Estimation of Total Phenol, Flavonoid and Tannin Contents in Galls of *Ficus benghalensis* Leaf

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Abstract

The total phenolic, total flavonoid and total tannin contents in leaf galls of *Ficus benghalensis* were studied with the aim of drawing the standards.; here non structural total phenolic content (TP) determined by Folin- Ciocalteu reagent and flavonoid content (TF) was determined by aluminum chloride colorimetric method. The TP found to be, 157.25±0.015mg (gallic acid equivalent/g) and TF 151.95±0.085mg (quercetin equivalent/g). In addition, total tannin content (TT) determined by back titration with potassium permanganate and, was found as 106.41±0.000mg (tannin equivalent/g). In study TP, TF and TT are significant and prove that, galls are rich in estimated phytoconstituents and may have pharmacological importance.

Key words *Ficus benghalensis*, phenolic, flavonoid, tannin, phytoconstituents

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Introduction

Ficus benghalensis is grown in Maharashtra, India as a wild. It is known as 'Banyan Fig tree' in English and it belongs to the family Moraceace.

Fig. 1: *Ficus benghalensis* leaf galls

Ficus benghalensis 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 benghalensis* has been extensively used in traditional medicine for a wide range of ailments. Its bark, fruits, leaf, roots, aerial roots, latex and seeds are medicinally used in different forms, sometimes in combination other herbs (Gopukumar ST and Praseetha PK, 2015).

It is a medicinal plant which is endowed with curative properties. Its curative property is due to the presence of its phytoconstitues as; stem bark showed the presence of leucoanthocyanins, glycosides, β-sitosterol, lupeol, ketone, α- amyrin acetate. In trunk bark, lupeol, β-sitosterol and stigmasterol were present. Fruit of *Ficus benghalensis* contains glauanol, β-sitosterol, gluanol acetate, glucose, tiglic acid, and esters of taraxasterol, lupeol acetate, friedelin, higher

hydrocarbons. A thermo stable aspartic protease was isolated from the latex of the plant. The stem bark and fruit also showed presence of glauanol acetate. The leaf of this plant contains sterols, triterpenoids, alkaloids, tannins and flavonoids (Khaliq HA, 2017).

But, till neither the use of *Ficus benghalensis* leaf galls (Fig 1) has been reported nor has its phytochemistry study 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' (Ushir YV *et al*, 2015). Here, the logic was made that, during growth of the galls the phytoconstituents present in the plant tissue were imparted in galls. This Phytochemistry part is intended to establish parameters of leaf galls of the plant. These will be used as diagnostic features in pharmacological study of *Ficus benghalensis* leaf galls.

Materials and Methods

Procurement of Plant Material

Fresh leaf galls of *Ficus benghalensis* were collected from Dhamangaon, Igatpuri (Dist. Nashik), Maharashtra, India in Aug-Sep 2018. Plant sample was authenticated by Prof. Rajesh T. Wankhede, Dept. of Dravyguna, S.M.B.T. Ayurvedic College and Hospital, Nashik.

Preparation of Plant Material

Fresh mature leaf galls of *Ficus benghalensis* are shade dried and powdered was prepared by passing through sieve # 44, and kept in air tight polythene bags for further study.

Chemicals and Instruments

UV/Vis double beam spectrophotometer (SCHIMADZU made, Japan) and 1 cm pathlenght quartz cells were used for absorbance. Solvents and reagents were procured from Loba Chemicals, Mumbai, India.

Extraction

Methanol used for extraction, 100 g of the powder was extracted using Soxhlets apparatus for 6 hrs. The extract dried and kept in container for further study

Determination of TP

The TPC of the *Ficus benghalensis* leaf galls methanol extracts was determined using the Folin- Ciocalteu reagent. The diluted methanolic extract (0.5 ml of 1:10 g mL^{-1}) or gallic acid (standard phenol compound) was mixed with Folin - Ciocalteu reagent (5 ml, 1:10 diluted with distilled water), and aqueous sodium carbonate (4 ml, 1 M). The reaction mixture was kept in dark at ambient conditions for 0.30 h to complete the reaction. The absorbance at 765 nm was measured (Roya K and Fatemeh G, 2013). Gallic acid was used as standard and the results were expressed as mg gallic acid equivalent/g *Ficus benghalensis* leaf galls.

Determination of TF

Total flavonoid content was determined using aluminum chloride ($AlCl_3$) according to a known method, using quercetin as a standard. Plant extract (0.5 ml of 1:10 g mL^{-1}) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10 % aluminum chloride, 0.1 ml of 1 M

potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes; the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 µg mL^{-1} in methanol (Stanković MS, 2011). The results were expressed as mg quercetin equivalent/g *Ficus benghalensis* leaf galls.

Estimation of TT

TTC was determined by back titration with potassium permanganate solution. Accurately weighed 0.5 g (W) of plant material boiled with 20 ml water for 15 minutes and then filtered. The step was performed four times; final volume was made to 100 ml. 10ml filtrate and 10 ml indigo carmine solution was added. Diluted this mixture to 100 ml with water. Lastly, back titrated against 0.1 N $KMNO_4$ still the golden yellow color is obtained. Obtained volume (T2) subtracted from initial volume (T1) of 0.1 N $KMNO_4$ and used in percentage calculation. TT calculated by applying the following factor; 1 ml 0.1 N $KMNO_4$ ≈ 0.00416 g tannins and percentage quantity of total tannins= [(T2-T1) X actual normality X 0.004157 X 1000]/W X 0.1 (Mahmoodi MH *et al.*, 2013).

Results and Discussion

The leaf galls of *Ficus benghalensis* methanol extract have the abundant quantity of phenols which expressed in term of gallic acid (the standard curve equation: Y = 0.005X + 0.147, r2 = 0.997), flavonoids is expressed in term of quercetin (the standard curve equation: Y = 0.003X + 0.115, r2 = 0.987) and tannins (Table-1). Phenolic, flavonoid and tannins compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases.[10] The *Ficus benghalensis* leaf galls may have high scavenging property due to hydroxyl groups existing in the phenolic compounds.

Table 1: TP, TF and TT of *Ficus benghalensis* Leaf Galls on dry weight basis

Parameter	Values mg/g	Equivalent/g
TP	157.25±0.015	Gallic acid
TF	151.95±0.085	Quercetin
TT	106.41±0.000	Tannins

TP-Total Phenolic Contents; TF-Total Flavonoid Contents; TT-Total Tannin Contents

The total flavonoid contents indicate there might be a chance to search new flavonoids in leaf galls of *Ficus benghalensis*. The determination of the present study directs that *Ficus benghalensis* leaf galls could be a significant source of natural antioxidant that could have extreme importance as pharmacological or therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative various diseases.

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