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RESEARCH ARTICLE .....!!!

## FREE RADICAL SCAVENGING ACTIVITY AND PHYTOCHEMICAL INVESTIGATION OF *FICUS BENJAMIN* FRUIT

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### KEYWORDS:

*Ficus benjamina*,  
Antioxidant activity,  
Hydrogen peroxide.

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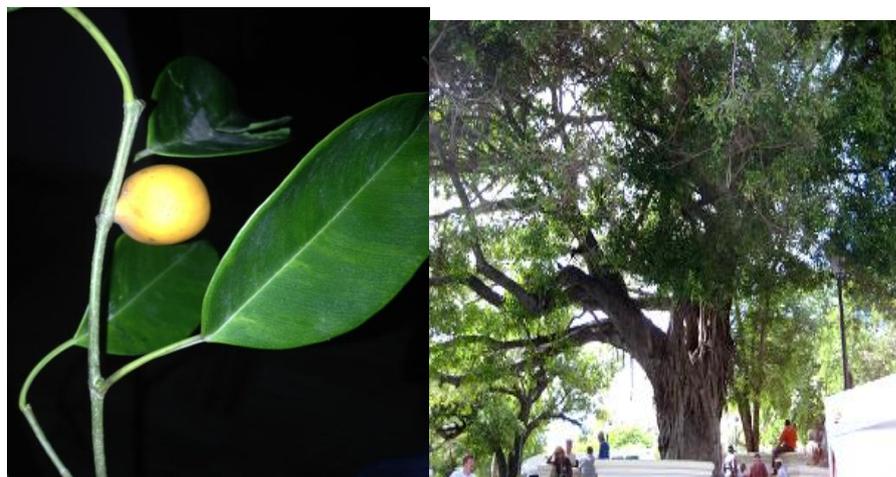
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### ABSTRACT

*Ficus benjamina* also known as the Benjamin's fig or *Ficus* tree is a species of *ficus*, family Moraceae native to Asia and Australia. The present study was designed to investigate the anti-oxidant property of *Ficus benjamina* fruit. The dried coarse powder of *F. benjamina* was exhaustively extracted with ethanol and the resulting crude ethanolic extract was assayed for antioxidant activity. The extract showed strong anti oxidant property which suggests the use of plant for therapeutic purpose, supporting traditional claims.

**INTRODUCTION:**

*Ficus benjamina* is a species of flowering plant belonging to the family Moraceae, generally grows in Asia and Australia. *Ficus benjamina* or *Ficus* tree are often sold in market as ficus. *Ficus benjamina* is evergreen tree up to the height 30 meter, branches are drooping branchlets and leathery leaves 6–13 cm, oval in shape with an acuminate tip, Petioles are 5 to 10 mm long. Fruits are orange in colour and became pulpy when mature, spherical in shape and 1 cm in diameter. Usually, its small fruit were eaten by birds <sup>[1]</sup>. *Ficus benjamina* is popular ornamental plant grows in mild temperature, due to its superior growth and it is tolerable to high and low temperature conditions <sup>[2]</sup>. Leaves bark, and fruits consist of cinnamic acid, lactose, quercetin, caffeic acid and stigmasterol. Caffeic acid shows cytotoxic activity against T-lymphoblastic leukemic cell which exhibit antibacterial activity against *B. cereus* and *P. aeruginosa*<sup>[3]</sup>. The fruit of other species (*F. carica*) also have commercial importance or eaten as food. However, they are important source of food for wildlife and traditionally they are use as medicine because of the presence of laxative substances, flavonoids, sugars, vitamins A and C and enzymes. Many studies reveal that the figs are skin allergens, and the latex is a serious eye irritant <sup>[4,5]</sup>.



**Fig 1:-** *Ficus benjamina* fruit and tree

Increasing research on herbal medicine reveals its importance in treating many problems. Most of studies report the presence of antioxidant agent along with dietary constituents with antioxidant properties which is acting as naturally occurring cancer preventing agents. Therefore, there is an urgent need to develop efficient therapeutic agent.

Antioxidants also play a vital role in preventing cancer causing agents and use in therapy to treat cancer. The antioxidant property is due to the presence of the phenolic components. Several plants reported to possess antioxidant properties but *Ficus* constituted one of the largest genera of

medicinal plant. In India most of the species of the *Ficus* are *Ficus bengalensis*, *Ficus carica*, *Ficus benjamina* and *Ficus elastic*. *Ficus benjamina* is a species of flowering plant which usually grow in tropical and subtropical region .Various part of the plant like bark, leaves, fruits and latex are medicinally important <sup>[6-9]</sup>.

According to USDA data, dried figs are richest source of fibers, copper, potassium and vitamin K. Figs shows antioxidants that inhibit the oxidative mechanism by scavenging the oxygen free radicals which is responsible for causing cancer. *Ficus benjamina* also shows the anti-inflammatory, anti-bacterial, hypoglycemic and laxative effect but the present review is correlated with the study of antioxidant effect of *Ficus benjamina*<sup>[10]</sup>.

#### TAXONOMICAL CLASSIFICATION:

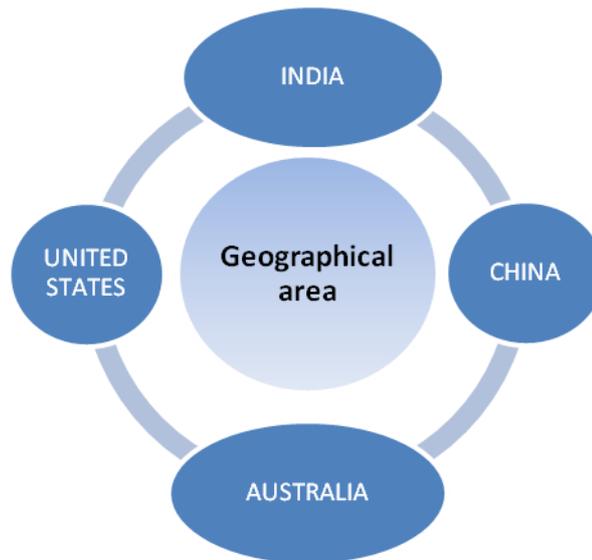
Kingdom		PLANTAE
<b>Phylum</b>		TRACHEOPHYTA
<b>Class</b>		MANGOLIOPSIDA
Order	ROSALES	
	Family	MORACEAE
Genus	FICUS	
	Species	BENJAMINA

#### SYNONYMS:

- *Ficus benjamina* var. bracteatacorner
- *Ficus benjamina* var. comosaking
- *Ficus benjamina* var. nuda (Mig)
- *Ficus comosa* Roxb
- *Ficus cuspidatocaudata* Hayata
- *Ficus dictyophylla* Wall
- *Ficus lucida* Aiton
- *Ficus nitida* Thunb.
- *Ficus nuda* (Mig)
- *Ficus papyrifera* Griff.
- *Ficus reclinata* Desf.
- *Ficus striata* Roth
- *Ficus umbrina* Elmer
- *Urostigmabenzaminum* var. *nudum* Mig

## GEOGRAPHICAL DISTRIBUTION:

*Ficus* tree are adapted to grow in a poor conditions .It is mostly found in India to southern China, Malaya, Australia, some of these species also found in West Indies and in the State of Florida and United State (US). In Manila, it is planted across the road sides for shade. It also grows in most of islands and forests at low and medium altitude. It is tolerable to high and low temperature and adapted to normal conditions <sup>[11]</sup>.



**Fig2:**Geographical areas of *Ficus benjamina*

## VERNACULAR NAMES:

**Table 1:-** Common names of *Ficus benjamin*

Malaysia	jawijawi, kelatsega
English	benjamina tree, weeping fig, java fig
China	chui ye rong
India	ponlchi, putrajuvi (tamil), sunonija(hindi) , juripakri (assam)
Nepal	Kabra
German	Birkenfeige
Indonesia	Waringin (java)

## PHYTOCHEMISTRY:

The phytochemistry of *Ficus benjamina* has revealed the presence of various active constituents which are listed below:-

- Bark of the plant consists of 4.4 % of tannin.
- Latex contain 30% of caoutchouc and 59% of resin. Wax contains cerotic acid.
- Fruit of this plant contain carotenoid which is responsible for the orange pigment of fruits. It also consist of flavonoides, Vitamin A and C
- Extraction of leaves, barks and fruit reveals the presence of cinnamic acid, lactose, quercetin, caffeic acid and stigmasterol which possesses anti-inflammatory activity<sup>[12-14, 3]</sup>.

## LITERATURE SURVEY:

The plant is well known due to its medicinal potential. Its latex and some fruit extracts are used treat skin disorders, inflammation, vomiting, leprosy, malaria and cancer. The plant is also used as antimicrobial, antipyretic, hypotensive and anti-dysentery remedy. The leaves and twigs are used as insecticides. The leaves, bark and fruits of *Ficus benjamina* contain various bioactive constituents like cinnamic acid, lactose, naringenin, quercetin, caffeic acid and stigmasterol<sup>[15-17]</sup>.

The genus *Ficus*, consists of 800 species and about 40 genera of family, Moraceae. Many *Ficus* species has significant pharmacological activities and have commercial importance<sup>[18]</sup>. Some of the species of *Ficus benjamina* were listed below:

### *Ficus carica*

The fig is one of the earliest fruit trees cultivated by man. Figs are having a definite laxative effect due to the bulk of seeds and fibre combined with some specific solvent present in the juice. The dry fruits of anjeer are supplement food for diabetics. Sweets in the markets with sugar free for diabetics contain *ficuscarica*. The paste of fruit is applied in swellings, tumours and inflammation for relieving pain. The enzyme ficin present in the fig latex is responsible for its anthelmintic activity and can be given with great benefit in worm infestations. Figs are a good source of potassium, a mineral that helps to control blood pressure. *Ficus carica* have numerous bioactive compounds such as Mucilages, flavinoids, vitamins, enzymes, nicotinic acid, and tyrosin. Stigmasterol, psoralen, taraxasterol, rutin ,sapogenin, lepeolacetate and oleanolic acid sistosterol are present in the leaf<sup>[19-21]</sup>.

### *Ficus benghalensis*

This tree is commonly known as Indian banyan. The plant is a large evergreen tree distributed all over India from sub Himalayan region and in the deciduous forest of Deccan and south India. It is mainly grown in gardens and road sides for shades<sup>[22,23]</sup>. Extracts obtained from various parts of

the tree are cooling, alterative and demulcent. Astringency in various part of plant is due to the presence of tannins. It is also used as a blood purifier in skin diseases; urinary and urogenital disorder <sup>[24, 25]</sup>.

### ***Ficus elastic***

It is commonly known as Rubber tree. It is best for removing chemical toxins from the indoor environment. The latex contains 10-30%; resin, 1.58%; a bitter substance; albuminoid; and magnesium salts. Wax contains cerotic acid <sup>[26]</sup>. Leaves consist of quercitrin, oleanolic acid and ursolic acid. It is used as anti-inflammatory, antioxidant due to presence of polyphenolic compounds, anthelmintic and anti-microbial <sup>[27]</sup>.

### ***Ficus racemosa***

It is known as cluster fig tree. In India the tree and its fruit are called gular in the north and atti in the south <sup>[28]</sup>. Bark is reddish grey or grayish green with soft surface, 0.5-1.8 cm thick. Bark highly efficacious in threatened abortion and also recommended in urological disorders, diabetes, hiccup, leprosy, dysentery and piles. Leaves are dark green, 7.5-10 cm long, glabrous. They are useful in dysentery and diarrhoea. The infusion of bark and leaves is also employed as mouth wash to spongy gums <sup>[29-32]</sup>.

### ***Ficus macrophylla***

It is commonly known as the Australian banyan, is a large evergreen banyan tree. It is mostly grown in public parks and gardens in warmer climates such as California, Portugal, Italy, northern New Zealand, and Australia. Traditionally, used as the fibres for fishing nets <sup>[33]</sup>. Latex obtained from the tree yields a rubber of very fair quality <sup>[34]</sup>.

## **MATERIALS AND METHODS:**

### **Collection of plant material**

The fruit of *Ficus benjamina* were collected from the Garden of Rayat Institute Of Pharmacy, Railmajra, Punjab (India) during the month of March and further authenticated by Guru Nanak Dev University, Amritsar, Punjab. The plant material was dried under the shade at room temperature then made a powder in mixing at below 40°C temperature. The powder was sieved for removing the dust particles.

### **Preparation of extracts and fractions**

The fruit chosen for study were washed to remove dust. The collected fruit were chopped into small pieces, and dried under shade for 5 days then ground into coarse powder with mechanical grinder and stored in an airtight container. Extraction was done by simple maceration process by taking 60 g of powder and then mixed with 250 ml of ethanol for 5 days at room temperature 25 °C with

occasional shaking. The solvent was filtered off using muslin cloth and the residue macerated again with the fresh solvent <sup>[35]</sup>. Both the solvents were combined and filtered by using Whatmann filter paper. The extract is concentrated under reduce pressure and the concentrated extract was stored in refrigerator throughout the investigation.

### Drugs and chemical

Ascorbic acid, methanol, Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), Potassium dihydrogen phosphate, Sodium chloride, Disodium hydrogen phosphate.

### Instruments

Digital weighing balance (Kern), UV spectrophotometer (Shimadzu-1700 Japan), pH meter (Control dynamic pH meter), Micro Pipette (Riviera).

### Phytochemical screening of the extract

Phytochemical screening was carried out for various constituents such as: flavonoids, tannins, alkaloids, terpenoids, carbohydrates, anthraquinone glycosides according to standard procedure <sup>[36]</sup>.

### Test for alkaloids

1. *Hagers reagent test*: Hagers reagent (picric acid solution) was added to the extract, yellow colored precipitates confirm the presence of alkaloids.
2. *Mayer's reagent test*: Mayer's reagent (potassium iodide solution) was added to the extract, cream-colored precipitates confirm the presence of alkaloids.
3. *Wagner's reagent test*: Wagner's reagent (solution of iodine in KI) was added to the extract, reddish brown precipitates confirm the presence of alkaloids.
4. *Dragendroff's test*: Dragendroff's reagent (potassium bismuth iodide solution) was added to the extract, reddish brown precipitates confirm the presence of alkaloids.

### Test for sterols

1. *Moleschott's test*: A few mg of the extract was heated with 1mL mixture of sulphuric acid and water (5:1) red violet color confirms the presence of sterols.
2. *Hess's test*: A few mg of the extract was dissolved in 1mL of chloroform and equal volume of sulphuric acid, a blood red color confirms the presence of sterols

### Test for tannins

1. *Ferric chloride test*: A few mg of the extract was dissolved in 2ml of distilled water and filtered. To the filtrate ferric chloride solution was added. A bluish green/brownish green color confirms the presence of tannins.

**Test for carbohydrates**

1. *Molish test*: A few mg of the extract was taken in 50% v/w ethanol. Two drops of 20% solution of  $\beta$ -naphthol in ethanol was added and about 1mL of concentrated sulphuric acid was allowed to flow down the sides of the test tube. A red or violet colored ring at the junction of two layers confirms the presence of carbohydrates.

**Test for anthraquinone glycosides**

1. *Bontrager's test*: A small amount of the extract was mixed in ether and after filtration aqueous ammonia or caustic soda was added. Appearance of pink, red or violet color in aqueous layer after shaking, confirms the presence of the anthraquinone glycoside.

**Test for flavonoids**

1. *Concentrated nitric acid test*: Concentrated nitric acid was added to the extract, crimson or magenta color confirms the presence of flavonoids
2. *Aqueous sodium hydroxide test*: Aqueous sodium hydroxide was added to the extract, a red or purple color confirms the presence of flavonoids.

**Evaluation of Antioxidant activity of methanolic fruit extracts of *Ficus benjamina*****Hydrogen peroxide free radical scavenging method.**

Hydrogen peroxide free radical scavenging activity was determined according to the method<sup>[37]</sup>. The methanolic fruit extract of *Ficus benjamina* (MEFB) solution in various concentrations (100-300  $\mu\text{g mL}^{-1}$ ) was mixed with 2.4 mL of 0.1 M phosphate buffer (pH 7.4), and then 0.6 mL of a 43 mM solution of  $\text{H}_2\text{O}_2$  in the same buffer were added. After 10 min the absorbance values of the reaction mixtures were recorded against a blank solution containing phosphate buffer without  $\text{H}_2\text{O}_2$  at 230 nm using a spectrophotometer (Shimadzu UV- 1700 Pharma spec). For each concentration, a separate blank sample was used for background subtraction. Ascorbic acid was used as a standard and mixture without sample was taken as a control. All tests were done in triplicate. The percentage scavenging of  $\text{H}_2\text{O}_2$  was calculated as:

$$\text{Percentage scavenging of } \text{H}_2\text{O}_2 = [(A_0 - A_1)/A_0] \times 100$$

Where:

$A_0$  is the absorbance of the control

$A_1$  is the absorbance of the extract/standard

**RESULT AND DISCUSSION:****Plant Authentication**

The fruit of *Ficus benjamina* were collected from the Garden of Rayat Institute Of Pharmacy, Railmajra, Punjab (India) during the month of March, 2016. It was authenticated by Guru Nanak Dev University (GNDU), Amritsar, Punjab.

**Phytochemical screening**

The phytochemical screening of fruit extracts of *Ficus benjamina* indicates the presence of alkaloids, carbohydrates, flavanoids, sterols and tannin.

Plant constituent/test	Methanolic extract
Alkaloids	++
Carbohydrates	+
Sterols	+
Flavanoids	++
Tannin	++
Anthraquinone glycoside	-

**Table 2:-**Phytochemical screening of *Ficus benjamina* fruit extract.

(-) indicates absence, (+) indicates minor presence (++) indicates high presence of the corresponding constituent.

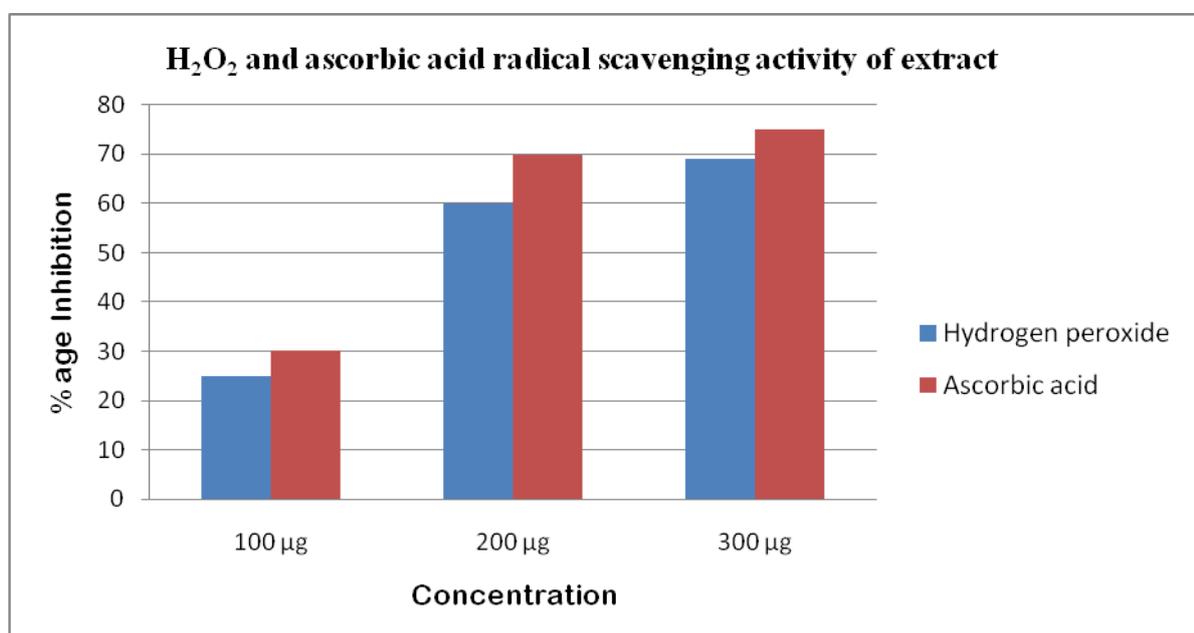
**Free radical scavenging activity by H<sub>2</sub>O<sub>2</sub>**

Antioxidant potential of methanolic fruit extract of *Ficus benjamina* was evaluated by Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) radical scavenging activity. The reduction capability of Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) radical was determined by the decrease in its absorbance at 517 nm. Maximum free radical scavenging activity of methanolic fruit extract of *Ficus benjamina* was shown at a dose of 200µg/ml is 60.12 ± 0.57 by Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) model as shown in Table. Ascorbic acid was used as standard to compare the free radical scavenging activity of methanolic fruit extract of *Ficus benjamina*.

**Table 3:-**Percentage inhibition of Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and Ascorbic acid.

S.No	Concentration ( $\mu\text{g/ml}$ )	Absorbance (nm)	Percentage inhibition of H <sub>2</sub> O <sub>2</sub> radical	
			Methanol extract	Ascorbic acid
1	100	0.209	25.09 $\pm$ 0.35	30.00 $\pm$ 0.45
2	200	0.159	60.12 $\pm$ 0.57	70.00 $\pm$ 0.69
3	300	0.099	69.03 $\pm$ 0.61	75.10 $\pm$ 0.67

Values are mean of triplicate experiments and represented as Mean  $\pm$  S.E.M.

**Fig 4:-** H<sub>2</sub>O<sub>2</sub> and ascorbic acid radical scavenging activity of extract**CONCLUSION:**

The present investigation was for evaluation of methanolic extract of *Ficus benjamina* fruit for the antioxidant activity and it justifies the traditional use of this plant in the treatment of various types cancer. Further study has to be required.

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