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Ananth V
Research Scholar, Department of Botany, Bishop Heber College Autonomous, Tiruchirappalli, Tamil Nadu, India

Anand Gideon V
Associate professor and Head, Department of Botany, Bishop Heber College, (Autonomous), Tiruchirapalli, Tamil Nadu, India

John Britto S
The Rapinat Herbarium and Centre for Molecular Systematics, St Joseph's College Autonomous, Tiruchirappalli, Tamil Nadu, India

Correspondence
Ananth V
Research Scholar, Department of Botany, Bishop Heber College Autonomous, Tiruchirappalli, Tamil Nadu, India

Pharmacognostical and preliminary phytochemical profile of the leaf extracts of *Embelia ribes* Burm. F.

Ananth V, Anand Gideon V and John Britto S

Abstract
Embelia ribes Burm. f. is a valuable medicinal plant used in Indian system of medicine. This article deals with gross morphology, anatomy and preliminary phytochemicals presence of alkaloids, flavonoids, amino acids, proteins and carbohydrates.

Keywords: *Embelia ribes*, microscope, preliminary phytochemical

Introduction
Plants are the invaluable sources of medicines, especially in traditional systems of health care. The recent global resurgence of interest in herbal medicines has led to an increase in the demand for them. The research on the analysis and quality control of herbal medicines now is in the direction of an integrative and comprehensive approach, in order to better address the inherent holistic nature of herbal medicines.
Embelia ribes Burm. f. of family Primulaceae. (APG IV 2016) is seen in hills of India up to 1500m elevation extending from outer Himalayas to Western Ghats. It is an endangered medicinal plant. Due to its over exploitation it is reported in red list data book as vulnerable [Ravikumar & Ved 2000].

Taxonomic Classification

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Ericales
Family	Primulaceae
Genus	*Embelia*
Species	*E. ribes*

Binomial name
Embelia ribes Burm. f.

Geography
The distribution range of this species extends from India, Sri Lanka, Singapore, Malaysia and South China. Within India it is seen in the Central Himalayas, Arunachal Pradesh, Assam, Maharashtra, Tamilnadu, Kerala, Karnataka and Andhra Pradesh.

Materials and methods
Collection and Authentication
Plant materials of *E. ribes* Burm.f. (Leaves and stem) were collected from the Western Ghats of Tamil Nadu and Kerala. They were authenticated by Dr. S. John Britto, Director and Head, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous) Tiruchirappalli, Tamil Nadu, S. India. (Voucher specimen no: RHT 68490).

Preparation of Extract
The powdered plant parts (leaves and stem) 10 g of each were extracted in 100 ml of Acetone, ethanol, methanol, aqueous with continuous shaking on mechanical shaker for 24/hrs at room temperature. The extracts were then filtered through Whatmann No.1 filter paper and stored airtight.

Macroscopic Studies
The macroscopic study of plants provides the morphological description of the plant and the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug.

Microscopic Studies
Microscopic study involves anatomy and is done by taking appropriate section of the plant parts under study. Detailed microscopic characters were studied by taking free hand thin transverse sections of leaf, petiole and stem. Surface preparation of the leaves was done by peeling method to observe epidermal cells. The leaf was examined transversely through lamina and midrib. Microphotographs were taken in digital microscope attached with computer system.

Phytochemical Screening
Qualitative Phytochemical tests were conducted to test the powdered form of the plant samples (methanol, ethanol, acetone and aqueous) using standard methods of Harborne (1978) [6] and Edeoga *et al.,* (2005) [5].

Preliminary Phytochemical Analysis
Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids and terpenoids were carried out for all the extracts by the method described by Mukherjee (2002) [8].

Detection of Alkaloids
Solvent free extract 50 mg was stirred with few ml of dil. HCl and filtered. The filtrate was tested carefully with various alkaloidal reagents as given below.

a. **Mayer's Test:** To a few ml of filtrate, one or two drops of Mayer's reagent were added by the side of the test tube. A white creamy precipitate indicated the test as positive.
b. **Wagner's Test:** To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish brown precipitate confirmed the test as positive.
c. **Hager's Test:** To a few ml of filtrate 1 or 2 ml of Hager's reagent (Saturated aqueous Solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

Detection of Carbohydrates and Glycosides
The extract (100mg) was dissolved in 5 ml water and filtered. The filtrate was subjected to the following tests:

a. **Molish's Test:** To 2 ml of filtrate two drops of alcoholic solution of α- napthol was added, the mixture was shaken well and 1 ml of con H_2SO_4 was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.
b. **Benedict's Test:** To 0.5 ml of filtrate, 1 ml of Bendict's reagent was added. The mixture was heated on a boiling water bath for 2 mins. A characteristic coloured precipitate indicated the presence of sugar.

Detection of Glycosides
50 mg of extract was hydrolysed with concentrated HCl for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following tests:

a. **Borntrage's Test:** To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.
b. **Legal's Test:** 50 mg of the extract was dissolved in pyridine. Sodium nitro prusside solution was added and made alkaline using 10% NaOH. Presence of glycosides was indicated by pink colour.

Detection of Saponins
To 1ml of extract was added 2ml of distilled water and shaken vigorously and allowed to stand for 10 min. There was the development of foam on the surface of the mixture. Then shake for 10 minutes, it indicated the presence of saponins.

Detection of Proteins and Amino Acids
The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through Whatmann no: 1 filter paper and filtrate was subjected to tests for proteins and amino acids.

a. **Millon's Test:** To 2 ml filtrate, few drops of Millon's reagent were added. A white precipitate indicated the presence of proteins.
b. **Biuret Test:** An aliquot of 2 ml of filtrate was heated with 1 drop of 2 % $CuSO_4$ solution. To this 1 ml of ethanol (95%) was added, followed by excess of KOH Pellets. Pink colour in the ethanolic layers indicated the presence of proteins.
c. **Ninhydrin Test:** 2 drops of Ninhydrin solution (10 mg of Ninhydrin in 200 ml of acetone) was added to 2 ml of aqueous filtrate. A characteristics purple colour indicated the presence of amino acids.

Detection of Phytosterols
a) Liebermann – Burchard's Test
The extract (50 mg) was dissolved in 2 ml acetic anhydride. To this one or two drops of concentrated H_2SO_4 were added slowly along the sides of the test tube. An array of colour change showed the presence of phytosterols.

Detection of Fixed Oils and Fats

a. **Spot Test:** A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil.
b. **Saponification Test:** A few drops of 0.5 N alcoholic KOH Solution were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on water bath for 2 hours. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Detection of Phenolic Compounds and Tannins

a. **Ferric Chloride Test:** The extract (50 mg) was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.
b. **Lead Acetate Test:** The extract (50 mg) was dissolved in distilled water and to this 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.
c. **Gelatin Test:** The extract (50 mg) was dissolved in 50 ml of distilled water 2 ml of 1% solution of gelation containing 10% sodium chloride was added to it. White precipitate indicated the presence of phenolic compounds.
d. **Alkaline Reagent Test:** An aqueous solution of the extract was heated with 10% NH_4OH solution. Yellow fluorescence indicated the presence of flavonoids.
e. **Magnesium and Hydrochloric Test:** The extract (50 mg) was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated HCl were added (dropioire). If any pink to crimson developed, presence of flavanol glycoside was inferred.

Detection of Gum and Mucilages
The extract (100 mg) was dissolved in 10ml of distilled water and to this 25 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums mucilages.

Detection of Volatile Oil
In volatile oil estimation apparatus, 50 g of powdered material (crude drug) was taken and subjected to hydro distillation. The distillate was collected in graduated tube of the assembly where in the aqueous portion automatically separated out from the volatile oil.

Test for Steroids
10 ml of all extract of the test plant was evaporated to a dry mass and the mass dissolved in 0.5 ml of chloroform. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades shows the presence of steroidal compounds.

Test for Starch
To 3 ml of the extract was added a few drops of dilute iodine solution. Blue colour indicated the presence starch. Colour disappears on boiling and reappears on cooling.

Test for flavonoids

a. **Shinoda Test:** To 2 ml of the extract and a few fragments of magnesium ribbon were added and to it con. Sulphuric acid was added drop wise. Pink scarlet or crimson red appeared.
b. **Zinc Chloride Reduction Test:** To 2 ml of the extract a mixture of zinc dust and con. HCl were added. A red colour was obtained after few minutes.
c. **Alkaline Reagent Test:** To 2 ml of the extract sodium hydroxide solution was added to give a yellow or red colour.

Result and Discussion
Macroscopic Studies
This large scandent shrub with long slender and flexible branches usually found in high altitude hilly areas above 1600-1950 m. Vines or lianes. Leaves narrowly elliptic to oblong, to 9x3 cm with scattered, minute, sunken glands; petiole to 1 cm. Panicle terminal or in upper axils; peduncle 12 cm, pupesent; pedicel to 1 mm. Calyx-lobes 5, coriaceous, 0.5 mm. Corolla greenish white, 2 mm wide; lobes 5, puberulous. Stamens 5, exerted; ovary ribbed. Drupe 4 mm wide. Flowers greenish, in peak during April-May.

Microscopic Studies
Leaves: T. S of leaves shows epidermis, cuticle, chlorophyll, vascular bundle, sclerenchyma, pallisade tissue, xylem, phloem and oil glands.

Stem: T. S of stem shows a circular outline, single layer of epidermis covered with a thin cuticle, epidermis, endodermis, chlorophyll, collenchymatous cortical tissue is present. Compound Starch grains, oil glands and crystals are identified in different parts. Fibers are towards vascular bundles, cambium, medullary rays and pith are seen.

Preliminary Phytochemical Screening
The preliminary phytochemical screening (acetone, ethanol, methanol, aqueous) of the extracts of *E. ribes* burm. f. (Leaves) revealed the occurrence of alkaloids, flavonoids, amino acids, proteins, carbohydrates.

Discussion
Macroscopic and microscopic analyses enable the pharmacologist to authenticate the taxonomic identification of the plant species. Plant morphology and anatomy play very important role to identify plants. The distinct presence of secondary metabolites and phytochemical compounds such as alkaloids, flavonoids, terphenoids, proteins, glycosides and phenols, as revealed in cells are indication of the presence of the above classes of compounds.

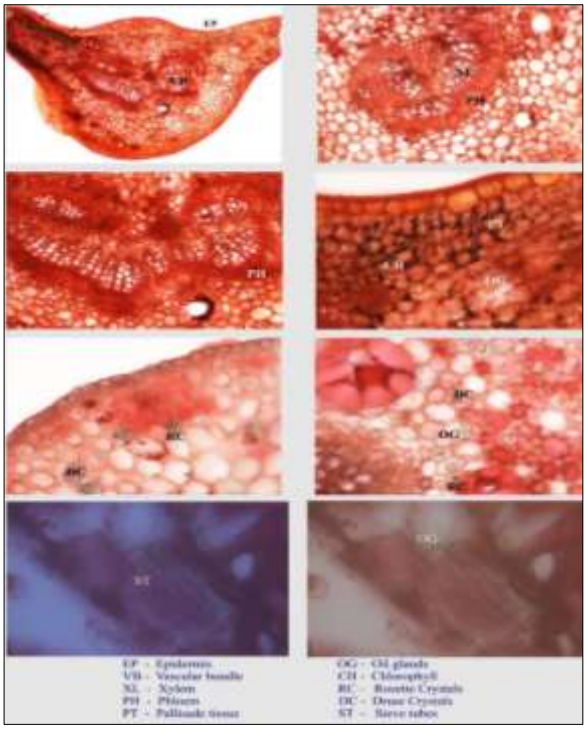

Fig 1: *Embelia ribes* Burm. f.-T.S. of leaf

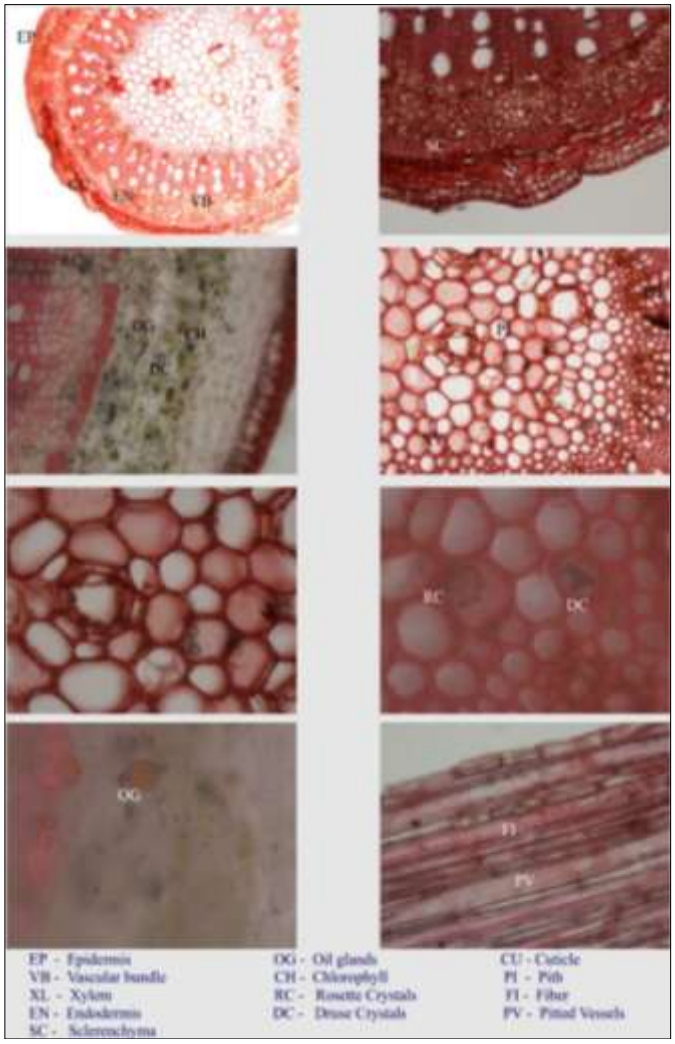

Fig 2: *Embelia ribes* Burm. f. T.S. of stem

Table 1: Preliminary Phytochemical Analysis of *E. ribes* Burm. f. Leaves

	Test	Acetone	Ethanol	Methanol	Aqueous
Alkaloids	Wager's	++	++	+++	++
	Hager's	+	+++	+++	++
	Mayer's	+	+	++	+
Flavonoids	Pew's	-+	++	++	++
	Shinoda	++	+	++	+
	NaOH	-	+	+	+
	Con.H_2SO_4	+	+	+	+
Phenols & Tannins	$FeCl_3$	+	+	+	+
	$K_2Cr_2O_7$	-	+	+	-
	Lead Acetate	+	+	+	+
	Braymers	+	+	+	-
Saponins	Foam	+	+	++	+
	$NaHCo_3$	-	-	-	+
Glycosides	Keller-Kiiani	++	++	+++	+
	Glycosides	++	++	++	++
	Libermann	+	+	+	+
Carbohydrates	Molish	++	++	++	+
	Benedict's	+	+	+	++
	Emodins	+	+	+	+
Anthocyanin	Borntrager's	+	-	+	-
	Quinones	+	-	+	-
Sterols	Salkowski's	-	+	+	+
	Triterpenoids	+	+	++	-
Protein	Biuret	++	++	+++	++
	Con. H_2SO_4	++	+++	+++	+
	Xanthoprotein	+	+	+	-
	Terpenoids	+	+	+	+

Conclusion

In this macroscopic and microscopic investigation of plants external morphological and internal structure are presented in various levels. The preliminary phytochemical studies are studied have proved the presence of alkaloids, flavonoids, tannins, protein, glycosides etc.

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