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Cecidological and pharmacognostical study of *Ficus racemosa* leaf galls

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Abstract
Pharmacognostical parameters for the leaf galls of *Ficus racemosa* Linn. Were studied with the aim of drawing the Pharmacognostical standards. The cecidology, macroscopy, microscopy, powder characteristics, physical standards and thin layer chromatographic studies of *Ficus racemosa* L. leaf galls included in research work. The study also deals with the phytochemical screening of with various extracts. During the cecidologic study it has been observed that, *Ficus racemosa* L. leaf galls have three stages; development, maturation and senescence stage. Microscopically the leaf galls show variation in their growing stages. Commonly cuticle, lignified cells, cortex, starch grains, nutritive tissue and larval chamber were identified. The results showed that the moderate presence of terpenes, flavonoids, steroids, phenols and tannins. The cecidological and pharmacognostical standardization studies have been reported for the *Ficus racemosa* L. leaf galls first time.

Keywords: *Ficus racemosa*, Macroscopy, Microscopy, Cecidology Phytochemical

1. Introduction
Ficus racemosa vernacular name 'Audumbar tree' or 'umber tree' is grown in Maharashtra, India as an wild. The plant have a traditional importance as, is the tree of Lord Dutta digumber (God of Hindu). Hence mostly found near the temples Lord Dutta digumber and other gods temples of Hindu. It is known as 'Cluster Fig' tree in English and 'Ambar' in Hindi and it belongs to the family Moraceace [1]. *Ficus racemosa* is a large deciduous evergreen tree growing to a height of 20-25 meters. It is grown in villages for shades and its edible fruit.
Ficus Racemosa has been extensively used in traditional medicine for a wide range of ailments. Its bark, fruits, leaf, roots, latex and seeds are medicinally used in different forms, sometimes in combination other herbs [1].
It is a medicinal plant which is endowed with curative properties including Hypoglycemic, Antioxidant, Antidiarrhoeal, Memory Enhancing in Alzheimer's disease, Anticholinesterase activity, Anti-inflammatory, Antibacterial, Antidiuretic, Antipyretic, Hypolipidemic, Antifilarial, Hepatoprotective, Cardio-protective, Mosquito larvicidal, Gastroprotective, Renal anti carcinogenic, Anti-tussive, Wound healing, Treatment of cancer, Anti ulcer [2, 3].
Neither the use of *Ficus racemosa* leaf galls has been reported nor has its pharmacognostic evaluation been carried out. Although, galls or *cecidia* is an abnormal outgrowths of plant tissues. Plants galls are highly organized structure and study of plant galls is known as 'Cecidology'.[4] Galls are formed by microorganism, or feeding and egg-laying activity of some insects, mites, nematodes, viruses, fungi, bacteria. The most common are leaf, stem and flower galls produced by insects and mites. Eriophyidae mites are plant parasites forming various galls on foliage or other part of plant [4].
Here, this cecidology and Pharmacognosy is intended to establish, conventional botanical and modern pharmacognostical parameters of leaf galls of the plant. These will be used as diagnostic features in the authentication, evaluation and pharmacological study of *Ficus racemosa* leaf galls.

Materials and Methods
Procurement of Plant Material
Fresh leaf galls of *Ficus racemosa* were collected from Dhamangaon, Igatpuri (Dist. Nashik), Maharashtra, India in Aug-Sep 2014. Plant sample was authenticated by Prof. Rajesh T. Wankhede, Dept. of Dravyaguna, S.M.B.T. Ayurvedic College, Nashik.

Preparation of Plant Material
Fresh mature leaf galls are store in formalin solution and powder of leaf galls of *Ficus racemosa* was prepared by passing through sieve #44, and kept in air tight polythene bags for further study.

Chemicals and Instruments
Photomicroscope (OLYMPUS Pvt. Ltd., New Delhi; Model-CH 2OiBIMF) provided '3V-MICRO' video attachment eye piece device (Version8) with 10x, eye piece (12 mega pixel) with cells tracking function and 4x digital zoom camera was used. Solvents and reagents were procured from Loba Chemicals, Mumbai, India.

Macroscopical Examinations
The macro-morphological features of the plant leaf were observed under magnifying lens and simple microscope [5].

Microscopical Examinations
Fresh leaf galls of the species were studied using transverse sections. The different parts of leaf galls like epidermis and cortex were studied according to the methods of Brain and Turner [5]. As per the procedure of K. R. Khandelwal [6] the microscopy was studied. The transverse sections were prepared and stained with laboratory staining reagents. The different lens of photomicroscope as, OLYMPUS iNEA 5X, 10X/0.2; India, and 100X/1.25 oil India were used for capturing the photographs.

Histochemical Studies
The Sudan red III, Phloroglucinol+ HCl, dilute iodine solution, dilutes ferric chloride solution, etc. The reagent treated hard section of the plant tissue was observed and microscope to detect the presence of histochemical components [7].

Powder Microscopy
A little quantity of leaf gall powder was taken onto a microscopic slide; 1-2 drops of 0.1% w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope. The presence of starch grain was detected by the formation of blue color on addition of 2-3 drops of 0.01 M iodine solution [8]. The characteristic structures and cell components were observed and their photographs were taken using photomicrography.

Physical Evaluation
Moisture content of the powder was determined based on the loss of drying (LOD- Oven) method[9]. The ash values were determined, to find out about the physiological state and level of extraneous matter. Extractive values were determined according to the official methods prescribed in Ayurvedic Pharmacopoeia of India [10].

Phytochemical Investigation
The successive extractive values carry out and all extract studied for preliminary phytochemical investigation as per the procedure of C. K. Kokate [11].

TLC Finger Print Profile
To ascertain the presence of flavonoids and tannins the thin layer chromatography of the ethanolic extract was studied and R_f values were determined [12].

Results and Discussion
Macroscopic and Microscopic Examination
Macroscopically the fresh leaf galls of *Ficus racemosa* are 0.50-1.08-2.20 cm in length and 0.30-0.55-0.80 cm in width. The leaf galls were ovate shape; some of the leaf galls were found attached to each other physiologically (figure 1). The three stages of galls observed during their growth as; development, maturation and senescence (figure 2). In development stage galls were green in color, while reaching to maturation stage they become brown and finally at senescence stage color of galls is reddish brown.

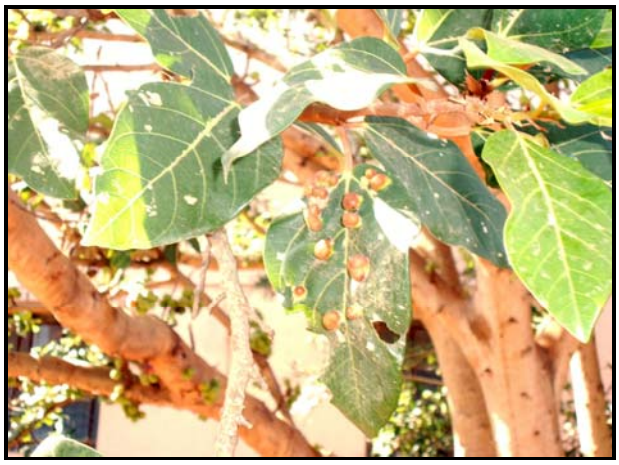

Fig 1: *Ficus racemosa* Leaf

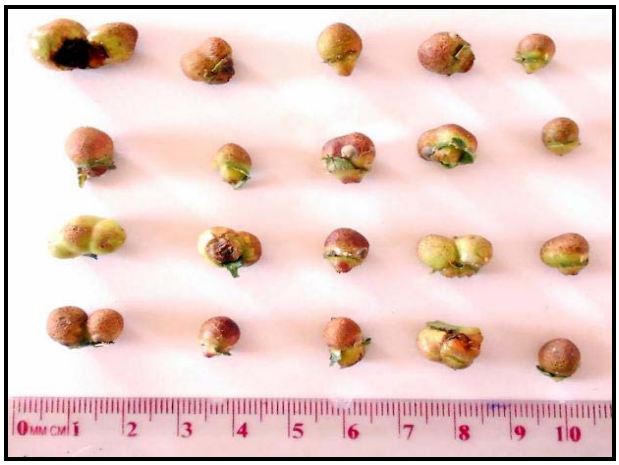

Fig 2: *Ficus racemosa* Leaf Galls

Trans-sections of Leaf Gall
Cuticle of the leaf gall is thin as compared to the cuticle of leaf of *Ficus racemosa*. Epidermis shows epidermal cells which are comparatively small to other cells, the palisade cells are also observed. The lignified sclerenchyma cells frequently present in between the palisade tissues. Cortex region of galls consist of spongy parenchymatous cells specifically at development stage of galls (Figure 3).

Whereas, at maturation and senescence stage the cortex tissues grown in two region; outer and inner cortex tissues. Outer cortex showed hyperplasic spongy parenchyma, fiber sclereids which are lignified. Inner cortex showed nutritive tissue and 3-6 larval chambers (figure 4).

Nutritive tissues have a nutrition material for larvae, these tissue are the parenchymatous cells might be enrich with carbohydrates and lipids. Larval chamber is fully surrounded by nutritive tissues and shows lignified sclerenchymatous zone.

Powder characteristics

The powder was dark brown in color, on microscopically examination, the powder showed epidermal cell, simple starch grains, Parenchyma, lignified tissues and spongy parenchyma (Figure 5).

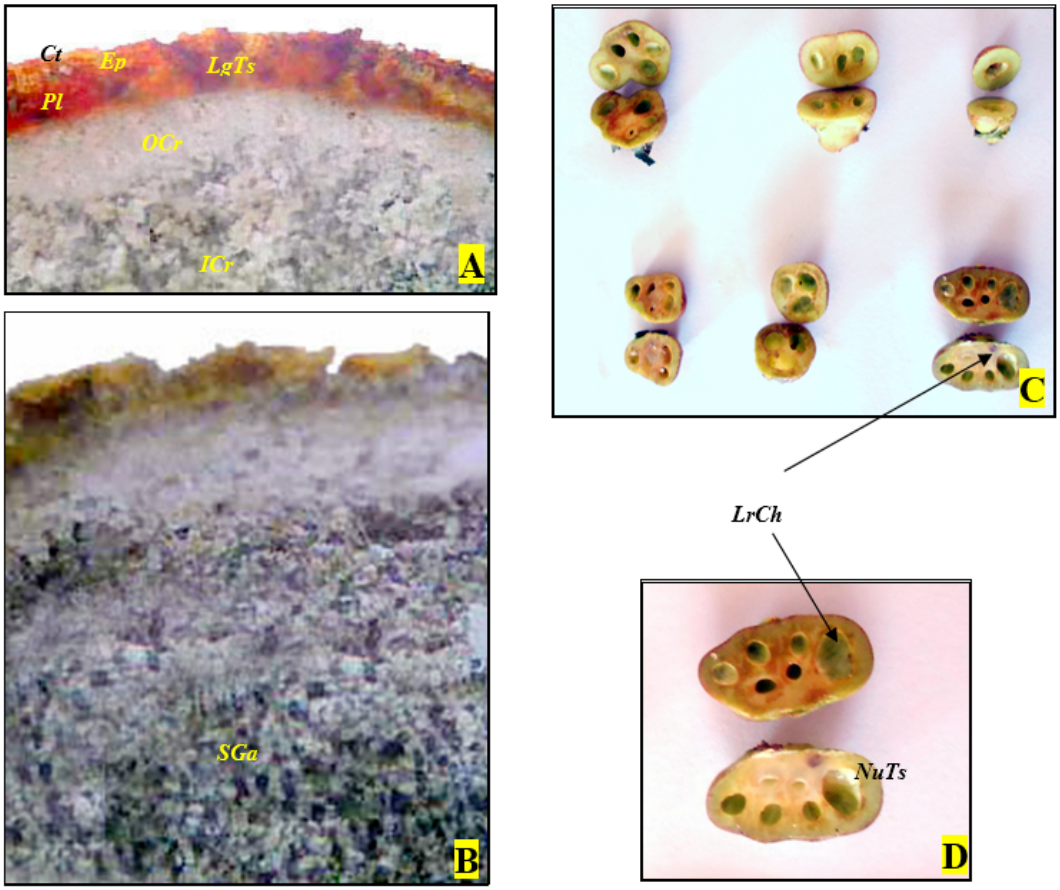

Fig 3: Transverse section of *Ficus racemosa* Leaf Gall A) and B) at development stage C) and D) Maturation and senescence stage. *Ct-Cuticle; Ep-Epidermis; LgTs- Lignified tissue; Pl-Palisade cells; OCr- Outer cortex; ICr- Inner cortex; SGa- Starch grain; LrCh- larval chamber; NuTs- Nutritive tissues*

Histochemical Studies

The counter idea about presence of phytoconstituent is obtained through this study like simple starch grains in inner cortex as indicated by bluish black stain when transverse section treated with dilute iodine solution (Table 1).

Table 1: Histochemical Studies of *Ficus racemosa* Leaf Galls

Reagent	Phytoconstituent	Histological zone in leaf galls	Inference
Sudan Red III	Terpenes	Cuticle	+
Phloroglucinol + HCl (1:1)	Lignin	Lignified tissues	-
Aqs. $FeCl_3$ Solution	Phenolics	Palisade parenchyma	+
Iodine Solution	Starch	Inner cortex	+
Libermann-Burchardlt reagent	Steroids	Spongy parenchyma, Nutritive tissues	+

+ positive; - negative

Physical Evaluation

The loss of drying seems to be lower than necessary to support the growth of pathological microorganisms to bring any change in the composition of the drugs. Physical constant as total ash value, sulphated ash, acid insoluble ash and water soluble ash of the drug gives an idea of the dusty matter or the inorganic composition and, other impurities present along with the drug. Extractive values are useful for the determination of exhausted or adulterated drugs (Table 2).

Table 2: Physicochemical Parameters of *Ficus racemosa* Leaf Galls

Parameter	% w/w Avg±S.D
Ash Values	
Total	08.00±0.053
Acid - insoluble	01.30±0.078
Water - soluble	02.46±0.031
Sulphated Ash	00.03±0.029
Extractive Values	
Pet. Ether Soluble (40-60°)	01.63±0.013
Ethanol Soluble (95%)	14.40±0.107
Water Soluble	18.00±0.075
Moisture content	02.60±0.280

Phytochemical Investigation

Revealed the presence of primary and secondary metabolites as; carbohydrates, mucilage, alkaloids, flavonoids, steroids, Tannins, substances and terpenoids. Flavonoids, steroids/triterpenoids, alkaloids and phenolics are known to be bioactive principles for treating various diseases and disorders [13].

TLC Fingerprint Profile

Thin layer chromatography of the ethanolic extract was carried out using Toluene: Ethyl acetate: Formic acid (7:3:1) as mobile phase by targeting tannins and flavonoids. The R_f values were recorded in Table 3.

Table 3: TLC Fingerprint for *Ficus racemosa* Leaf Galls

Mobile phase	Extract	Number of spot and their R_f value
Toluene: Ethyl acetate: Formic acid (7:3:1) Detection- 365nm	Ethanolic	0.02, 0.54, 0.73 and 0.93

Conclusion

The present study shows that, leaf galls have a specific anatomical and physiological structure. The phytochemical found in leaf galls are pharmacologically significant. Microscopically *Ficus racemosa* leaf gall varies at their growing stages. The *Ficus racemosa* galls are enriched in lignin and phenolic compound such as tannins, which was observed during its microscopy and phytochemical study. The lignifications in addition with the accumulation of phenolic compounds in gall parenchyma commonly occur in response to different types of injuries, and consists in a high mechanical resistance against the attack of predators and parasitoids.[14] Finally we conclude that, like to other part of the *Ficus racemosa* plant its leaf galls also consist a phytoconstituents which have pharmacological importance. The reported parameters will act as a important tool for the researcher to identify the leaf galls of *Ficus racemosa*. They can also aid the pharmaceutical manufacturers.

Although it is an abnormal growth of the plant, they have their own importance. Lastly, authors accept the fact that lot of work remain in this area, but the present article will act as a ladder for future researchers who venture in this field.

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