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Phytochemical Screening and Evaluation of Banyan Tree (Ficus benghalensis) Aerial Root Extract for Anti-Inflammatory Activity in Gingivitis

Amrita Bharti [1]*, Dr. Rishikesh Sharma[1]
1.Sarvepalli Radhakrishnan College of Pharmacy, NH-12, Narmadapuram Road, Jatkhedi, Misrod, Bhopal M.P. 462026

ABSTRACT

The aerial root of the Banyan Tree was tested and assessed for its anti-inflammatory effects in the case of Gingivitis. The collection and identification of the plant material involved gathering the main aerial parts of the Banyan tree from a local area. The outer surface of the aerial root of *Ficus benghalensis* is gray in color, while the cut surface is reddish-brown and rough, with longitudinal and transverse cracks, as well as rows of lenticels. The fracture surface shows a fibrous, tough bark, and the wood portion is short. The anatomy of the roots showed a well-defined secondary growth pattern. The physiochemical properties of *Ficus benghalensis* were analyzed, including loss on drying, ash values, and extractive values. The aqueous extract was found to contain flavonoids, phenolics, saponins, proteins, and carbohydrates. The total alkaloid content was calculated in terms of atropine equivalent (mg/100mg) based on a calibration curve. The total flavonoid content in the test samples was determined using a calibration plot ($Y = 0.0162x + 0.0044$, $R^2 = 0.999$) and expressed as milligrams of quercetin equivalent (QE) per gram of dried plant material. Group III, which received the extract (EMM) at a dose of 400 mg/kg from the aerial roots of *Ficus benghalensis*, showed a higher percentage of paw edema inhibition compared to Group II (ESM) and Group IV (EDM) that received other extracts of the same plant. However, both groups treated with the extract exhibited lower anti-inflammatory activity than the positive control.

Keywords: Phytochemical, Pharmacological, Banyan Tree, Anti-inflammatory, Gingivitis, Arial root.

*Corresponding Author Name: Amrita Bharti
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INTRODUCTION

Most oral health conditions are largely preventable and can be treated in their early stages. Most cases are dental caries (tooth decay), periodontal diseases, tooth loss and oral cancers. Globally, an estimated 2 billion people suffer from caries of permanent teeth and 514 million children suffer from caries of primary teeth.[1] Gingivitis is reversible with good oral hygiene; however, without treatment, gingivitis can progress to Periodontitis, in which the inflammation of the gums results in tissue destruction and boneresorption around the teeth. Periodontitis can ultimately lead to tooth loss.[2] Studies of the oral micro biome in periodontal disease typically focus on small populations in developed countries with advanced dental healthcare systems, which may not be representative of the natural history of periodontal disease in the absence of treatment.[3] Gingivitis is an inflammatory condition of the gingival tissue or the gums most commonly due to a bacterial infection.[4] It is inflammation of the gingiva with the attachment of the connective tissue to the tooth remaining at the original level, that is, without attachment loss.[5] However, the chronic form of gingivitis that is caused by plaque is considered to be the most common variant. Gingivitis is characterized by swelling and redness in the affected area, along with bleeding upon probing.[6] It is associated with an inflammatory reaction upon the pro-inflammatory cytokines, which are known to be responsible for causing a balance between humoral and cell mediated immune responses.[7] The primary treatment of gingivitis is the removal of plaque and tartar. Dental professional may recommend chlorhexidine mouthwashes in conjunction with brushing and flossing.[8] Some herbs, such as comfrey and Ephedra, can cause serious harm. Some herbs can interact with prescription or over-the-counter medicines.[9] Herbs generally refers to the leafy green or flowering parts of a plant (either fresh or dried), while spices are usually dried and produced from other parts of the plant, including seeds, bark, roots and fruits.[10] Aerial roots of banyan tree have the ability to prevent bad breath, tooth decay, and bleeding problems.[11] Aerial roots of banyan trees have astringent and anti-bacterial properties which prevent bacterial infection and also effective for other oral health problems. Aerial root acts as natural toothpaste and also helps with bad breath.[12]

MATERIALS AND METHOD

Plant Material Collection and Authentication

The aerial parts and leaves of the Banyan tree (Ficus benghalensis) were gathered from Bhopal (Madhya Pradesh) and its neighboring local regions. To eliminate external debris, the collected plant components were washed thoroughly 2 to 3 times using running tap water. The cleaned leaves were then spread out and shaded-dried at room temperature for a duration of one week to

ensure complete moisture evaporation. Once completely dried, a ball mill was utilized to pulverize the samples into a fine powder. This processed powder was then preserved in appropriately labeled, airtight plastic containers for subsequent experimental procedures.

Figure 1: *Ficus benghalensis* aerial root of Banyan tree.

Macroscopic Evaluation

The morphological and macroscopic identification of the medicinal plant material was conducted by analyzing parameters such as shape, size, texture, color, surface characteristics, fracture type, and the visual appearance of the cut surface. For this assessment, harvested segments of the aerial roots measuring 8–10 cm in length were utilized.

Microscopic Examination

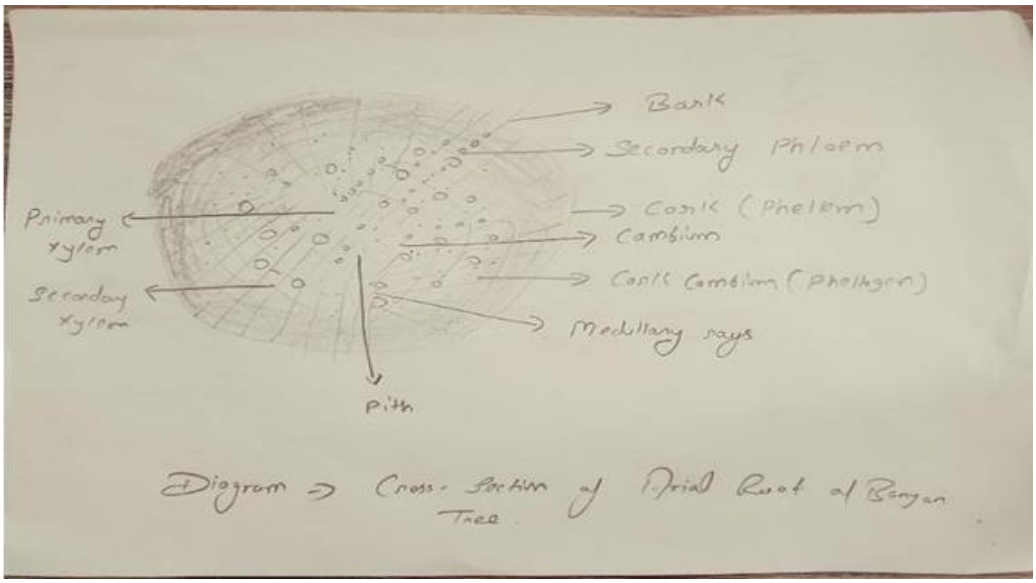

Figure 2: Cross-section of *Ficus benghalensis.*

Freshly harvested aerial root specimens of Ficus benghalensis were thoroughly rinsed with distilled water and subsequently fixed using a 2.5% glutaraldehyde solution. Transverse sections

(semi-thin) were obtained freehand using a sharp razor. These sections underwent a systematic dehydration process using an ascending series of ethanol gradients, ranging from 35% up to 100%. Following dehydration, the specimens were mounted on clean glass slides using a drop of DPX mountant and evaluated directly under a light microscope at low magnification.

Phytochemical Analysis

Plants serve as a rich source of diverse secondary metabolites that offer therapeutic benefits against various human health disorders. The genus Ficus represents one of the largest groups in the plant kingdom, characterized by a promising array of bioactive compounds. The species Ficus benghalensis contains several major chemical classes, including phenols, flavonoids, alkaloids, sterols, tannins, terpenoids, and saponins.

Quantitative Estimation of Phytochemicals

Estimation of Total Flavonoid Content: The total flavonoid content within the extract was determined using the aluminum chloride colorimetric assay, with quercetin serving as the reference standard to generate the calibration curve. To prepare the stock solution, 5.0 mg of quercetin was dissolved in 1.0 ml of methanol, followed by serial dilutions to obtain standard concentrations between 40 and 200 μg/ml. For the assay, 0.6 ml of either the diluted extract or the standard quercetin solution was mixed with 0.6 ml of 2% aluminum chloride. The reaction mixture was incubated for 60 minutes at room temperature. Subsequently, the absorbance was recorded at 420 nm against a reagent blank using a Varian UV-Vis spectrophotometer.

Estimation of Total Alkaloid Content: A 1 mg portion of the plant extract was dissolved in methanol, treated with 1 ml of 2 N HCl, and filtered. The filtrate was transferred to a separating funnel, where it was mixed with 5 ml of bromocresol green solution and 5 ml of phosphate buffer. This mixture was sequentially extracted with chloroform (1, 2, 3, and 4 ml) via intense agitation, collected in a 10-ml volumetric flask, and brought to volume using chloroform. Standard reference solutions of atropine were prepared concurrently at concentrations of 40, 60, 80, 100, and 120 μg/ml using the identical procedure. The absorbance of both the test samples and standard dilutions was measured at 470 nm against a reagent blank via a UV/Visible spectrophotometer. The total alkaloid content was quantified as mg of Atropine Equivalents (AE) per 100 mg of the extract.

In-Vivo Anti-Inflammatory Evaluation of Banyan Aerial Root Extract

Experimental Animals

Healthy, young adult albino rats (weighing 100-120 g) of both sexes and similar age groups were selected for the study. The animals were housed in polyacrylic cages under standard laboratory

conditions, receiving a standard pellet diet and water ad libitum. Prior to the commencement of the study, the animals were allowed to acclimatize to the laboratory environment for one week. The experimental protocol received clearance from the Institutional Animal Ethics Committee (IAEC) of SRK University, Bhopal, M.P. (Approval No. ABCD/IAEC/July 2015/07), and all procedures adhered strictly to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Acute Oral Toxicity Study (OECD 425)

Acute oral toxicity was monitored on Wistar albino rats following the guidelines outlined in OECD 425. The animals were subjected to overnight fasting (with water available ad libitum) prior to dosing. In this limit test, three selected animals were orally administered a single dose of 2000 mg/kg body weight of the Ficus benghalensis extract via an oral gavage cannula. The animals were monitored individually for mortality and clinical signs of gross toxicity—such as tremors, convulsions, depression, circling, or behavioral changes—continuously for the first 4 hours, at the 24-hour mark, and daily thereafter. The administered dose of 2000 mg/kg was found to be safe and well-tolerated as no mortality occurred. Consequently, a working dose of 400 mg/kg body weight was designated for subsequent pharmacological evaluations.

Carrageenan-Induced Paw Edema Model

The in-vivo anti-inflammatory evaluation was conducted using the carrageenan-induced paw edema model. A cohort of 30 albino rats was randomized into five distinct groups, with each group comprising six animals. Acute localized inflammation was induced across all groups via a sub-plantar injection of 0.1 ml of a freshly prepared 1% carrageenan suspension in normal saline into the left hind paw.

Experimental Design

The formulated plant extract (0.3g), vehicle base, or standard drug was applied topically to the plantar region of the hind paw with gentle manual rubbing. This application was performed for each respective animal group one hour prior to and one hour following the carrageenan challenge. The volume of the paw edema was quantified utilizing a plethysmometer at 30-minute intervals over a post-injection period of 4 hours. The mean paw edema volume for each treated group was determined and statistically evaluated against the control group. The percentage inhibition of edema was calculated using the formula:

% Edema inhibition = (1-Vt/Vc) X 100

Where, Vt = Mean edema volume of test, Vc = Mean edema volume of control.

RESULTS AND DISCUSSION

Microscopic Characteristics of Ficus benghalensis Aerial Roots

Anatomical investigation of the aerial roots demonstrated a distinctive secondary growth configuration. The outermost region was composed of bark layers, followed by the phloem and phellogen. The baseline arrangement of the primary vascular bundle was indicated by radially oriented, exarch primary xylem positioned in the central zone. The strands constituting the secondary vascular tissues exhibited a collateral arrangement.

The initiation of cambial activity led to the development of medullary rays that traversed between the phloem and xylem elements through the cambium. The xylem vessels displayed a nearly circular cross-sectional outline. Taken together, these anatomical features present the structural profile of a characteristic dicotyledonous root undergoing secondary thickening. No structural abnormalities or anomalous secondary growths were identified.

The current structural analysis indicates that the anatomical configuration and cellular arrangements of these roots remain highly uniform, displaying minimal structural alterations in response to variations in their chemical and metabolic profiles. Organoleptic attributes of the pulverized Ficus benghalensis aerial roots were also documented.

Physicochemical Characterization of powder of aerial root of *Ficus benghalensis*

Physiochemical parameters of *Ficus benghalensis* (aerial root).

Table 1: Determination of following Physicochemical parameters.

S. No.	Parameters	Observed value (%w/w)
1.	**Loss on Drying**	12.25
2.	**Ash values**	
	Total ash	3.35
	Water soluble ash	3.30
3.	**Extractive values**	
	Water soluble extract	2.70

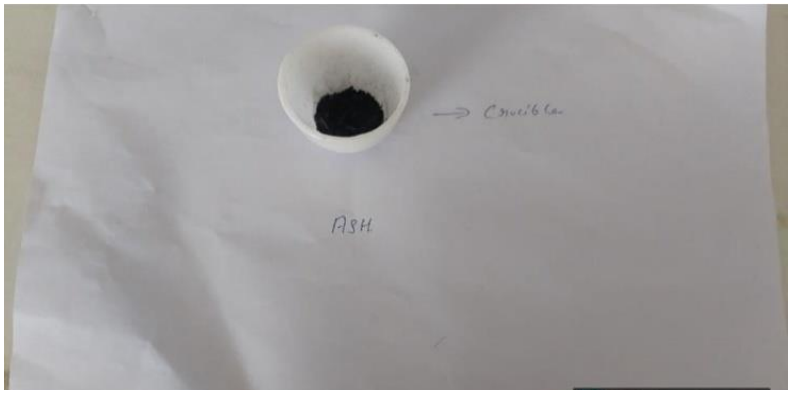

Figure 3: Determination of Ash value.

Extraction of Powdered Aerial Root of *Ficus benghalensis*:

The aqueous extract was standardized with respect to physio-chemical parameters like, color, consistency, pH and extractive value Qualitative chemical tests were carried out to determine the presence of phytoconstituents. The aqueous extract was found to contain flavonoids, phenolics, saponins, proteins and carbohydrates.

Table 2: Conditions used during Extraction of *Ficus benghalensis.*

Method	Solvent	Temperature	Pressure	Time	Volume Consumed	Polarity of natural Product
Soxhlet Apparatus	Aqueous	Under heat	Atmospheric pressure	6hrs	Moderate	Dependent of extracting solvent
Maceration	Aqueous	Room temperature	Atmospheric pressure	7day	Large	Dependent of extracting solvent
Decoction	Water	Under Heat	Atmospheric	15min	Nine	Polar compound

Table 3: Physico-chemical evaluation of the aqueous extract.

Physicochemical properties	Aqueous extract
Nature	Semisolid
Color	Dark brown
pH	8.5-9.8
Extractive value % w/w	25.37%
Water soluble extractive of the *Ficus benghalensis* %w/w	26.5%

Table 4: Properties of Extracts obtained by different methods.

S. No.	Properties	Extraction by Soxhletion Method (ESM)	Extraction by Maceration Method (EMM)	Extraction by Decoction Method (EDM)
1	Color	Dark Yellowish	Yellow Color	Brown Color
2	Extraction sustained	6 Hrs.	7 days	15 min
3	Temperature	30-40°C	Room temperature	100°C Heat mantle
4	Solvent	250 ml	250 ml	200 ml
5	Aerial root powder	20 gm	5 gm	5 gm

Qualitative Phytochemical Evaluation of *Ficus benghalensis* extract

Phytochemical investigation of *Ficus benghalensis* aerial roots.

Table 5: Quantitative Phytochemical tests of *Ficus benghalensis* Aqueous extracts.

Name of the test	ESM	EMM	EDM
Test of the Alkaloids			
a) Dragendroff's Test	+	+	+
b) Wagner's Test	+	+	-
c) Mayer's Test	+	-	+
d) Hager's Test	+	+	+
Test of the Tri-terpenoids			
a) Salkowski Test	–	–	–
Test of the Carbohydrates			
a) Molisch's test	–	–	–
b) Fehling's test	–	+	–

c) Benedict's test	–	–	–
Test of the Cardiac glycosides			
(a) Baljet test	-	-	-
Test of the Flavonoids			
(a) Shinoda test	+	+	+
(b) Alkaline reagent test	+	+	+

The *Ficus benghalensis* extracts of aerial roots were evaluated for the detection of its phytochemical constituents. The solvents are used aqueous (water) for extraction. The extracts were tested for carbohydrates, triterpenoids, alkaloids, cardiac glycosides, flavonoids. In *Ficus benghalensis* aerial roots, the aqueous extract shows the absence of triterpenoids whereas the aqueous extracts show the presence of, alkaloids, and flavonoids. From the results, it was confirmed that flavonoids and alkaloids were present in the aqueous extracts. The *Ficus benghalensis* was used for the qualitative test of aqueous extract and the data was represented in.

Table 6: Identification test of Constituent.

Name of compound	Name of the test	Color
Alkaloids	(a) Dragendroff's test	Orange–brown
	(b) Wagner's Test	ppt Reddish
	© Mayer's Test	brown ppt
	(d) Hager's test	Cream Color
		Yellow Color
Tri-terpenoids	(a) Salkowski Test	Pink Color
Carbohydrates	(a) Molisch's test	Voilet Color
	(b) Fehling's test	Dark red Color
	© Benedict's test	Dark red Color
Cardiac glycosides	(a) Baljet test	Orange Color
Flavonoids	(b) Shinoda test	Pink Color
	© Alkaline Reagent test	Colorless

Quantitative tests Evaluation of *Ficus benghalensis* extract

Estimation of Total flavanoid content (TFC): The concentration of total flavonoid content in the test samples was calculated from the calibration plot ($Y = 0.002x + 0.007$ $R^2 = 0.998$) and expressed as mg quercetin equivalent (QE)/g of dried plant material. All the determinations were carried out in triplicate.

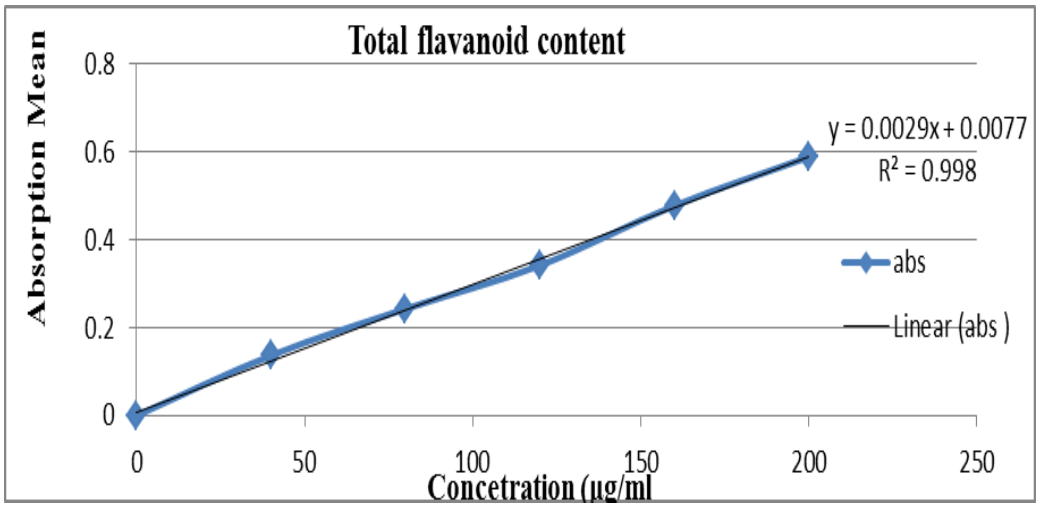

Figure 4: Graph of calibration curve of quercetin.

Estimation of Total alkaloid content (TAC):

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: y = 0.008x + 0.010, R2=0.999, where X is the Atropine equivalent (AE) and Y is the absorbance.

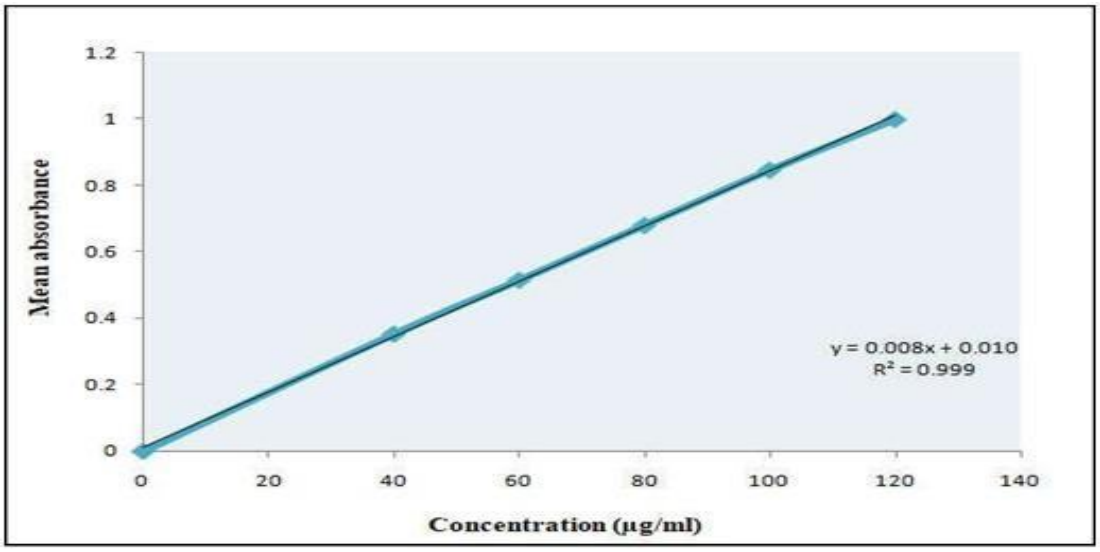

Figure 5: Graph of calibration curve of Atropine.

Table 7: Quantitative Estimation of *Ficus benghalensis* extracts obtained by different extraction methods.

S. No.	Aqueous Extract	Total flavanoid content (mg/100mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	ESM	0.756	0.632
2.	EMM	0.642	0.612
3.	EDM	0.609	0.578

Anti-inflammatory Activity

Table 8: Anti-inflammatory activities of *Ficus benghalensis* extracts.

Groups	% Inhibition of Edema							
	0.5 Hr	1.0 Hr.	1.5 Hr.	2.0 Hr.	2.5 Hr.	3.0 Hr	3.5 Hr.	4.0 Hr.
Group I (Saline Water)	0	0	0	0	0	0	0	0
Group II Plant Extract(ESM)	10.3± 0.43	18.4± 1.83	25.8 ±2.16	37.3± 1.47	43.0 ±2.12	50.3± 1.32	58.1± 2.46	62.1± 1.02
Group III Plant Extract(EMM)	09.2± 0.34	16.3± 1.03	24.3± 2.13	37.2± 1.95	43.1± 2.68	51.2± 0.71	58.1± 1.59	68.1± 2.74
Group IV Plant Extract (EDM)	11.5± 1.24	15.3± 1.63	22.1± 2.83	28.2± 2.67	35.3± 0.81	42.8± 1.63	49.3± 0.91	54.3± 1.85
Group V Diclofenac Sod.	14.8± 0.89	21.3± 0.71	29.8± 1.83	37.3± 1.20	46.8± 3.71	58.9± 2.51	68.1± 2.15	76.2± 2.94

Means ± SD; (n = 5) ns ≥ 0.05 compared to control (Ordinary One way ANOVA test).

Statistical Analysis:

The results of the Anti-inflammatory activities of the aqueous extract of *Ficus benghalensis* in terms of % Inhibition of Edema were statically presented as shown below

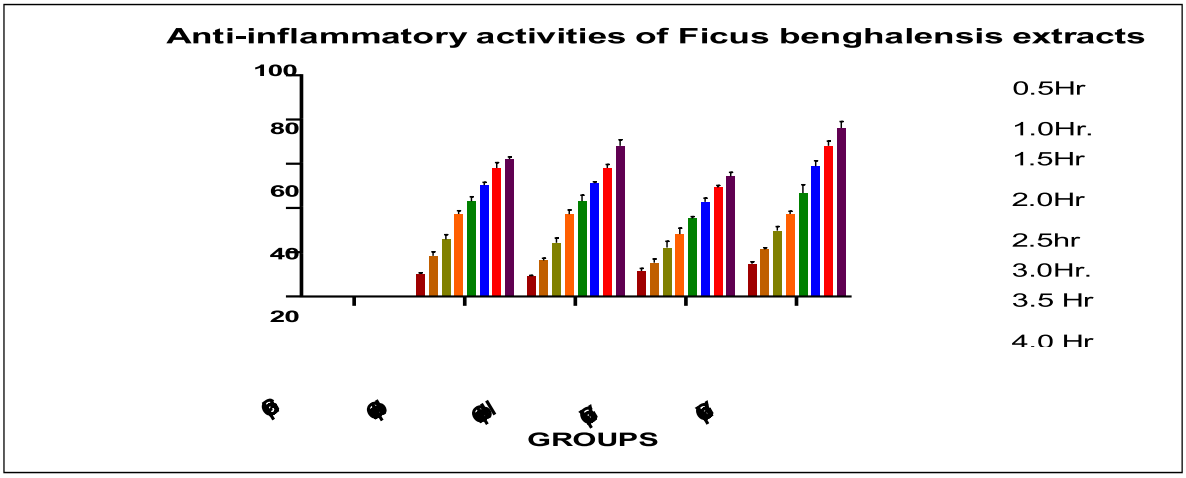

Figure 6: Anti-inflammatory activities of *Ficus benghalensis* extract.

Apart from that, all the three aqueous extracts obtained using soxhelation, maceration and decoction showed a similar trend of edema development. Additionally, the aqueous extracts of *Ficus benghalensis* produced significant anti-inflammatory activity at fixed dose label 400mg/kg.

The aqueous extracts obtained from three different extraction methods of aerial roots of *Ficus benghalensis* successfully controlled the thickness of paw due to edema when compared with that of the negative control group treated with standard drug Diclofenac sodium. The Group III treated with Extract (EMM) 400 mg/Kg of aerial roots of *Ficus benghalensis* possessed higher percentage of paw edema inhibition then Group II treated with extract (ESM) and Group IV treated with extract (EDM)of aerial roots of *Ficus benghalensis.* However, all two groups treated with extract exhibited lower anti-inflammatory activity than the positive control.

CONCLUSION

The *Ficus benghalensis* is a well-known bioactive plant in Ayurvedic system of medicine. The present investigation was aimed at determining the anti-inflammatory activity of aerial root of *Ficus benghalensis* extract. This study confirms the ayurvedic claim that the aqueous extract of *Ficus benghalensis aerial* roots possesses significant anti-inflammatory activity. The anti-inflammatory property of aqueous extract of *Ficus benghalensis* may be due to the presence of flavonoids and alkaloids as presented in quantitative and qualitative test results. However, further studies are required to isolate and identify the possible phytoconstituents of *Ficus benghalensis* responsible for the activity, which would facilitate the future use of isolated phytoconstituents of *Ficus benghalensis* in inflammation related disease.

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