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PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL EVALUATION OF EXTRACT ARIAL ROOT OF BANYAN TREE FOR ANTI-INFLAMMATORY ACTIVITIES OF GINGIVITIS

Kaveri Soni[1]*, Dr. Nishi Prakash Jain[2], Dr. Prashant Soni[3] and Ankita Chourasiya[4]

[1]Student, RKDF College of Pharmacy, Bhopal.

[2]Principal, RKDF College of Pharmacy, Bhopal.

[3]Principal, Dr. APJ Abdul Kalam College of Pharmacy, Bhopal.

[4]Assistant Professor, RKDF College of Pharmacy, Bhopal.

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***Corresponding Author**
Kaveri Soni
Student, RKDF College of Pharmacy, Bhopal.

ABSTRACT

Arial root of Banyan Tree was screened and evaluated for Anti-inflammatory activities in the condition of Gingivitis. Collection and identification of plant material Aerial parts of Banyan tree major were collected from local area. The external surface of the aerial root of *Ficus benghalensis* is Gray, the cut surface is reddish-brown, rough due to longitudinal and transverse cracks and transverse rows of lenticels, fracture, and bark portion is fibrous, tough, and short in wood portion. Anatomy of the roots revealed a well-characterized secondary growth pattern. Physiochemical parameters of *Ficus benghalensis* were loss on drying, ash values, extractive values determined. The aqueous extract was found to contain flavonoids, phenolics, saponins, proteins and carbohydrates. Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve. The concentration of total flavonoid content in the test samples was calculated from the calibration plot ($Y = 0.0162x + 0.0044$, $R^2 = 0.999$) and expressed as mg quercetin equivalent (QE)/g of dried plant material. 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.

KEYWORDS: Phytochemical, harmacological, Banyan Tree, Anti-inflammatory, Gingivitis, Arial root.

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]

MATERIAL AND METHOD

Collection of plant material: Collection and identification of plant material Aerial parts of Banyan tree major were collected from local area. The plants were collected from local area of Bhopal, and its surroundings. Then *Ficus benghalensis* washed with tap water about 2-3 times. For evaporating the water content, the washed plants leaves were kept in for drying for 1week for room temperature under the shade. After drying, sample was grounded to get fine powder with the help of Ball mill. Then for the future use with proper labeling, the powder stored in air tight plastic container.

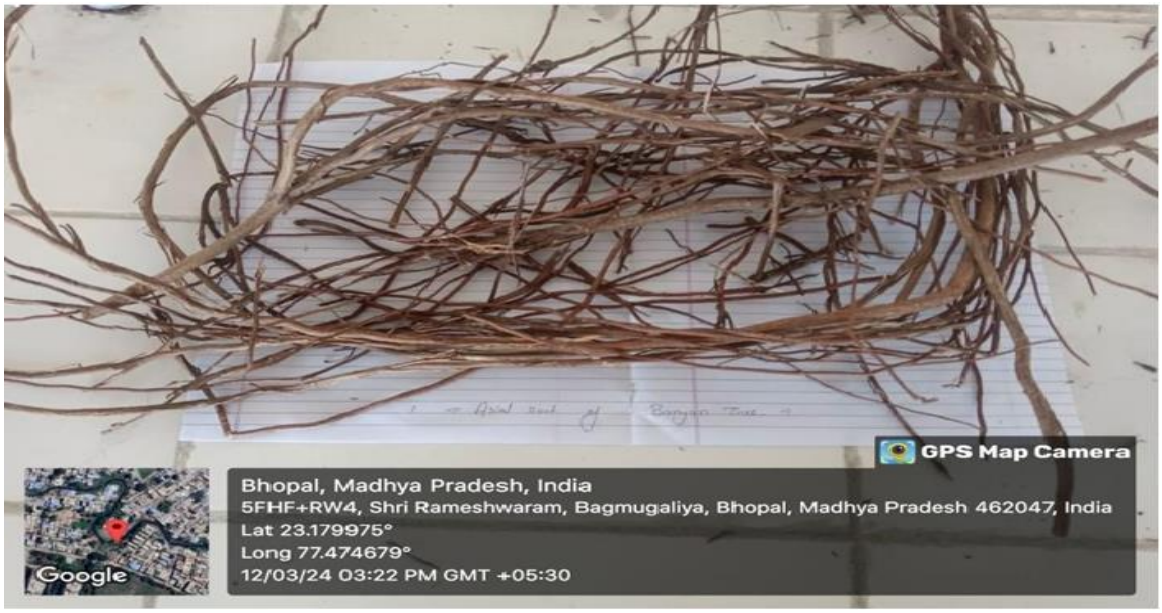

Figure 1: *Ficus benghalensis* aerial root of Banyan tree.

Macroscopic characteristics: Macroscopic characteristics identification of medicinal plant materials is based on its shape, size, surface, texture, fracture, color, and appearance of the cut surface. The collected cut pieces of aerial roots were 8–10 cm long and macroscopic characteristics were evaluated.

Microscopic characteristics: Fresh specimens of aerial roots of *Ficus benghalensis* were collected and washed thoroughly with the distilled water. Then, the samples were fixed in 2.5% glutaraldehyde. Freehand semi-thin transverse sections were done with the help of a razor. The sections were allowed to dehydrate by passing it from various gradations of ethanol starting from 35% to 100%. After the dehydration, sections were mounted over a drop of DPX taken in a clean slide and directly observed under light microscope under low magnification.

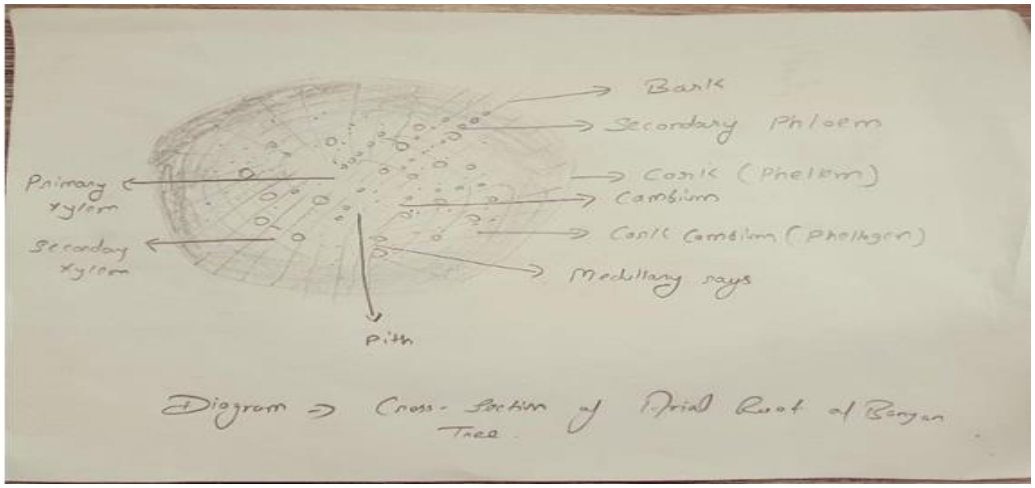

Figure 2: Cross-section of *Ficus benghalensis.*

Phytochemical Constituent

Plants are the source of various phytochemical constituents that are functional as a remedy for health defects that occur in humans, and the diversity of the plant metabolites benefits humans in treating those conditions. *Ficus benghalensis* species is one of the largest generals of the plant kingdom, with promising phytoconstituents from various classes of compounds, including phenols, flavonoids, sterols, alkaloids, tannins, saponin, terpenoids, etc.

Quantitative Tests of Extracts for Phytochemical

(A) Estimation of total flavonoids content: The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the sample. For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 ml methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol (40–200 μg/ml). An amount of 0.6 ml diluted standard quercetin solutions or extracts was separately mixed with 0.6 ml of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 420 nm wavelength with a Varian UV-Vis spectrophotometer.

(B) Estimation of total alkaloids content: The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same

manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

***In–vivo* anti-inflammatory activity of extract of Arial root of banyan tree**

Animals: Healthy young adult albino (100-120 gm) of either sex and of approximate same age were used throughout the study were housed under standard laboratory conditions in polyacrylic cages, and were provided with pelleted food and water *ad libitum*. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. Animal studies were approved by Institutional Animal Ethics Committee (IAEC) of SRK University, Bhopal, M.P. with Approval Number, ABCD/IAEC/ July 2015/07, and carried out in accordance with the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute oral toxicity study (OECD guideline 425): Acute oral toxicity study was evaluated as per OECD guidelines (425) on Wistar albino rats. Before experimentation rats were fasted overnight with water *ad libitum*. Three animals were selected which receives dose of 2000mg/kg. All three animals were received dose of 2000 mg/kg body weight of extract of *Ficus benghalensis* extract by gavage using oral canula (limit test). Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours, and thereafter, 24 hours, Administered dose was found tolerable (as no death found). Therefore, 400 mg/kg dose level was selected for further activity.

Carrageenan induced paw edema model in rats: *In-vivo* Anti-inflammatory study of plant extract was conducted by Carrageenan induced paw edema model using 30 albino rats and divided into five groups of six animals on each. In all groups, acute inflammation was induced by sub-planter injection of 0.1 ml of freshly prepared 1 % suspension of Carrageenan in normal saline in left hind paw of the rats.

Anti-Inflammatory Activity (Experimental design): The plant extract (0.3g) or base or standard were applied topically to the planter surface of hind paw with gentle rubbing with index finger to each rat of respective group one hour before and one hour after the Carrageenan challenge. The paw edema volume was measured using plethysmometer at every 30 minute intervals for 4 hour after injection of Carrageenan. The average paw edema

volume of all the groups were calculated and compared with that of control. The percent inhibition of edema was calculated by using following formula.

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

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

RESULT AND DISCUSSION

Microscopic character of aerial root of *Ficus benghalensis*: Anatomy of the roots revealed a well-characterized secondary growth pattern. Outer most layer consists of bark followed by the phloem and phellogen. The structure of primary vascular bundle in this stage was revealed by the radially arranged exarch primary xylem located in the central region. The strands of secondary vascular tissues were arranged collaterally. Cambial activity causes the formation of medullary rays which runs between the xylem and the phloem through the cambium. Xylem vessel shows almost circular in outline. Overall, the anatomical structures revealed the picture of a typical dicot root showing a secondary growth. No other kind of special character, as well as an anomaly, was observed.

The present anatomical study was predictable to conclude that in this case there is hardly any change in their anatomical structures and cellular pattern in relation to their metabolic and chemical profile. Organoleptic features of powder of aerial roots of *Ficus benghalensis.*

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

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

Table No. 1: Determination of following Physicochemical parameters.

S. No.	Parameters	Observed value (%w/w)
1.	**Loss on Drying**	11.75
2.	**Ash values**	
	Total ash	3.45
	Water soluble ash	3.33
3.	**Extractive values**	
	Water soluble extract	2.90

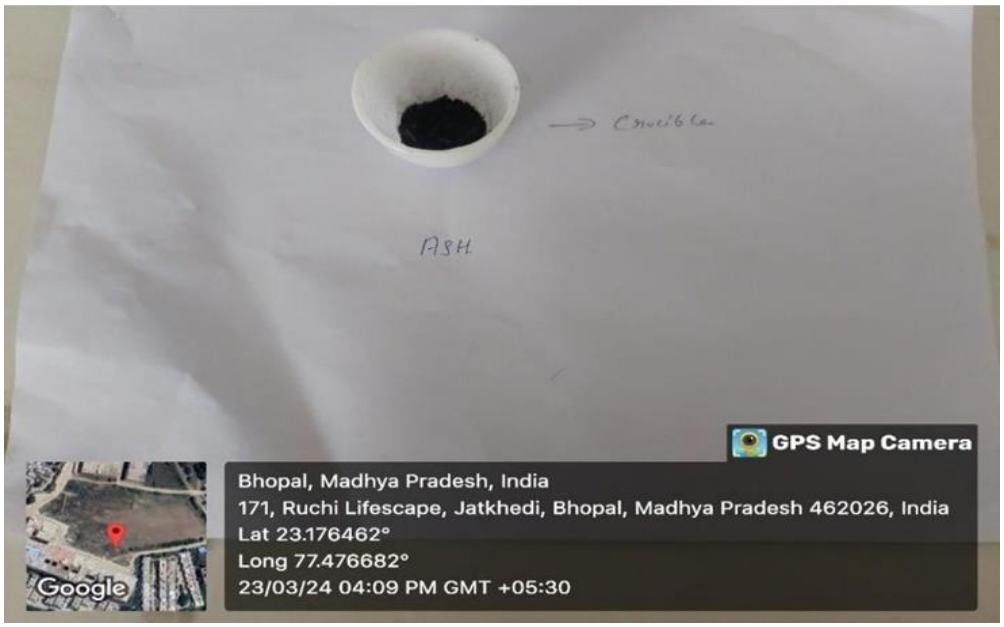

Figure 3: Determination of Ash value.

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 No. 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 No. 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	24.37%
Water soluble extractive of the *Ficus benghalensis* %w/w	27%

Table No. 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 No. 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 No. 6: Identification test of Constituent.

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

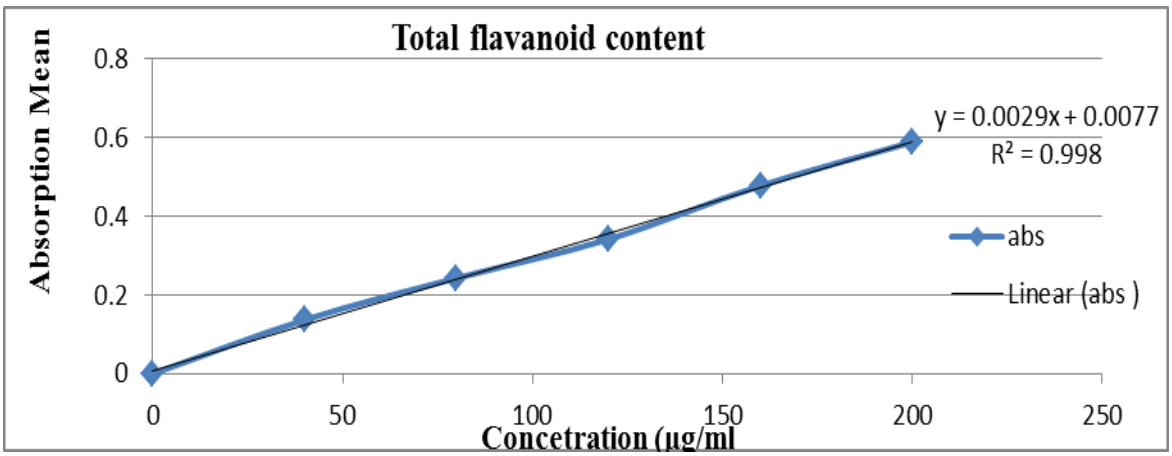

Figure 4: Graph of calibration curve of quercetin.

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.

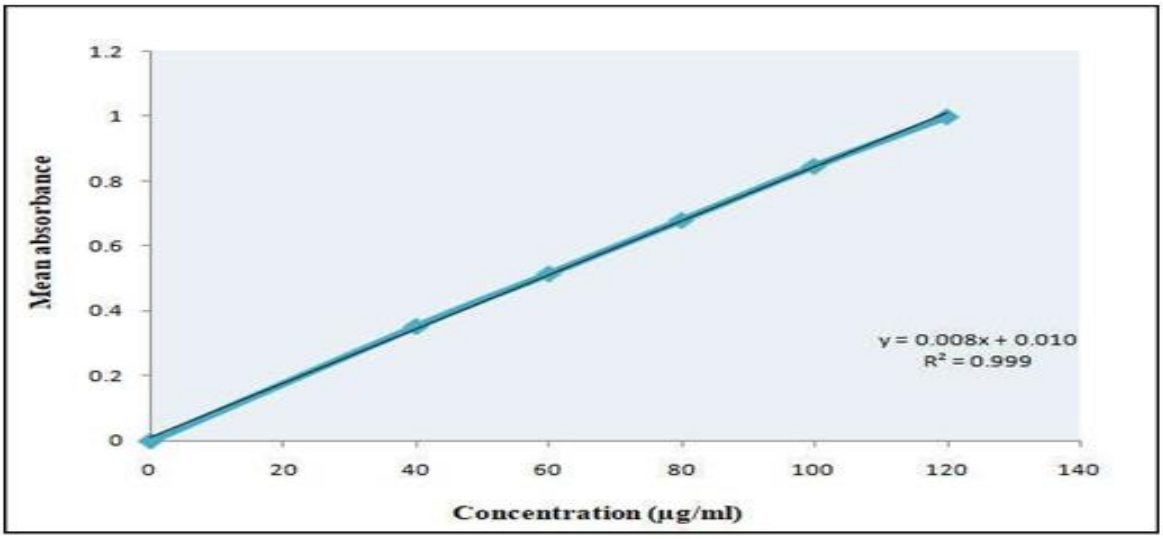

Figure 5: Graph of calibration curve of Atropine.

Table No. 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

7.9 Anti-inflammatory Activity

Table No. 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

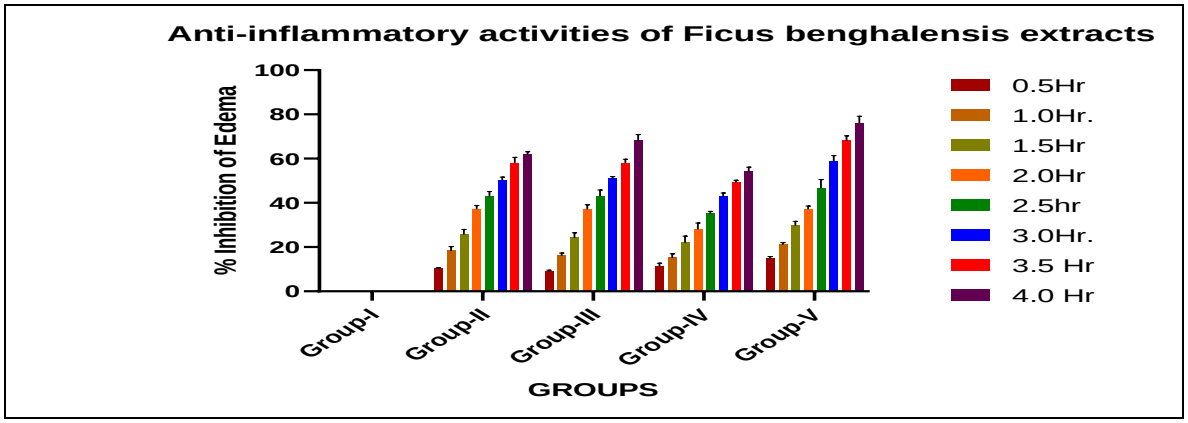

Figure 6: Anti-inflammatory activities of *Ficus benghalensis* extract.

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 antiinflammatory 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 diseases.

CONFLICTS OF INTERESTS

There are no conflicts of interests.

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