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PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTIOXIDANT ACTIVITY IN LEAF GALL OF *FICUS GLOMERATA* ROXB. (MORACEAE)

SAVITHA RS, AKSHATHA JV. AND NALINI MS*.

Assistant Professor Department of Studies in Botany, University of Mysore, Manasagangothri, Mysore–570 006, Karnataka, India.

ABSTRACT

Ficus glomerata Roxb., (Moraceae) is a large deciduous tree and plant parts such as root, bark, leaves, fruits and galls are used in therapeutics. The leaf gall of *F. glomerata* is induced by the insect *Pauropsylla depressa*. In the present study, the solvent extracts of leaves as well as the gall portion at various developmental stages were screened for the presence of phytochemicals in comparison to the normal leaves. The presence of flavonoids, terpenoids, saponins were detected in normal and galled leaves, while most phytochemicals were present in the gall portion of the leaves. The gall stages as well as the gall leaves were tested for total phenolic content and DPPH radical scavenging assay. Results indicated ~0.7-fold increase in the phenolic content of gall leaves (90µg/ml GAE) over the normal leaves (62.5µg/ml GAE). Comparison of phenolic contents among various stages of gall development namely, young, medium and mature indicated high phenolic content in young galls (123µg/ml GAE). Young galls depicted 80% radical scavenging activity. This is the first report on the antioxidant activity in the insect-induced galls of *F. glomerata*.

KEY WORDS - *Ficus glomerata*, Leaf gall, Phytochemicals, Total phenolics, Radical scavenging, DPPH.

NALINI MS
Assistant Professor Department of Studies in Botany, University of Mysore, Manasagangothri, Mysore–570 006, Karnataka, India.

Corresponding author

INTRODUCTION

The genus *Ficus* is found throughout the world as moderate woody plants or trees. *Ficus* belongs to the family Moraceae, which constitutes large taxa of over 50 genera and nearly 1400 species[1]. *F. glomerata* extracts have also been reported to possess significant medicinal and pharmacological properties like anti-microbial, anti-cancer and anti-oxidant activity[2]. Root, bark, leaves, fruits and galls are part of the tree used for therapeutic activity. The powdered leaves are mixed with honey and are given in bilious infections. *Ficus glomerata* is a medium tall tree, growing 10-16 m in height. The rich green foliage provides a good shade. The bark is reddish grey and often cracked. The leaves are dark green, 7.5-10 cm long, ovate or elliptic. Leaf galls are commonly seen in *F. glomerata*. The insect *Pauropsylla depressa* causes leaf gall of *F. glomerata*[3]. The normal leaves of *F. glomerata* are dark green in colour with no bulbous outgrowths or masses. The galled leaves are globular, yellowish or reddish brown in colour. The galls measure about 15-30 mm in diameter. There are no reports of galls of *Ficus glomerata* from Karnataka and also the phytochemical analysis of different developmental stages of gall and their antioxidant activity. Therefore, the present investigation is taken up to evaluate the phytochemical constituents and antioxidant potentials.

MATERIALS AND METHODS

Collection of samples

Healthy and gall leaves of *Ficus glomerata* representing different development stages, were collected from Mysore, Karnataka state. A herbarium specimen of the material has been prepared and maintained at the DOS in Botany, University of Mysore. The leaves were washed and gall portions were separated from the leaves and air dried. The samples were homogenized to fine powder and weighed, labeled and stored in an air tight zip lock polythene covers.

Microscopic observations of gall tissue

Different developmental stages of gall samples such as small, medium and mature were taken separately and dissected vertically into two halves and observed under stereo-trinocular microscope (Lawrence & Mayo India Ltd., Bangalore). Different stages of insect development were observed in the dissected gall and photographed.

Phytochemical analysis

Extraction of the sample

Twenty grams of dried powder of normal leaves, gall leaves and gall portion of the plant, *F. glomerata* was taken and solvent extracts (non polar and polar) prepared by modified Kupchan partition method[4]. These solvent extracts were used for subsequent phytochemical tests.Phytochemicals were tested according to the standard procedure for qualitative detection[5].

Tannins

Small quantity (1mg) was mixed with one ml of water and heated on a water bath. The mixture was filtered and two drops of ferric chloride ($FeCl_3$) was added to the filtrate. A green solution indicated the presence of tannins.

Saponins

One ml of aqueous extract of the plant parts were taken and 2 ml of distilled water and shaken vigorously, a stable persistent froth indicated the presence of saponins.

Flavonoids

A few drops of 1% ammonia solution were added to the aqueous extract of plant parts and concentrated sulphuric acid was also added, a yellow coloration indicated the presence of flavonoids.

Terpenoids

Five ml of aqueous extract of plant parts were taken separately and mixed with 2 ml of chloroform, to this mixture 3 ml of concentrated sulphuric acid was added carefully. The appearance of reddish brown layer indicated the presence of terpenoids.

Steroids
Ten ml of chloroform was added to the 20 mg of plant parts and then filtered. Two ml of acetic anhydride was added to this extract and then concentrated sodium hydroxide was added. A green ring indicated the presence of steroids.

Anthraquinones
Dried plant parts (0.5 g) was boiled with 10% HCl (v/v) for a few minutes in a water bath, the contents were filtered cooled. To this filtrate equal amount of chloroform was added and a few drops of 10% NH_3 was added to the mixture and heated. Formation of rose pink color indicated the presence of anthraquinones.

Phlobatannins
One ml of aqueous extract of plant parts were taken and boiled with 2% HCl solution which gives a red precipitate, this indicated the presence of phlobatannins.

Glycosides
Two ml of aqueous extract of plant parts were taken to this one ml of glacial acetic acid and $FeCl_3$ and concentrated sulphuric acid was added carefully which gives reddish blue coloration at the junction of two layers of solution and formation of the bluish green color at the upper layer, which indicates the presence of glycosides.

Reducing sugars
One ml of aqueous extract of plant parts was boiled with a few drops of Fehling's solution A and B for a minute. An orange red precipitate indicated the presence of reducing sugars.

Alkaloids
About 0.2 g of material was heated with 2% H_2SO_4 solution for a two minute and filtered. To this filtrate a few drops of Dragendorff's reagent was added. An orange red precipitate indicates the presence of alkaloids.

Evaluation of antioxidant activities of the extracts

Estimation of phenolic content in leaves/gall stages
Total phenolic content of various samples were estimated by Folin-Ciocalteu (FC) method employing Gallic acid as standard (1 mg/ml) as per the procedure[6] with some modifications. Different concentrations of standard as well as the extracts (50-250 µg/ml) were taken in test tubes and one ml of FC reagent (1:1 dilution) was added, 3-5min later 2.0 ml of 20% sodium carbonate was added and the mixture was allowed to stand for 45 min under dark condition. After the specified incubation period, the absorbance of standard and samples were read at 765 nm using a spectrophotometer. The concentration of total phenolics was expressed in terms of µg/ml GAE (Gallic acid equivalence).

DPPH radical scavenging assay
Different aliquots of standard (1mg/ ml) and aqueous extracts of plant sources 5-25µg were taken and the total volume was made up to 250µl with water/ methanol respectively. To this one ml of DPPH (4mg/ 100ml) was added and the tubes were kept in dark for incubation at room temperature for 20 min. The absorbance was checked against the blank at 517nm. Percentage of free radical scavenging activity was calculated based on the extent of reduction in the color[7]. The percentage of radical scavenging was calculated as follows:

$$\% \text{ radical scavenging} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c= absorbance of control and A_s= absorbance of test sample.

RESULTS

Leaf gall of Ficus glomerata
Gall on *Ficus glomerata* initiates as small bulbous growths on newly formed leaves (Fig. 1). The size of the gall increases as the leaf expands. Initially the galls are solitary, later more than one may be found which coalesced to clumps.

Figure 1
Gall on Ficus glomerata leaves

Microscopic observation

Vertical section of the young gall under stereo binocular microscope showed an egg within the green chamber. Gradual maturation occurred during this period of time larval stages were observed in moults (Fig. 2). In the maturation period necrosis appeared and a small exit hole was formed through which the adult fly emerged.

Figure 2
Developmental stages of insect within the gall chamber

a. Gall chamber b. Larva with a moult c. Close up of larva d. Adult fly

Phytochemical analysis

Phytochemical analysis of galled leaves, galled portion and normal leaves of different solvent extracts showed the presence of saponins, flavonoids, terpenoids, reducing sugars in all three samples. Normal and gall leaves contained tannins; whereas glycosides were present only in galled portion (Table 1). Samples extracted in solvents showed marked differences in phytochemicals. Saponins were abundant in samples extracted in polar solvents methanol and water. Flavonoids were found in both aqueous and hexane extracts of all three samples. Terpenoids were detected in hexane extracts, while only gall portion extracted in methanol contained the same. The chloroform extract of the galled portion indicated positive for the

presence of glycosides. Alkaloids, phlobatannins and anthraquinones were absent in the solvent extracts of samples.

Table 1
Phytochemical evaluation of different extracts

Phytochemicals	Normal leaves				Gall leaves				Gall portion			
	H	C	M	Aq	H	C	M	Aq	H	C	M	Aq
Saponins	-	+	+	-	-	+	+	+	-	+	+	+
Flavonoids	+	-	-	+	+	-	-	+	+	-	-	+
Terpenoids	+	-	-	-	+	-	-	-	+	-	+	-
Steroids	-	-	-	-	-	+	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	+	-	-
Reducing sugars	+	-	-	-	-	-	-	+	+	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	-	-	-	+	-	-	-	-	-	-	-	+

M= Methanol, H= Hexane, C= Chloroform, Aq= Aqueous, + = Positive for the test, - = Negative for the test.

Evaluation of antioxidant activity

The total phenolic content in the gall leaves was detected at 90 µg/ml GAE in comparison with normal leaves (62.5 µg/ml GAE) (Fig. 3). A 0.7-fold increase was observed in the phenolic content of gall leaves over the normal leaves. A comparison of phenolic contents among various stages of gall development *viz.*, young, medium and mature indicated high phenolic content in young galls (123 µg/ml GAE) when compared to medium (73.5 µg/ml GAE) and mature gall (60 µg/ml GAE) (Fig. 4). The radical scavenging potentials of young gall was 80% (Fig. 5).

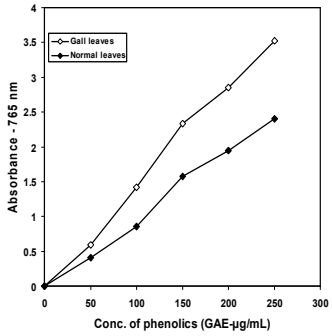

Figure 3
Total phenolic content in normal and gall leaves of Ficus glomerata

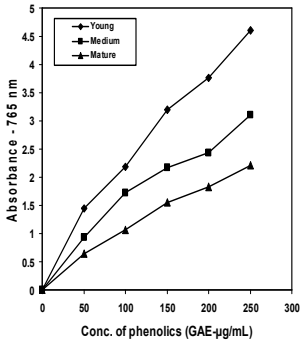

Figure 4
Comparison of total phenolic content in gall tissue of Ficus glomerata

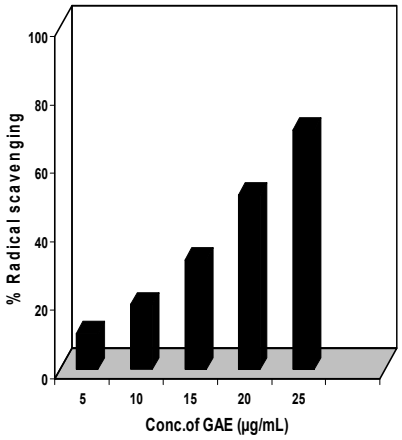

Figure 5
Free radical scavenging activity in young galls of Ficus glomerata

DISCUSSION

Ficus plants are well known in traditional herbal medicine. A study on the leaf gall of *F. glomerata* at various stages of development, their phytochemicals and evaluation of antioxidant activity was carried in the present investigation. Mani[8] reported the existence of leaf gall in 11 species of *Ficus* and later *Gynakothrips ficorum* was identified as the causal agent of leaf gall on *Ficus laevigata*[9]. The gall leaves differed from the non-galled/normal leaves. This is supported by the studies on the morphology of galled leaves of *Ficus glomerata* collected from Loha, Nanded, Kandhar locality in Maharastra[10]. In present study the phytochemical screening of normal leaves, gall leaves and gall portion were done. Various phytochemicals like saponins, tannins, flavonoids, reducing sugars etc., was present in both normal leaves and gall leaves. The phytochemical screening of normal leaves and gall leaves of *F. glomerata* conducted earlier indicated the presence of flavonoids and tannins whereas the presence of coumarins and quinones only in the galled leaves[11]. Our study demonstrates that the phytochemicals differ in plants sampled from different agro climatic zones. The gall portion contained terpenoids and glycosides in addition to flavonoids and tannins both are known to possess anti-diabetic and anti inflammatory activities. In the present study, the antioxidant activity was evaluated by the estimation of total phenolic content as well as radical scavenging activity. The galled leaves showed high phenolic contents, which is indicative of any plant-insect interaction[12]. Phenolic compounds show many biological activities such as anti ageing, anticancer, anti-inflammatory and antioxidant properties. Thus, the antioxidant potentials of medicinal plants rich in phenolic compounds have been elucidated[13]. Young galls showed 80% radical scavenging potentials suggesting that galls are good sources of antioxidants. This is the first report of the presence of leaf gall of *Ficus* in Karnataka, as well as evaluation of antioxidant activity in the stages of gall development. Further studies are needed to evaluate the biochemical changes as well the characterization of antioxidants in the insect galls.

CONCLUSION

Ficus glomerata is a moderate sized avenue tree found throughout India. It is popular in indigenous system of medicines like ayurveda, siddha, unani and homoepathy. A comparison of phytochemical constituents of normal leaves, gall leaves and gall portion of *F. glomerata* revealed differences in the phytochemical constituents of three samples of solvent extracts. The present study revealed that antioxidant activity was correlated to high

phenolic content in young stages of gall growth as well as in the gall leaves. Phenolic compounds present in the gall tissues may have contributed to the radical scavenging potentials. Therefore, galls are indeed a good source of phytochemicals that are equated with various biological activities.

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