Journal of Basic and Clinical Pharmacy

www.jbclinpharm.com

# A COMPARATIVE EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF THE BARK OF *FICUS BENGALENSIS* IN PLANTS OF DIFFERENT AGE

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ABSTRACT: *The medicinal plants have been selected for thorough studies from indigenous folk medicines, Ayurvedic, Unani and Siddha systems of medicines. The aim of this study deals with the comparative evaluation of anti-inflammatory activity of the bark of Ficus bengalensis in plants of different age. The anti-inflammatory activity was evaluated by rat paw edema model induced by carrageenan for acute inflammation and cotton pellet granuloma model for chronic inflammation. Indomethacin was used as a standard drug. The various extracts were studied for their anti-inflammatory activity in carrageenan-induced hind paw edema in rats and the paw volume was measured plethysmometrically from 0 to 3h after injection. We have determined the anti-inflammatory activity of various extracts of the bark of Ficus bengalensis with oral administration doses of 300 and 600 mg/kg/day of body weight to healthy animals. Positive results for flavonoids, sterols, and triterpene, tannins and saponins compounds were investigated by phytochemical analysis. The ethanolic extract of younger plant showed a greater anti-inflammatory effect compared with the standard drug indomethacin. Present studies besides confirming anti-inflammatory activity of the ethanolic extract of younger more potent than mature plant help to identify from the comparative study of the bark of Ficus bengalensis.*

KEYWORDS: Anti-inflammatory, Ficus bengalensis, Moraceae, Indomethacin

received on 25-03-2010

modefied on 18-04-2010

accepted on 01-05-2010

available online 15-05-2010

www.jbclinpharm.com

## INTRODUCTION

*Ficus* Linn. (Moraceae) constituted one of the largest genera of flowering plants with about 800 species of free-standing trees, hemi-epiphytes, and shrubs primarily occurring in subtropical and tropical regions world-wide. The genus is remarkable for the large variation in the habits of its species [1]. Many species are cultivated for shade and ornament in gardens. Several species produce edible figs of varying palatability. Some species are producing latex/rubber yielding tree. The fig is a very nourishing food and used in industrial products. It is very energizing, rich in vitamin, mineral elements, water, and fats. In India, the most important species of *Ficus* are *F. bengalensis*, *F. carica*, and *F. elastica*. It is propagated by seeds. Thus, the *Ficus* germplasm is characterized by a great diversity since a high number of varieties accessions has been identified [2]. *Ficus bengalensis* (Moraceae) is commonly known as a Banyan tree or Vata or Vada tree in ayurveda. *Ficus bengalensis* is a remarkable tree from India that sends down its branches and great number of shoots, which take root and become new trunks. This tree is considered to be sacred in many places in India. Earlier, glucoside, 20-tetratriaconthene-2-one,6-heptatriacontene-10-one,pentatriacontan-5-one, beta sitosterol-alpha-Dglucose, and meso-inositol have been isolated from the bark of the *Ficus bengalensis* [3,4]. The tree *Ficus bengalensis*, Linn (Moraceae), commonly known as Banyan tree [5] contains flavonoids [6], tannins and carbohydrates [5]. The uses reported are anti atherosclerosis, antioxidant [7], antidiarrhoeal [8] etc. The barks were reported to have antidiabetic activity [9].

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In some places the aerial roots were also used with barks as drugs by the traditional medical practitioners. Although inflammatory and related immune responses are normal defence mechanisms essential to health, they play potentially harmful roles in diseases such as rheumatoid arthritis and asthma [10]. Inflammation is a complex process regulated by many different mediators, including prostaglandins [11, 12]. Nonsteroidal anti-inflammatory compounds can relieve the pain and inflammation associated with elevated levels of prostaglandins in the body [13]. Similar compounds are proposed to be the agents responsible for the action of traditional herbal remedies associated with the reduction of pain, fever and inflammation. The fruit extracts of *Ficus bengalensis* exhibited antitumor activity in the potato disc bioassay [14].The leaves contain 9.63% crude protein, 26.84% crude fibres, 2.53% CaO, and 0.4% phosphorous. It yields latex containing caoytchoue (2.4%), resin, albumin, cerin, sugar, and malic acid. It is used in ayurveda for the treatment of diarrhea, dysentery, and piles [8, 15] and as a hypoglycemic [16, 17]. The extracts of Ficus bengalensis were also reported to inhibit insulinase activity from the liver and kidney [18]. It was also found to inhibit the lipid peroxidation [19]. Various extracts of *Ficus bengalensis* were screened for its anti-allergic and anti-stress potential in asthma by milk-induced leucocytosis and milk-induced eosinophilia [20]. Other species of Ficus viz. *Ficus Racemosa* [21] *Ficus inspida, Ficus religiosa, Ficus elastica and Ficus Indica* [22, 23] were found to have anti-inflammatory activity. Based on this, an attempt has been made to evaluate the inflammation potency of *Ficus bengalensis*.

## MATERIAL AND METHODS:

### Drugs

Indomethacin (Micro labs, Bangalore), Carrageenan (Sigma Chemicals), Ethanol AR (Thomas Baker Chemical Pvt. Ltd.), Petroleum ether AR (60-80 °C, MCC) were used during the experimental protocol.

### Animals

Wistar albino rats (120-200g) of either sex supplied from Yash Farms, Pune India were used. The animals housed under standard laboratory conditions maintained at 25 ± 10 °C and under 12/12 h light/dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India ltd) and water *ad libitum*. The experimental protocol has been approved by the institutional animal ethics committee and by the animal regulatory body of the Indian Government (Registration No.652/02/a/ CPCSEA, dated 25/01/1999).

### Plant material

The younger plant fresh bark of *Ficus bengalensis* was collected from the foothills of the Satpuda ranges in the district of Jalgaon (MS) in the month of May and June 2008. The plant was identified and authenticated by the Joint Director of Botanical Survey of India, Pune dated 09/09/2008 and letter No.BSI/WC/Tech/2008/411.

The mature plant fresh bark of *Ficus bengalensis* was collected from the Nashik district, Maharashtra, India. The plant was identified and authenticated by the Joint Director of Botanical Survey of India, Pune dated 09/09/2008 and letter No. BSI/WC/Tech/2008/412.

### Preparation of extracts

The powdered plant material (450g) was repeatedly extracted in a 5000 ml round bottomed flask with 2000 ml solvents starting with petroleum ether, chloroform and ethanol. The reflux time for each solvent was 40 cycles. The extracts were cooled at room temperature, and evaporated to dryness under reduced pressure in rotary evaporator.

### Toxicity studies

The extracts were given at the doses of 300 and 600 mg/kg/day of body weight. All the animals found to be safe at dose of 5000 mg/kg (as per OECD Guidelines).

## EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

### Carrageenan-Induced Paw Edema method

The albino rats of either gender were divided into eight groups of six animals each. Group I received 0.2 ml of 2% w/v carboxy methyl cellulose suspension orally for 7 days as a control group, Group II received 300 mg/kg body weight of ethanolic extract of *Ficus bengalensis* (EEFB-I) orally for 7 days, Group III received 600 mg/kg body weight of ethanolic extract of *Ficus bengalensis* (EEFB-II) orally for 7 days, Group IV received 300 mg/kg body weight of chloroform extract of *Ficus bengalensis* (CEFB-I) orally for 7 days, Group V received 600 mg/kg body weight of chloroform extract of *Ficus*

**Table I: Effect of Ficus bengalensis (Younger plant) with carrageenan induced paw edema in rats**

| Treatments | Dose mg/kg | Mean paw volume in ml | | | | | | Percent inhibition |
|---|---|---|---|---|---|---|---|---|
| | | 0 mins | 15 mins | 30 mins | 60 mins | 120 mins | 180 mins | |
| Control | 2%CMC | 1.60 ± 0.005 | 1.53 ± 0.009 | 1.45 ± 0.006 | 1.44 ± 0.005 | 1.43 ± 0.008 | 1.42 ± 0.006 | Vc 1.47 |
| EEFB-I | 300 | 0.85 ± 0.005 | 0.87 ± 0.007 | 0.91 ± 0.007 | 0.93 ± 0.006 | 0.94 ± 0.005 | 0.12 ± 0.005 | 37.64 |
| EEFB-II | 600 | 0.27 ± 0.005 | 0.35 ± 0.006 | 0.47 ± 0.008 | 0.49 ± 0.007 | 0.55 ± 0.008 | 0.60 ± 0.005 | 69.04 |
| CEFB-I | 300 | 0.92 ± 0.008 | 0.93 ± 0.005 | 0.95 ± 0.005 | 1.1 ± 0.007 | 1.31 ± 0.006 | 1.41 ± 0.005 | 24.94 |
| CEFB-II | 600 | 0.93 ± 0.005 | 0.95 ± 0.008 | 0.96 ± 0.009 | 0.97 ± 0.007 | 1.1 ± 0.003 | 1.12 ± 0.005 | 31.63 |
| PEEFB-I | 300 | 1.30 ± 0.005 | 1.31 ± 0.007 | 1.34 ± 0.005 | 1.36 ± 0.005 | 1.42 ± 0.007 | 1.45 ± 0.008 | 7.25 |
| PEEFB-II | 600 | 1.12 ± 0.006 | 1.21 ± 0.005 | 1.25 ± 0.005 | 1.31 ± 0.005 | 1.42 ± 0.01 | 1.56 ± 0.006 | 10.77 |
| Indometh-acin | 10 | 0.72 ± 0.007 | 0.79 ± 0.01 | 0.85 ± 0.005 | 0.74 ± 0.005 | 0.55 ± 0.008 | 0.28 ± 0.005 | 81.08 |

Figures in parenthesis indicated oedema inhibition percentage, N=6 animals per group, $p<0.05$ by Student's t-test

**Table II: Effect of bark of Ficus bengalensis on cotton pellet granuloma in rats[a]**

| Treatments | Dose | Weight of cotton-pellet (mean ± S.E.M.) | Inhibition (%) |
|---|---|---|---|
| Control | 2%CMC | 41.5±0.5 | - |
| EEFB-I | 300 mg/kg | 33.5±0.3** | 19.27 |
| EEFB-II | 600 mg/kg | 25.3±0.6* | 39.03 |
| CEFB-I | 300 mg/kg | 35.4±0.5 | 14.69 |
| CEFB-II | 600 mg/kg | 29.7±0.5 | 28.43 |
| PEEFB-I | 300 mg/kg | 36.8±0.6 | 11.32 |
| PEEFB-II | 600 mg/kg | 33.9±0.6 | 18.31 |
| Indomethacin | 10 mg/kg | 10.5 ±0.5* | 75.64 |

[a]N=6 animals per group, *$p<0.001$by Student's t-test, **$p<0.05$ by Student's t-test

*bengalensis* (CEFB-II) orally for 7 days, Group VI received 300 mg/kg body weight of petroleum ether extract of *Ficus bengalensis* (PEEFB-I) orally for 7 days, Group VII received 600 mg/kg body weight of petroleum ether extract of *Ficus bengalensis* (PEEFB II) orally for 7 days, and Group VIII received 10 mg/kg of body weight of indomethacin intraperitoneally for 7 days as a standard drug. Acute inflammation was induced in all groups by injecting 0.1 ml of 1% (w/v) carrageenan into the sub-plantar region of the right hind paw of the rats. On the 7th day, paw volume was measured 1h prior to carrageenan injection using a plethysmometer and at form 0 to 3h after the carrageenan injection [24, 25]. This method is for the same with the younger and mature age plant of the *Ficus bengalensis*

Mean increase in the paw volume was measured and percentage inhibition was calculated.

Percentage of inhibition =100 (1-Vt/Vc)

where, Vc = edema volume in control and Vt = edema

Volume in test/standard compound. [Table I and III]

## COTTON PELLET GRANULOMA METHOD

This study was carried out by cotton pellet implantation method in rats [26]. This method used here was with light ether anesthesia, sterile cotton pellets (10mg) were implanted subcutaneously in the axilla and groin regions of the rats [27]. The animals were treated orally with various extracts at different doses (300 and 600 mg/kg) daily for 7 consecutive days. Animals in the control group received either normal saline with CMC. Indomethacin (10mg/kg, orally) was given to animals in the reference groups. They were sacrificed on the 8th day, the cotton pellet removed, freed from extraneous tissue and dried overnight at 60°C and weighed. The inhibition percent of the dry weight of the granuloma were calculated and compared [Table II and IV].

**Table III: Effect of Ficus bengalensis (Mature plant) with carrageenan induced paw edema in rats**

| Treatments | Dose mg/kg | Mean paw volume in ml | | | | | | Percent inhibition |
|---|---|---|---|---|---|---|---|---|
| | | 0 mins | 15 mins | 30 mins | 60 mins | 120 mins | 180 mins | |
| Control | 2%CMC | 0.79 ± 0.01 | 1.05 ± 0.008 | 1.32 ± 0.007 | 1.69 ± 0.008 | 1.77 ± 0.007 | 1.48 ± 0.009 | ------ |
| EEFB-I | 300 | 0.78 ± 0.01 | 1.17 ± 0.007 | 1.41 ± 0.004 | 1.35 ± 0.009 | 0.83 ± 0.005 | 0.71 ± 0.007 | 55.03 |
| EEFB-II | 600 | 0.73 ± 0.007 | 0.83 ± 0.007 | 0.91 ± 0.007 | 0.82 ± 0.006 | 0.67 ± 0.008 | 0.51 ± 0.005 | 65.54 |
| CEFB-I | 300 | 0.85 ± 0.009 | 1.30 ± 0.008 | 1.37 ± 0.006 | 0.95 ± 0.008 | 0.90 ± 0.009 | 0.88 ± 0.005 | 22.9 |
| CEFB-II | 600 | 0.75 ± 0.01 | 1.15 ± 0.008 | 1.32 ± 0.008 | 1.33 ± 0.008 | 0.82 ± 0.007 | 0.75 ± 0.009 | 40.10 |
| PEEFB-I | 300 | 0.77 ± 0.005 | 1.11 ± 0.004 | 1.29 ± 0.008 | 1.62 ± 0.008 | 1.59 ± 0.006 | 1.41 ± 0.008 | 04.73 |
| PEEFB-II | 600 | 0.74 ± 0.007 | 1.15 ± 0.004 | 1.41 ± 0.007 | 1.58 ± 0.008 | 1.46 ± 0.007 | 1.37 ± 0.01 | 07.43 |
| Indometh-acin | 10 | 0.72 ± 0.005 | 0.79 ± 0.007 | 0.85 ± 0.006 | 0.74 ± 0.006 | 0.55 ± 0.008 | 0.28 ± 0.008 | 81.08 |

Figures in parenthesis indicated oedema inhibition percentage, N=6 animals per group, p<0.05 by Student's t-test

**Table IV: Effect of bark of *Ficus bengalensis* on cotton pellet granuloma in rats[a]**

| Treatments | Dose | Weight of cotton-pellet (mean ± S.E.M.) | Inhibition (%) |
|---|---|---|---|
| Control | 2%CMC | 43.2 ± 0.6 | - |
| EEFB-I | 300 mg/kg | 37.2 ± 0.4** | 14.12 |
| EEFB-II | 600 mg/kg | 28.4 ± 0.2* | 34.25 |
| CEFB-I | 300 mg/kg | 38.1 ± 0.3 | 11.80 |
| CEFB-II | 600 mg/kg | 35.5 ± 0.5 | 17.82 |
| PEEFB-I | 300 mg/kg | 39.6 ± 0.2 | 08.33 |
| PEEFB-II | 600 mg/kg | 36.7 ± 0.6 | 15.04 |
| Indomethacin | 10 mg/kg | 10.5 ± 0.5* | 75.69 |

[a]N=6 animals per group, *p<0.001by Student's t-test, **p<0.05 by Student's t-test

## STATISTICAL ANALYSIS

Statistical analysis was carried out using Graph Pad Prism 4.0 (Graph Pad software San Diego, CA). Results were expressed as Mean ± SEM. Statistical significance was calculated by Student's t-test <0.001 was considered as significant.

## RESULTS AND DISCUSSION:

### YOUNGER AGE PLANT

#### Carrageenan-induced rat paw edema method-Acute inflammatory study

The carrageenan-induced paw edema results of younger age plant were showed in Table I. When ethanolic extract of *Ficus bengalensis* at 300 mg/kg body weight per day (EEFB-I) given orally as a suspension the paw volume was reduced by 37.64%, whereas the case of the ethanolic extract of *Ficus bengalensis* at 600 mg/kg body weight per day (EEFB-II) shows a 69.04% inhibition after 3 h, which indicatee that the effect of ethanolic extract of *Ficus bengalensis* is reflected in a dose-dependent manner. Both EEFB-I and EEFB-II showed an inhibitory effect on carrageenan-induced paw edema thus exhibiting anti-inflammatory effect against acute inflammation. Chloroform extract of *Ficus bengalensis* at 300 mg/kg body weight per day (CEFB-I) reduced the paw volume by 24.94% and chloroform extract of *Ficus bengalensis* at 600 mg/kg body weight per day (CEFB-II) exhibited a 31.63% reduction in paw volume after 3 h. in contrast petroleum ether extract of Ficus bengalensis at 300 mg/kg body weight per day (PEEFB-I) reduced the paw volume by 7.25% and petroleum ether extract of *Ficus bengalensis* at 600 mg/kg body weight per day (PEEFB-II) exhibited a 10.77% reduction in paw volume after 3 h. Therefore, petroleum ether extract of *Ficus bengalensis* did not possess significant anti-inflammatory activity when compared with control and indomethacin-treated animals (Table I). It may be due to the absence of flavonoids in the petroleum ether extract.

### COTTON PELLET GRANULOMA METHOD

#### Chronic inflammation study

The results of cotton pellet granuloma were given in Table II. The paw volume were reduced by 19.27% with ethanolic extract of *Ficus bengalensis*

at 300 mg/kg body weight per day (EEFB-I) when given orally as a suspension whereas in case of ethanolic extract of *Ficus bengalensis* at 600mg/kg body weight per day (EEFB-II) showed 39.03% inhibition after 3 h. This indicated that the effect of ethanolic extract of *Ficus bengalensis* is reflected in dose dependent manner. Both EEFB-I and EEFB-II showed inhibitory effect on cotton pellet granuloma thus, exhibiting anti-inflammatory effect against chronic inflammation. In case of chloroform (CEFB-I) and Petroleum ether extract (PEEFB-I) of *Ficus bengalensis* at 300mg/kg body weight per day reduced the paw volume 14.69% and 11.32%. Chloroform (CEFB-II) and petroleum ether extract (PEEFB-II) of *Ficus bengalensis* at 600mg/kg body weight per day exhibited 28.43% and 18.31% reduction in paw volume after 3 h. As a result, chloroform and petroleum ether extract of *Ficus bengalensis* did not possess significant anti-inflammatory activity when compared with control and indomethacin treated animals (Table II). It may be due to absence of flavonoids in the petroleum ether extract.

## MATURE AGE PLANT

### CARRAGEENAN-INDUCED RAT PAW EDEMA METHOD

#### Acute inflammation study

In this study, the results of carrageenan-induced paw edema were given in Table III. the paw volume was reduced by 55.03% with ethanolic extract of *Ficus bengalensis* at 300 mg/kg body weight per day (EEFB-I) when given orally as a suspension, whereas the case of the ethanolic extract of *Ficus bengalensis* at 600 mg/kg body weight per day (EEFB-II) showed a 65.54% inhibition after 3 h. which indicated that the effect of ethanolic extract of *Ficus bengalensis* was reflected in a dose-dependent manner. Both EEFB-I and EEFB-II showed an inhibitory effect on carrageenan-induced paw edema thus, exhibiting anti-inflammatory effect against acute inflammation. Chloroform extract of *Ficus bengalensis* at 300 mg/kg body weight per day (CEFB-I) reduced the paw volume by 22.9% and chloroform extract of *Ficus bengalensis* at 600 mg/kg body weight per day (CEFB-II) exhibited a 40.10% reduction in paw volume after 3 h. Petroleum ether extract of *Ficus bengalensis* at 300 mg/kg body weight per day (PEEFB-I) reduced the paw volume by 4.73% and petroleum ether extract of *Ficus bengalensis* at 600 mg/kg body weight per day (PEEFB-II) exhibited a 7.43% reduction in paw volume after 3 h. Therefore, petroleum ether extract of *Ficus bengalensis* did not possess significant anti-inflammatory activity when compared with control and indomethacin-treated animals (Table III). It may be due to the absence of flavonoids in the petroleum ether extract.

### COTTON PELLET GRANULOMA METHOD - Chronic inflammation study

The results of cotton pellet granuloma were given in Table IV. The paw volume were reduced by 14.12% with ethanolic extract of *Ficus bengalensis* at 300mg/kg body weight per day (EEFB-I) when given orally as a suspension whereas in case of ethanolic extract of *Ficus bengalensis* at 600 mg/kg body weight per day (EEFB-II) showed 34.25% inhibition after 3 h. which indicated that the effect of ethanolic extract of *Ficus bengalensis* was reflected in dose dependent manner. Both EEFB-I and EEFB-II showed inhibitory effect on cotton pellet granuloma, thus exhibiting anti-inflammatory effect against chronic inflammation. In case of chloroform (CEFB-I) and petroleum ether extract (PEEFB-I) of *Ficus bengalensis* at 300 mg/kg body weight per day reduced the paw volume 11.80% and 8.33%. chloroform (CEFB-II) and petroleum ether extract (PEEFB-II) of *Ficus bengalensis* at 600 mg/kg body weight per day exhibited 17.82% and 15.04% reduction in paw volume after 3 h. As a result chloroform and petroleum ether extract of *Ficus bengalensis* did not possess significant anti-inflammatory activity when compared with control and indomethacin treated animals (Table IV). It may be due to absence of flavonoids in the petroleum ether extract.

## DISCUSSION

Among several traditional claims, the usefulness of *Ficus bengalensis* in fever, inflammation and pain had been emphasized more in literature. Hence, it was considered that investigations for these medicinal properties might give scientific authentication to the traditional claims, still this plant has not been subjected to the systemic pharmacological screening mentioned above so far. In the present study, the anti-inflammatory activity of various extracts of the bark of *Ficus bengalensis* has been established. The test extracts with two different

doses of 300 and 600 mg/kg/day were found to inhibit the carrageenan-induced rat paw edema, significantly, which has significant predictive value for anti-inflammatory agents by inhibiting the mediators of acute inflammation. Inflammation has different phases. The first phase was caused by an increase in vascular permeability, the second one by infiltrate of leucocytes, and the third one by granuloma formation. Anti-inflammatory activity was determined by inhibition of carrageenan induced inflammation, which was one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan induced edema was bi-phasic. The first phase was attributed to the release of histamine, serotonin and kinins, and the second phase was related to the release of prostaglandins and bradykinins [28-32] .We observed that EEFB-I and EEFB-II showed significant inhibition against carrageenan-induced paw edema in the dose dependent manner. But chloroform and pet ether extract failed to possess the anti-inflammatory effect may be due to absence of flavonoid [33]. This response tendency of the extract in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the ethanolic extract. This anti-inflammatory effect of EEFB-I and EEFB-II may be due to the presence of flavonoids. It has been reported that a number of flavonoids possessed anti-inflammatory activity [34]. The presence of flavonoids might be responsible for the anti-inflammatory activity in ethanolic extract. Thus, it is concluded that the ethanolic extract of bark of *Ficus bengalensis* produced significant anti-inflammatory activity in dose dependent manner.

Besides the carrageenan-induced rat paw edema model, the production of protanoids has been through the serum expression of COX-2 by a positive feedback mechanism. Therefore, it is suggested that the action mechanism of test extracts may be related to the prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of non-steroidal anti-inflammatory drugs in the inhibition of inflammatory process induced by carrageenan.

The cotton Pellet granuloma, which has certain advantages for natural product testing. First, the response is local, and involves the skin. Thus, the topical application avoids drug metabolism and excretion. Secondly, this model uses very small amounts of drugs. Like-wise, the granulomatous tissue formation is related to the chronic inflammatory process, which is characterized by several phases [35].

## CONCLUSION:

From these investigations, it may be concluded that the various extracts of *Ficus bengalensis* showed anti-inflammatory effects similar to those observed for non-steroidal drugs such as, phenylbutazone and indomethacin. The activity of the *Ficus bengalensis* extracts and young bark extracts was significantly higher than that of the mature bark extracts. It is important to point out that the presence of flavonoids may be responsible for the anti-inflammatory activity. Further investigations are under process in our laboratory to isolate and characterize the specific active components of the plant extract which is responsible for observed pharmacological actions.

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