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Study of preliminary phytochemical and pharmacognoceutical profile of *Embelia ribes* fruit

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Abstract
Over centuries medicinal plants being used around the globe for healthcare regime. Plants being screened for potential therapeutic properties and pharmaceutical applications. *Embelia ribes* is one of the valuable medicinal plants used since from ancient time in Indian medicinal system. In present study morphological, anatomical and preliminary phytochemical analysis of *Embelia ribes* fruits is explored. In our study we found that Carbohydrates, Alkaloid, Saponins, Phenols and tannins, Flavanoids, Glycosides, Proteins, and Steroids are present in the various extracts of *Embelia ribes.*

Keywords: *Embelia ribes*, preliminary phytochemical, pharmacognosy, microscopic

Introduction
Medicinal plants are the invaluable backbone of traditional healthcare system around the world. Still it plays vital role for around 85% of the world's population [1] and major resources for herbal medicines. Ethnic groups of the world have their own traditional knowledge and experiences for healthcare system. Ayurvedic, Unani and Chinese traditional medicine systems uses medicinal plants from ancient times for treating various diseases and fortifying body system [2]. Numerous bioactive compounds have therapeutic values and medicinal plants are repository for such compounds. The wide array of therapeutic properties of medicinal plants includes anti-inflammatory, anti-fungal, anti-bacterial, antioxidant, anticancer and antiallergic. The recent advancement in the field of identification of plant based molecules has up surged interest in herbal medicines and its development. India, being varied in temperature and geographical conditions has a treasure for number of medicinal plants. The major research goal in the field of medicinal plants is of comprehensive and integrative manner to better address nature of medicinal plants.
Embelia ribes, a traditional medicinal plant belongs to Myrsinaceae family and widely used in ayurvedic preparations; commonly known for Krmighna meaning antihelmentic property. It possesses potential anti-inflammatory [6], antioxidant [6], cytotoxic [6], anti-bacterial [6], anti-fungal [6] and wound healing activity [5, 3]. Due to over exploitation, high demands in market, sterility of fruits, low response in tissue culture and destructive harvesting *E. ribes* is threatening for its existence [4]. It is a woody climber and distributed in hills up to an altitude of 1500 meter over Maharashtra, Karnataka, Tamilnadu and Kerala of Western Ghats [4]. Fruits or berries of *E. ribes* which are used in ayurvedic formulations are globular, red to black in colour with warty or wrinkled surface and size varies in between 2-4mm. A principle phytochemical constituent present in *E. ribes* berries is Embelin, which possesses medicinal properties, along with Embelin, embolic acid, tannins, christembine, alkaloids and volatile oils plays vital role. Current study emphasizes on preliminary phytochemical screening of *Embelia ribes* fruit extract and its pharmacognostical profile.

Materials and Methods
Plant collection and identification
Embelia ribes is distributed over a hilly region and grows well in humid climate. The fruits of *E. ribes* were collected from Kelshi, Koyna region of Maharashtra (17°23'20"N 73°39'26"E) and identified by Dr. Suresh Jagtap, Taxonomist and Associate professor (IRSHA, Pune). Fruits were collected and shade dried for further analysis.

Preparation of extract
The dried fruits of *E. ribes* were powdered and 5 gm powder was extracted in 100ml of ethanol, acetone, ethyl acetate, chloroform, hexane and aqueous solvent with soxhlet apparatus for 6hrs. The extracts were filtered through Whatmann No.1 filter paper and stored.

Fig 1: Embelia ribes fruits: collected from Kelshi; Koyna, Maharashtra

Preliminary phytochemical screening
The different solvent extracts of *Embelia ribes* (ethanol, acetone, ethyl acetate, chloroform, hexane and aqueous) were subjected to various chemical tests to detect various phyto-constituents in extracts [7, 8, 9, 10]

Detection of carbohydrates
The extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were subjected to test for detection of carbohydrates.

Molish's test: In a test tube,to 2ml of the filtrate few drops of Molish's reagent was added and 1 ml conc. sulphuric acid was slowly added from side of the test tube. A pinkish red to violet ring indicated the presence of carbohydrates.

Barfoed's test: To 1ml of the filtrate, 1ml of Barfoed's reagent was added. The mixture was heated for 2 minutes. A reddish black precipitate indicated presence of carbohydrates.

Benedict's test: To 1ml of filtrate, 1ml of Benedict's reagent was added. The mixture was heated for 2 minutes and cooled. A characteristic colour change along with precipitate indicated presence of carbohydrates.

Fehling's test: To1ml of extract equal volume of Fehling's solution A and B were added. The mixture was heated for 1-2 minutes and formation of a red precipitate showed presence of carbohydrates.

Detection of alkaloids
Solvent free extracts were added with few ml of dilute HCl and filtered. The filtrate was tested with alkaloid reagents.

Wagner's test: 1 ml of filtrate was taken in a test tube and few drops of Wagner's reagent (iodine solution in potassium iodide) were added. A reddish brown precipitate indicated presence of alkaloids.

Dragendroff's test: To 2 ml of filtrate, 1 ml of Dragendroff's reagent (solution of potassium bismuth iodide) was added and mixed, presence of orange precipitate indicated presence of alkaloids.

Mayer's test: 1 ml each of extract and Mayer's reagent were mixed, a white creamy precipitate indicates presence of alkaloids.

Detection of saponins
Froth test: 1ml of extract was mixed with 2 ml of distilled water in a test tube and shaken vigorously and allowed to stand for 10 minutes. The formation of foam indicated presence of saponins.

Detection of Phenolic Compounds and Tannins
Ferric chloride test: To 2ml of extract, neutral 5% of fresh ferric chloride solution was added. A reddish brown precipitate was occurred and it indicated presence of phenolic compounds.
Gelatin test: 2 ml of 1% gelatine solution prepared in 10% sodium chloride was added to 1 ml of extract. Formation of white precipitate indicated presence of tannins.

Lead acetate test: To1ml of extract 3 ml of 10% lead acetate solution was added. Formation of dirty green and white precipitate indicated presence of phenolic compounds.

Magnesium and Hydrochloride Test: To 1 ml of extract few fragments of magnesium ribbon and concentrated HCl were added slowly. Crimson red to slight pink colour were observed which indicated presence of flavanol glycoside.

Detection of flavanoids
Alkaline reagent test: To1ml of extract 1% sodium hydroxide was added, presence of red or yellow colour indicated presence of flavanoids.

Shinoda Test: To 1 ml of extract, few fragments of magnesium ribbon were added and concentrated sulphuric acid was added slowly through side. Crimson red was observed.

Zinc chloride reduction test: To 1 ml of extract, pinch of zinc dust was added and then concentrated hydrochloric acid along the side of the tube was added. Pinkish white precipitate was obtained.

Detection of glycosides
Keller-Killani Test: 2 ml glacial acetic acid with few drops of 2% of $FeCl_3$ was added to 2 ml of extracts and then 1 ml of concentrated H_2SO_4 was added through wall of test tube. A brown colour ring was observed which showed entity of cardiac steroidal glycoside.

Borntrager's test: The extracts were hydrolyzed with concentrated HCl for 2 hours in water bath, filtered and hydrolysed for the test. To 2 ml of filtrate hydrolysed, 3 ml of chloroform was added and shaken well. After stabilizing, chloroform layer was separated and 10% ammonia solution was added. Formation of pink colour was observed; it indicated the presence of glycosides.

Detection of proteins
The extract was dissolved in 10 ml of distilled water and filtered; filtrate was subjected to following tests:

Biuret Test: 1 ml of extract was heated with 2% $CuSO_4$ solution and to this 1 ml of 1% NaOH solution was added. Formation of pink to violet colour indicated presence of proteins.

Ninhydrin Test: To 2 ml of extract, few drops of Ninhydrin solution was added, a purple colour indicated presence of amino acids.

Detection of steroids
Salkowski's Test: 0.5 ml of extracts was dissolved in 2 ml of chloroform and concentrated H_2SO_4 was added through wall of test tube. A reddish brown ring was observed.

Macroscopic studies
The macroscopic studies of plants provide morphological visualization and description of it and it is the simplest way to establish the identity of a particular plant. Fruit samples were spread and examined for size, shape, colour, odour and taste.

Microscopic studies

Through microscopic analysis, fruits were viewed and studied for the pharmacognostics and morphological studies. Intact fruits/berries, seeds and entire pericarp was studied using Leica stereomicroscope (EZ4HD) equipped with CCD camera. To study the anatomical characters, fine sections of fruits were taken by sharp razor blade and sections were stained with 1% safranin. The stained sections were mounted on glass slide with glycerine and images were taken on Leica optical microscope (DM2500) equipped with CCD camera.

Result and Discussion

Preliminary Phytochemical Screening

Qualitative phytochemical tests of *Embelia ribes* were carried out for the identification of various phyto-constituents.

Results are depicted in table 1. The preliminary phytochemical screening has revealed the presence of carbohydrates, alkaloids, flavnoids, phenolic compounds, tannins, proteins and glycosides. These phytochemicals are the major sources of the plant for herbal medicinal properties.

Table 1: Preliminary Phytochemical Screening

	Test	Ethanol Extract	Hexane Extract	Chloroform Extract	Acetone Extract	Ethyl acetate	Aqueous Extract
Carbohydrates	Molish's	+	+	+	+	+	+
	Barfoed's	+	+	+	+	+	+
	Benedict's	+	+	+	+	+	+
	Fehling's	+	+	+	+	+	+
Alkaloids	Wagner's	+	+	+	+	+	+
	Dragendorff's	+	+	+	+	-	+
	Mayer's	+	-	+	+	-	+
Saponins	Froth	+	+	+	+	+	+
Phenols and tannins	Ferric chloride	+	+	+	+	+	+
	Gelatin	+	-	+	+	+	-
	Lead acetate	+	+	+	+	+	+
	Magnesium and Hydrochloric	+	-	-	+	-	+
Flavanoids	Alkaline reagent	+	+	+	+	+	+
	Shinoda	+	+	+	+	+	+
	Zinc chloride reduction	+	+	+	+	+	+
Glycosides	Keller- Kiliani	+	+	+	+	+	+
	Borntrager's	+	-	-	+	-	+
Proteins	Biuret	+	+	+	+	+	+
	Ninhydrin	+	+	+	+	+	+
Steroids	Salkowski's	+	+	+	+	+	+

Macroscopic studies

The fruits of *E. ribes* are oval in shape and measures about 2-4 mm in diameter. The berries vary in colour from brownish to dull black. As *E. ribes* fruits belong to drupe fruit type; they contain a thin, brittle pericarp and are single seeded. Fruits shows pedicle along with calyx; mostly fruit is with 5 sepals. On detachment of pedicle, a circular hollow scar is seen on fruit pericarp. The fruits have slightly aromatic odour, and astringent taste.

Microscopic studies

The fruits of *E. ribes* are single seeded and entire fruit pericarp is differentiated into three major regions as epicarp, mesocarp and endocarp. The fruit pericarp is warty or wrinkled (Fig. 2a & 2b); epicarp is very thin and consists single row of tabular cells of epidermis [3]. During the development from young fruit to mature fruit, endocarp becomes stony in nature and hence can be easily distinguishable from mesocarp and epicarp [11]. Cross section of dried fruit clearly shows a long pedicle, differentiated epicarp, a thick mesocarp, endocarp and single seed (Fig. 3) Transverse section of fruit which passes through mesocarp shows number of layers of cells (Fig. 4) and numerous fibrovascular bundles. Mesocarp contains oil bodies, abundant starch grain deposition which is oval to elliptical in shape [11]. Rarely a few prismatic crystals of calcium oxalate can be observed in mesocarp; inner part of mesocarp and endoderm is consists of stone cells. The matured fruit shows patches of brachysclereids in mesocarp (Fig.5a &b); brachysclereids resembles the parenchymatous cells and mostly present in fleshy part of fruit. These cells provide protection against mechanical and physiological stresses to the fruit.

Abbreviations: Emb: Embryo, Enc: Endocarp, Epc: Epicarp, Mes: Mesocarp, Ped: Pedicle, Sed: Seed,

Fig 2a & 2b: Embelia ribes: Dried fruits-stereomicroscopic view (x2) showing warty globular seeds

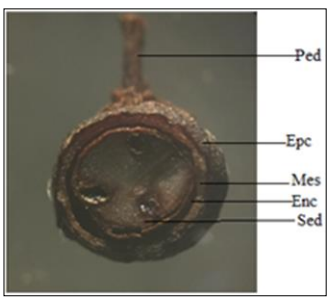

Fig 3: Embelia ribes: Cross section of dried fruit

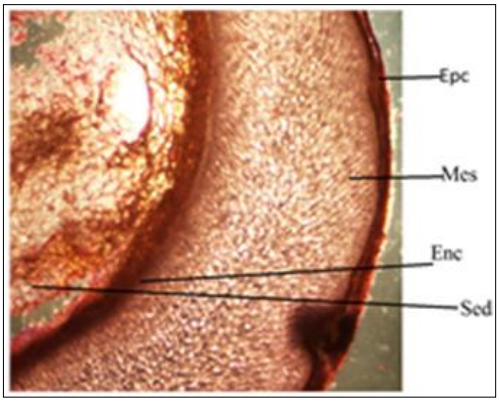

Fig 4: Embelia ribes fruits: T.S. of fruit showing mesocarp and seed portion

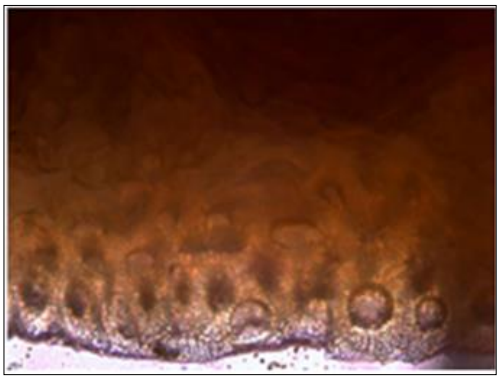

Fig 5a: Embelia ribes fruits: Polarised microscopy of T.S. of fruit showing brachy sclreids

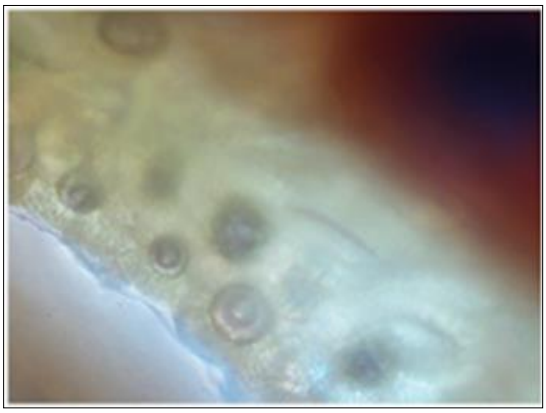

Fig 5b: Embelia ribes fruits: Polarised microscopy of T.S. passing through endocarp and showing sclereids structure

E. ribes fruits contain a single brownish or reddish black seed covered with thin membrane and yellowish spots (chitra tandula). (Fig. 6) The entire fruit is mostly occupied by seed and it is enclosed by stony endocarp [3, 11, 12] Embelin is the active constituent of *E. ribes* and it is present in between seed and pericarp; which can be easily observable. Seed coat composed of 2-3 layered reddish-brown coloured cells. The endosperm occupies major portion of seed (Fig.8) and endosperm cells are thick walled and irregular in shape. The endosperm cells contain fixed oils and protein masses; these protein masses help in seed development. Embryo is small when present, but most of the seeds are sterile. A small portion of embryo is appeared linear and centrally located in endosperm and it can clearly visible when cross section of fruit is taken (Fig. 7)

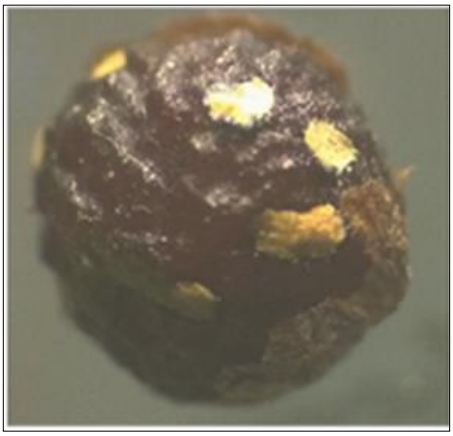

Fig 6: Embelia ribes fruits: Stereomicroscopic view of seed showing yellow spots (chits-tandula)

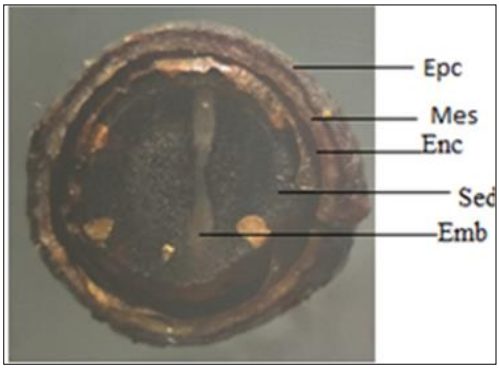

Fig 7: Embelia ribes: Cross section of dried fruit Stereondcroscopic view (x2)

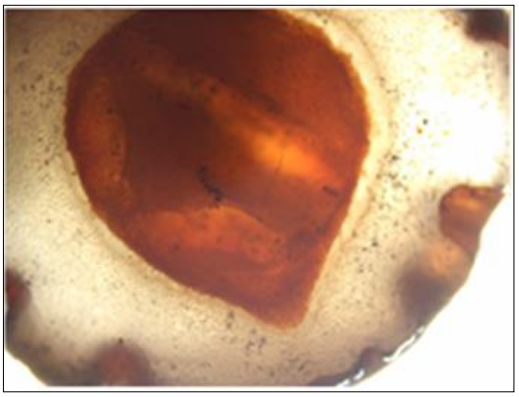

Fig 8: Embelia ribes fruits: Cross section of seed showing endosperm

Conclusion

The present study inferred presence of various phytochemical constituents of *Embelia ribes* in various extracts; due to presence of these phytochemical constituents *Embelia ribes* shows extensive medicinal properties. The macroscopic and microscopic study of *Embelia ribes* fruits are presented to provide morphological and anatomical characteristics of it.

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