



Biomarkers of *Embelia ribes* Burm. f. and its Adulterant *Piper nigrum* L.

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ABSTRACT: *Embelia ribes* Burm. f., locally known as false black pepper or Vidanga, belongs to the family Myrsinaceae. It resembles black pepper (*Piper nigrum*), which is used as an adulterant of *Embelia ribes*. These two plants are similar in fruit shape, color, and micromorphological characteristics; therefore, it is necessary to record the diagnostic features of both plants to establish their botanical identity. In the present investigation, pharmacognostic and phytochemical studies of the fruits of *Embelia ribes* and its adulterant *Piper nigrum* were carried out to identify and locate biomarkers. The results revealed that macroscopically, the fruit of *Embelia ribes* Burm. f. is distinct from *Piper nigrum* L. such as small beak-like persistent style at the apex of the fruit, reddish-brown seeds with yellow spots, and a ruminant endosperm. The fruit wall, or pericarp, is three-layered, viz., single-layered rectangular cells of epicarp, two-layered fleshy parenchymatous mesocarp, and stony endocarp. The fruit of *Piper nigrum* is globular and black in color with single-layered polygonal cells of outer epicarp, a broad and several-layered mesocarp, and a single-layered endocarp. Phytochemically, both fruits possessed distinct active constituents. *Embelia ribes* possessed embelin, which is a benzoquinone compound, while *Piper nigrum* contained an alkaloid, piperine.

Keywords: *Embelia ribes*, Embelin, *Piper nigrum*, Piperin, Thin Layer Chromatography.

INTRODUCTION

Embelia ribes is one of the most significant medicinal plants. Nearly every part of this plant contains varied chemical constituents and is utilized in the treatment of various diseases. *Embelia ribes* berries contain a few chemical constituents like embelin, volatile oil, fixed oil, resin, tannin, christembine, and phenolic acids like caffeic acid, vanillic acid, chlorogenic acid, cinnamic acid, and o-coumaric acid. 4.33% of the embelin content is observed within the berries of *Embelia ribes* Burm. f. It is a critically endangered medicinal plant known for its digestive, carminative, and laxative effects. *Embelia ribes* also has the following properties: antihelminthic, antibacterial, antioxidant, antidiabetic, anticonvulsant, anticancer, antihyperlipidemic, antifungal, antihyperhomocysteinemic, antitumor, anti-inflammatory, chemotherapeutic, contraceptive, anxiolytic, antidepressant, antimitotic, cardioprotective effect, antiobesity, and antihyperlipidemic are all treated with it (Pratik *et al.*, 2021). The cooked tender leaves and fruits are reported to have activity against infections of the kidney, ringworm, and other skin diseases and as a carminative to prevent flatulence in children (Choudhary *et al.*, 2021). According to Rout *et al.* (2021), the berries

of the *E. ribes* plant contain embelin, unstable oil, fixed oil, sap, tannin, and phenolic acids. Embelin, a benzoquinone, and embelinol, an alcohol, are both found in the seeds of the *Embelia ribes* plant (Sharma *et al.*, 2022; Wankhade *et al.*, 2021). Embelin extracted from berries of *E. ribes* with n-hexane by Soxhlet extraction was characterized using high-performance thin-layer chromatography, infrared, and liquid chromatography-mass spectrometry analyses. The presence of embelin was confirmed using the above-mentioned analytical techniques. Treatment with embelin showed amelioration of obesity biomarkers along with a substantial decline in the level of protein expression of nuclear factor erythroid 2-related factor and nuclear factor kappa-B in liver tissue. Treatment with embelin also normalizes the liver tissue levels of thiobarbituric acid reactive substances, glutathione, superoxide dismutase, and catalase. The histopathological analysis of liver tissue showed significant prevention of necrotic and inflammatory changes in embelin-treated HFD-fed mice (Guo *et al.*, 2020). Embelin occurs in golden yellow needles and is insoluble in water but soluble in alcohol, chloroform, and benzene. The decoction of dried fruits is used for fevers, and the paste is applied for skin diseases, while the fruit, in powdered form, when

taken with milk and followed by a purgative, has been one of the ancient remedies for eliminating tapeworms. It is also useful in flatulence, dyspepsia, and ringworm infections of the skin. Seed possessed antibiotic and anti-tubercular properties (Shelar *et al.*, 2009). It resembles black pepper (*Piper nigrum*), which is frequently used as an adulterant of *Embelia ribes*. These two plants are similar in their fruit shape, color, and micro-morphological characters. It is necessary to record the diagnostic features of both plants for their botanical identity. Hence, the present study is carried out to locate the biomarkers useful for its identification.

MATERIALS AND METHODS

The fresh aerial twig bearing flowers and fruits (Plates 1a, 1b, and 1c) of *Embelia ribes* and *Piper nigrum* (Plate 2a, 2b, and 2c) were collected from the natural habitat of the forest at Ayyapankoil in the Idukki District. The young drupaceous berries of *E. ribes* were green in color, and the ripened fruits were orange-red to black in color. The collected fruits were placed in sealed polythene bags and brought to the laboratory, and identified and authenticated with the Flora of the Presidency of Madras (Gamble, 1976).

Biomarkers

Anatomy. Freehand sections of dried, softened fruits of *Embelia ribes* and *Piper nigrum* were taken using the razor blade, and sections were stained with safranin (1%) and mounted on glass slides in glycerin. The diagnostic features of both fruits were identified and noted. Photomicrographs of the sections were taken using a

Trinocular Olympus CH20i microscope fitted with a Canon digital camera.

Powder Analysis. The finely powdered drugs were scanned under a microscope, and the powder characters were identified; photomicrographs were also taken.

Phytochemistry. The collected fresh fruits were shade-dried and completely dried by keeping them at 60°C in a hot air oven for 6 days. The dried materials were powdered with a mechanical grinder and stored in airtight polyethylene bags. Standard procedures (Harborne, 1984; Daniel and Denni 2016) are followed for the identification of the chemical constituents.

Thin Layer Chromatography of Embelin

Embelin is extracted directly by shaking 20g of powdered fruit in ether. The ether extract is concentrated, spotted on TLC, and separated using the solvent system chloroform: ethyl acetate: formic acid in the ratio of 5:4:1. Embelin gives a red color when sprayed with a 20% solution of aniline in alcohol.

Thin Layer Chromatography of Piperin

20g of finely powdered black pepper is refluxed in a 250ml round-bottom flask with 100ml of methanol for 30 minutes. The extract is filtered, and the filtrate is concentrated by distillation to 10 ml. The solution is spotted on TLC (silica gel G) plates and developed in toluene: ethyl acetate (70:30). The developed chromatograms are sprayed with concentrated sulfuric acid, and subsequent heating turns the alkaloids reddish brown.



Plate 1a: *Embelia ribes* Burm.f Habit.



Plate 1b: Twig with flowers.



Plate 1c: Twig with fruits.

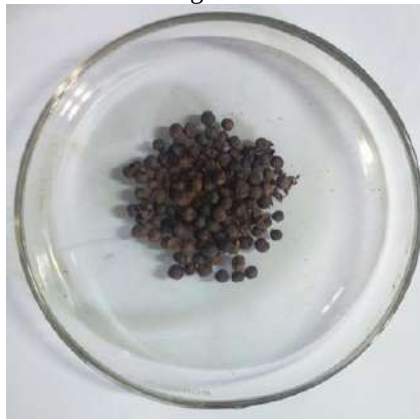


Plate 1d: Dried Fruits.



Plate 2a & 2b: *Piper nigrum* L. Habit



Plate 2c: Dried Fruits

RESULTS AND DISCUSSION

Pharmacognostic studies of *E. ribes* Burm. f.

Macroscopic characters

The dried mature fruit of *E. ribes* Burm. f. (Vidanga) It is brownish black with a warty surface and a small, beak-like, persistent style at the apex (**Plate 1d**). The pericarp is brittle, enclosing a single seed covered by a thin membrane. The entire seed is reddish and covered with yellowish spots.

Microscopic characters

Anatomy: T.S. of Fruit

The fruit wall, or pericarp, is differentiated into three layers: epicarp, mesocarp, and endocarp (Plates 1e and 1f). The epicarp has a wrinkled surface and is brownish-black when dried. Inside the endocarp, there is a single

seed (Plate 1i). In mature fruit, the epicarp is thin, the mesocarp is fleshy parenchymatous, and the endocarp is stony. The epidermis of the epicarp consisted of a single layer of rectangular cells, covered by a thick cuticle. The mesocarp is differentiated into outer and inner layers. The outer layer consists of parenchyma cells with patches of sclereids, fibrovascular bundles, oil bodies, starch grains, and a few prismatic and druse calcium oxalate crystals (Plate 1g). The inner layer is composed of stone cells. The seed coat consists of 2-3 layers of reddish-brown-colored cells. The seed was enclosed by a stony endocarp (Plate 1h).

Powder study: The dried powdered fruit is black-brown in color and contains prismatic and druse calcium oxalate crystals, starch grains, and reddish-brown colored parenchyma and stone cells (Plate 1j).

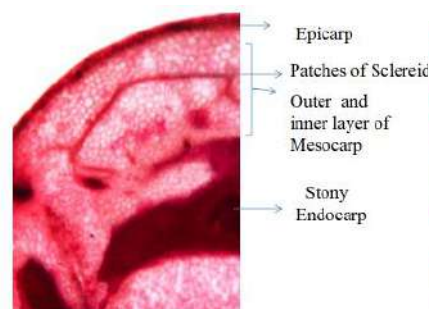


Plate 1e: T.S. of Fruit 100x

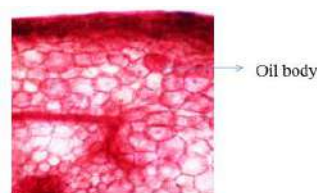


Plate 1g: T.S. of Fruit 400x

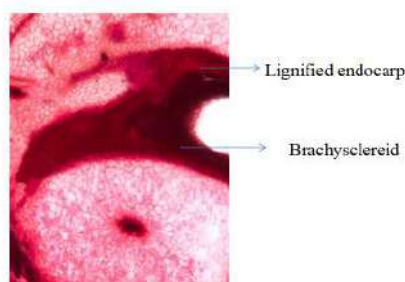


Plate 1f: T.S. of Fruit 100x

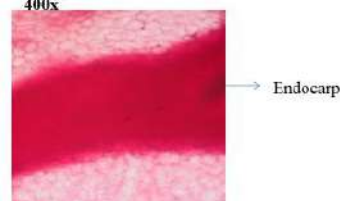


Plate 1h: T.S. of Fruit 400x

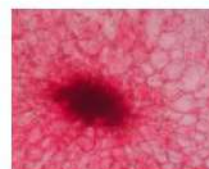


Plate 1i: Single Seed 400x

Phytochemical Analysis

Phytochemically, the fruit of *Embelia ribes* contained the active constituent embelin. In paper chromatography, embelin appears as a purple spot or band next to the solvent front when the paper is sprayed with a 10% sodium carbonate solution (**Plate 1k**). In thin-layer

chromatography, embelin gives a red color when the developed chromatograms are sprayed with a 20% aniline-alcohol solution (Plate 1l).

Pharmacognostic studies of *Piper nigrum* L.

Macroscopic characters

The dried fruit is a globular drupe, brown or black in color, which is aromatic and strongly pungent.

Microscopic characters

Anatomy: T.S. of fruit consists of three layers: outer epicarp, middle mesocarp, and inner endocarp. The outermost layer of epicarp is composed of polygonal cells with a distinct cuticle with dark brown to blackish contents. Just below the epidermis are 2-3 layers of thin-walled parenchyma cells intermingled with thick-walled isodiametric to radially elongated stone cells (Plate 2e). The mesocarp is the broad zone consists of outermost parenchyma cells containing small starch

grains and scattered secretory sacs with suberized walls containing oleoresin (Plates 2d and 2f). Below this are several layers of compressed cells, large oil cells, and two layers of small parenchyma cells. The endocarp consists of a single layer of small parenchyma cells. Spermoderm consists of 2-3 layers of compressed, elongated cells; beneath this is a pigment layer containing dark brown tannin substances (Plate 2e). There are scattered cells with rounded oleoresin cells containing globules of oil, masses of resin, and piperine (Plate 2g).

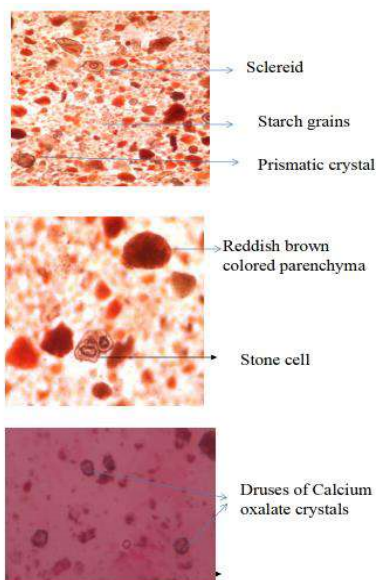


Plate 1j: Powder characters of *Embelia ribes* Burm. f.

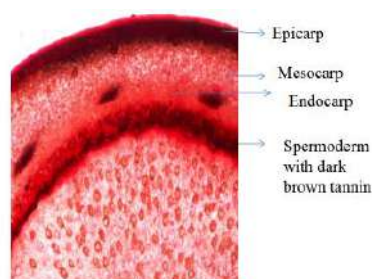


Plate 2d: T.S. of Fruit 100x

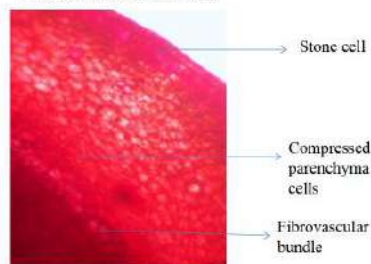


Plate 2e: T.S. of Fruit 400x

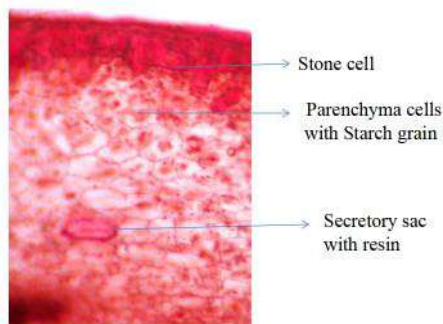


Plate 2f: TS of Fruit 400x

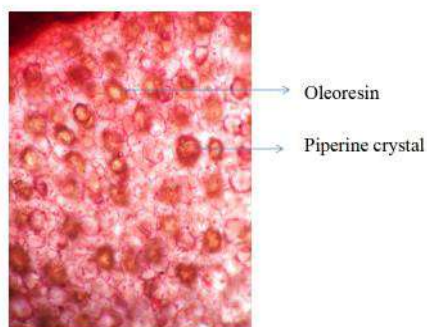


Plate 2g: T.S. of Fruit 400x

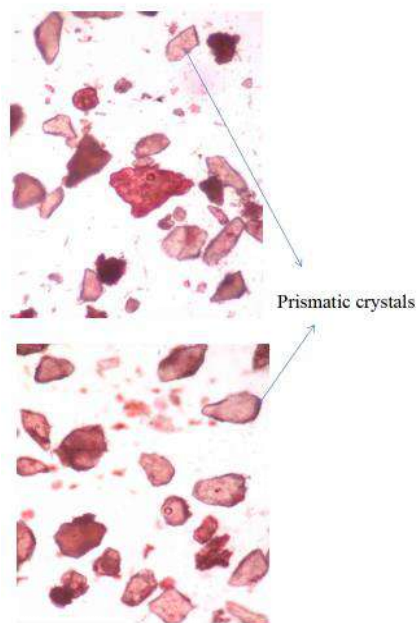


Plate 2h: Powder characters of *Piper nigrum*

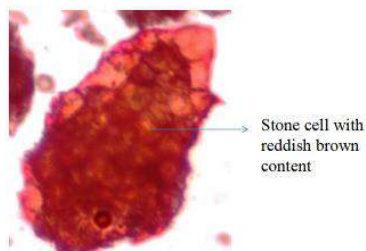
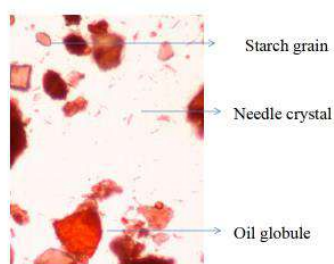


Plate 2i: Powder characters of *Piper nigrum*



Plate 1k: Paper Chromatography of Embelin.

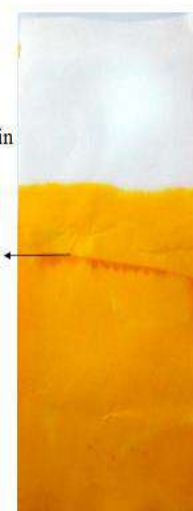


Plate 2j: Paper Chromatography of Piperin.



Before spray

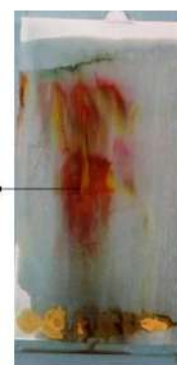


After spray

Plate 1l: Thin Layer Chromatography of Embelin.



Before spray



After spray

Plate 2k: Thin Layer Chromatography of Piperine

Powder study

The dried powdered fruit is dark gray in color, composed of starch grains of various shapes, prismatic crystals (Plate: 2h), needle-shaped piperine crystals, yellowish oil globules, and stone cells with reddish-brown contents (Plate: 2i).

Phytochemical Analysis of *Piper nigrum*

Phytochemically, the fruit of *Piper nigrum* contained the active constituent piperine. In thin-layer chromatography, the developed chromatograms were sprayed with concentrated sulfuric acid, and upon subsequent heating, the alkaloids turned reddish brown (Plate 2k). Biomarkers of *Embelia ribes* and its adulterant *Piper nigrum* are represented in Table 1.

In the present investigation, pharmacognostic and phytochemical studies on the dried fruits of *Embelia ribes* and its adulterant, *Piper nigrum*, were carried out. The results showed that macroscopically, the fruit of *Embelia ribes* is distinct from *piper nigrum* due to the presence of a small, beak-like, persistent style at the apex of the fruit, and the seed is reddish brown with yellow spots. Anatomically, the T.S. of *Embelia* fruit showed

the presence of single-layered rectangular cells of epicarp covered with thick cuticle; a two-layered mesocarp consisting of reddish-brown-colored parenchyma, sclereids, druses, prismatic crystals, stone cells, starch grains, and oil bodies; and a stony endocarp. Macroscopic and microscopic observations reported by Meena *et al.* (2013) ; Sudhakaran (2015) confirm the present results. The previous reports confirm the present results. The powdered drug of *Embelia ribes* contained reddish-brown-colored parenchyma, druses, prisms of calcium oxalate crystals, and starch grains.

While in *Piper nigrum*, the fruit is macroscopically globular and black. Anatomically, it contained outer polygonal cells with cuticle and dark brown to blackish contents, and below 2-3 layers of thin-walled parenchyma cells intermingled with radially elongated stone cells. The mesocarp consists of several layers of parenchyma cells with starch grains, scattered secretory sacs with resin contents, compressed parenchyma cells, fibrovascular bundles, and small parenchyma cells. The endocarp consists of a single layer of stone cells. In *Piper nigrum* similar results, such as fibrovascular, bundles,

stone cells, and perisperm containing starch grains, oil globules, etc., were reported by Madhushree and Vijay (2021). Perisperm consists of three regions with starch grains, rounded oleoresin, and piperine crystals. The *Piper nigrum* powder contained prismatic crystals, starch grains, needle-shaped piperine crystals, oil

globules, and stone cells with reddish-brown contents. Hytochemically, both fruits also possessed distinct active constituents. *Embelia ribes* possessed embelin, which is a benzoquinone compound, while *Piper nigrum* contained an alkaloid, piperine.

Table 1: Biomarkers of *Embelia ribes* Burm. f. and its adulterant *Piper nigrum* L.

Characters	<i>Embelia ribes</i>	<i>Piper nigrum</i>
Macroscopic Characters	Dried mature fruit is brownish- black. A small, beak like, persistent style is present at the apex.	The dried fruit is a globular drupe, brown or black. Beak- like persistent style absent.
Microscopic Characters	Pericarp: three layers The epicarp is composed of single -layered rectangular cells with a thick cuticle. Mesocarp-two- layered The outer layer consists of patches of sclereids and fibrovascular bundles, oil bodies, starch grains, and a few prismatic and druses of calcium oxalate crystals. Inner layer composed of stone cells. The endocarp is stony. Spermoderm and perisperm are absent. Ruminate endosperm.	Pericarp has three layers. The epicarp is composed of polygonal cells with cuticle, intermingled with radially elongated stone cells. The mesocarp is broad and several-layered parenchyma cells with starch grains, scattered secretory sacs with resin contents, compressed parenchyma cells, and small parenchyma cells. Fibrovascular bundles, small starch grains, oil bodies are present. Endocarp is single- layered with small parenchyma cells. Spermoderm and perisperm are present.
Powder Characters	The dried powdered fruit contained, sclereids, starch grains, prismatic crystals, reddish brown parenchyma, stone cells and druses of calcium oxalate crystals.	The dried powdered fruit dark gray in colour composed of prismatic crystals, starch grains, needle crystal, oil globule and stone cells with reddish brown content.
Phytochemical Constituents	The active constituent is Embelin (Benzoquinone).	The active constituent is Piperine (Alkaloid).

CONCLUSION

The results obtained from the present study revealed that the fruits of *Embelia ribes* Burm. f. and *Piper nigrum* L. are pharmacognostically as well as phytochemically distinct. Pharmacognostic and phytochemical markers are effectively used for the identification and authentication of the fruits of *Embelia ribes* Burm. f. from its adulterant species *Piper nigrum* L. This plant is endangered; hence, I recommend its conservation.

FUTURE SCOPE

Embelia ribes is an important Ayurvedic medicinal plant whose main active compound is embelin; it shows anti-inflammatory, antioxidant, antibacterial, anticancerous, and antidiabetic properties. Hence, I recommend that future research should focus on clinical validation, quality control, and conservation.

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Conflict of Interest. None.

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