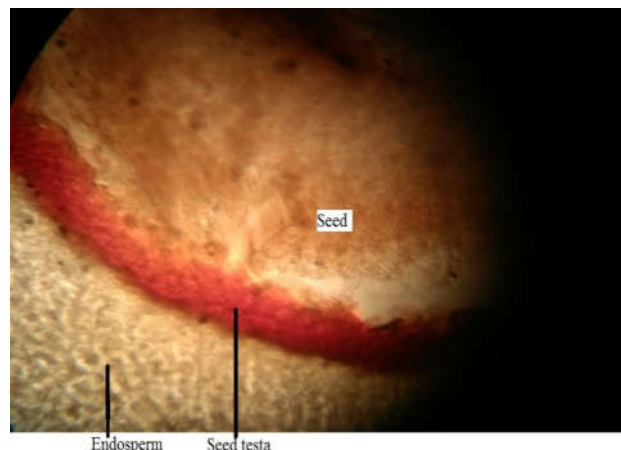
Coloured Bradyscleroid cells (Type of sclerenchyma)

Picture no 5 showing microscopic structures of fruits of *Embelia robusta* auct.Non roxb.auct.Non roxb

Fruit: Transverse section of fruit shows epicarp pink in colour consisting of 2-3 layers of tabular cells of epidermis, usually obliterated, in surface view, cells are rounded, mesocarp consists of a number of layers of brown coloured cells of irregular shape, inner part of mesocarp and endocarp composed of bradyscleroid cells.

Seed: Seed testa composed of 6-7 layered reddish-brown coloured cells. Perisperm consisting of bradyscleroids cells with no intercellular space & radially distributed on outer surface of endosperm. Endosperm occupies major portion of

seed. Numerous endosperm cells, irregular in shape are present with dense cytoplasm containing fixed oil and proteinous masses, embryo small when present, otherwise most of the seeds sterile.

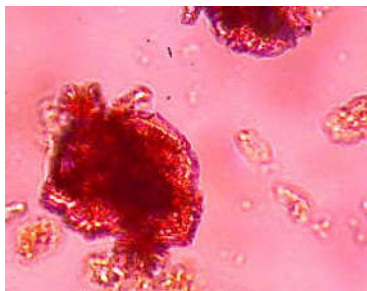


Enlarged endospermic cells

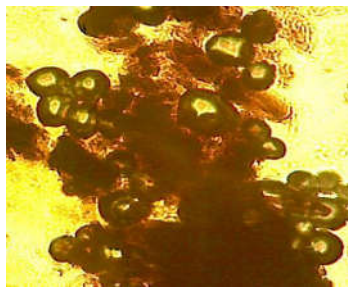


Picture no 6 showing microscopic structures of seeds of *Embelia robusta* auct.Non roxb.auct.Non roxb

Powder microscopy



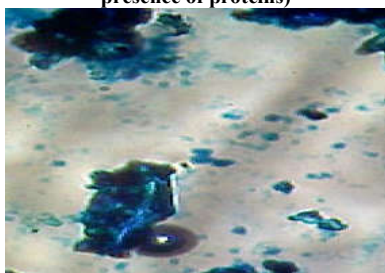
Eosin (red coloured crystalloids indicates presence of proteins)



Fec13 (green colour indicates the presence of tannins)



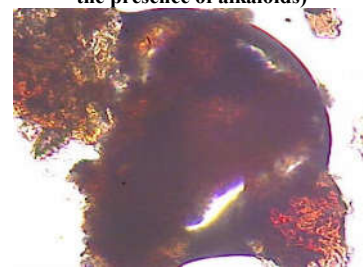
Iodine (reddish brown colour indicates the presence of alkaloids)



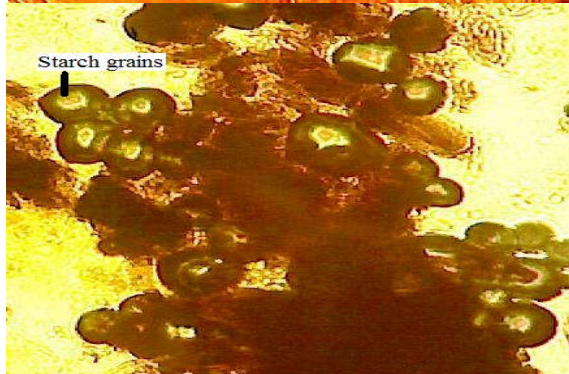
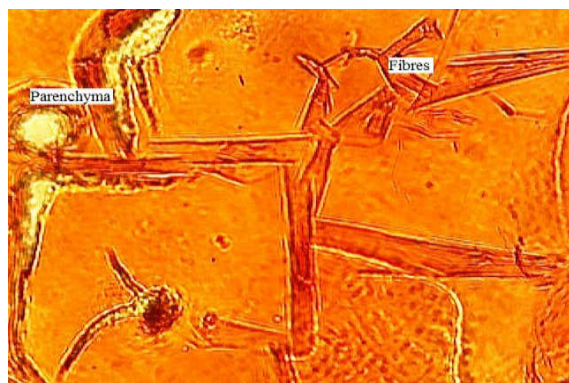
Methylene blue (deep blue colour indicates presence of mucilage)



Safaranine (red colour indicates presence of lignin)



Iodine (deep blue colour indicates the presence of lignin)



Fibres, parenchyma, lignified xylem vessels, starch grains were visible in powder microscopy. Powder microscopy depicts the presence of tannin, alkaloids, proteins, mucilage & lignin in *Embelia robusta* auct. Non roxb.

Physiochemical parameters

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. Results of these parameters are shown in the table 1.

Table 1 showing physiochemical parameters of *Embelia robusta* auct. Non roxb

S.no.	Test	Value
1.	Moisture content	8.42% W/W
2.	pH value	4.19
3.	Aqueous soluble extract	2.94% W/W
4.	Alcohol soluble extract	10.88% W/W
5.	Petroleum ether soluble extract	4.52% W/W
6.	Total ash	3.28% W/W
7.	Acid insoluble ash	0.60% W/W
8.	Water soluble ash	2.40% W/W

Chromatography

Thin layer Chromatography is a tool for separation and identification of chemical constituent.

Mobile solution

Toluene : ethyl acetate : formic acid
7 : 2.5 : 0.5

Stationary phase: silica gel-G

Fig no 7 showing powder microscopy of fruit of *Embelia robusta* auct. Non roxb



Visualization in day light



Visualization in iodine vapours



Visualization in Dragondroff's reagent

In alcoholic extract

Table no 2 showing Rf values of *Embelia robusta* auct. Non roxb. in different media

	In Day light	In Iodine vapours	In Dragondroff's reagent
Rf value	0.18, 0.58	0.13, 0.27, 0.57, 0.63, 0.75	0.58

Phytochemical analysis

The powder was used for the analysis of secondary metabolites by qualitative tests. The results are given in table 3. The efficacy of plants depends on the use of plant parts and its bioactive potency in turn depends upon the presence of secondary metabolite in a raw drug.

Carbohydrate test

Sr. no.	Name of test	Aqueous extract	Alcohol extract	Petroleum ether extract
A.	Molisch test	+ve	-ve	-ve
B.	Benedict test	-ve	+ve	-ve
C.	Barfoed's tests	+ve	+ve	+ve
D.	Fehling test	+ve	+ve	-ve

Alkaloids

A.	Dragondrof test	-ve	+ve	-ve
B.	Wagner's test	-ve	-ve	+ve
C.	Hager's test	-ve	+ve	-ve

Amino acids

A.	Ninhydrine test	+ve	+ve	-ve
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Proteins

A.	Biuret test	-ve	+ve	-ve
B.	Xanthoprotic test	-ve	+ve	-ve
C.	Millon's test	+ve	+ve	-ve

Saponin

A.	Foam test	-ve	+ve	-ve
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Glycosides

A.	Borntragar's test	-ve	+ve	-ve
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Phenolic compound

A.	Phenolic test	-ve	+ve	+ve
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Steroids

A.	Salkowaski reaction	+ve	+ve	-ve
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Flavonoids

A.	Shinod's Test	+ve	+ve	-ve
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Tannin

A.	FeCl ₃ test	-ve	+ve	+ve
B.	Lead acetate test	+ve	+ve	-ve
C.	Potassium dichromate test	+ve	+ve	-ve

Table no 3 showing phytochemical analysis of *Embelia robusta* auct. Non roxb.

DISCUSSION

Phytochemical analysis

Preliminary phytochemical screening showed the presence of Carbohydrate, Alkaloids, Amino acids, Proteins, Saponin, Glycosides, Phenolic compound, Steroids, Flavonoids, Tannin in *Embelia robusta* auct. Non roxb.

Embellin, a benzoquinone (Flavonoids), is an active chemical constituent of *Embelia robusta* auct. Non roxb. Qualitative phytochemical analysis shows that the flavonoids present in aqueous and alcoholic extract are responsible for its anti-oxidant and immunomodulatory property.

Presence of the phytoconstituents can be used as a major tool for obtaining quality control profile for a drug.

Physio-chemical analysis

The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. The moisture content of the drug i.e. *Embelia robusta* auct. Non roxb. is not too high, thus it could discourage bacterial, fungi or yeast growth.

Equally important in the evaluation of crude drugs, is ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. Only 3.28 % of inorganic substance and silica matter is present in *Embelia robusta* auct. Non roxb. Extractive value shows concentration of soluble chemical constituent in aqueous & alcoholic medium.

Alcoholic extract of *Embelia robusta* auct. Non roxb. has high concentration of alcoholic soluble chemical constituents. Ph. of *Embelia robusta* auct. Non roxb. is 4.19. As ph. is concerned it has acidic ph. But it is not very low. It is safe for local & internal use. It will not cause gastric irritation. Five chemical constituents are separated in mobile solution made up of toluene, ethyl acetate, formic acid in the ratio of 7: 2.5: 0.5. After visualization under iodine vapours Rf value of five constituents are 0.13, 0.27, 0.57, 0.63, 0.75.

CONCLUSION

In the present organoleptic, microscopic, physicochemical investigation and preliminary phytochemical screening of fruits of *Embelia robusta* auct. Non roxb., it provides useful information in regard to its correct identity and evaluation, and help to differentiate it from the closely related & original species of *Vidang* (*Embelia ribes* Burm f.). The other study viz.

chromatography analysis add to its quality control and quality assurance for proper future identification of the plant, and may serve as a standard monograph for identification and evaluation of fruit. Further research on this species may help in the isolation of therapeutically potent compounds which can be finally subjected to pharmacological activities, thus leading to opening up new avenues in the use of natural products for therapeutic purpose.

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