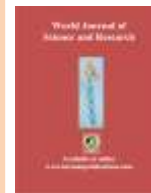


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Research Article

Biochemistry

A STUDY ON PHYTOCHEMICAL SCREENING AND ANTI-ARTHRITICS ACTIVITY OF *Ficus benghalensis* BARK EXTRACT

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ABSTRACT

The medicinal value of the chosen plant *Ficus benghalensis* bark has not been extensively worked out. Therefore, the present study was to investigate the phytochemical screening, histochemical and anti-arthritis activity of *Ficus benghalensis* bark extract. The phytochemical screening *Ficus benghalensis* bark showed that the presence of saponins, terpenoids, flavonoids, Triterpenoids, polyphenol and glycosides whereas tannin, steroids, alkaloids and anthroquinone were absent in ethanol and aqueous extracts was present only ethanol extract. Coumarins was present only aqueous extract. Significant amount of terpenoids (28mg/gm) saponin (26mg/gm), flavonoids (97mg/gm) and phenol (110mg/gm). In the present study, *Ficus benghalensis* bark were treated with specific chemicals and reagents. The increments in absorbance of test samples with respect to control indicated stabilization of protein (Egg & bovine albumin) denaturation by and reference diclofenac sodium. *Ficus benghalensis* bark exhibited anti- arthritis activities in dose dependent manner Overall, it can be concluded from the present study that *Ficus benghalensis* bark contains rich source of phytochemicals.

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INTRODUCTION

Arthritis is a musculoskeletal system disorder following mechanical and biological events that destabilize normal coupling between degradation and synthesis within articular cartilage. Arthritis can affect individuals of any age but is more predominant in the age range of 25 and 50 years with a peak in the age range of 40-50years (Kaur *et al.*, 2013). India (and South Asia more generally) is an important region in which to pursue

humoral constructions of arthritis and joint disorders. Many Indians suffer from joint pain and rheumatic problems: osteoarthritis is widespread and rheumatoid arthritis, the far less prevalent but more incapacitating form of the disease, affects an estimated ten million Indians, 80% of which are women (Times of India, 1999).

A number of natural products are used in the traditional medical systems in many countries. Alternative medicine for treatment of various

diseases is getting more popular. Making medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines. The majority of clinically important medicines belong to steroidal or non-steroidal anti-arthritis chemical therapeutic for treatment of arthritis. In order to investigate phytochemicals and *in vitro* anti-arthritis activity of the bark *Ficus benghalensis*.

MATERIALS AND METHODS

Collection of plant materials

The bark powder of *Ficus benghalensis* were purchased in December 2018 from siddha medicinal shop, Thanjavur, Thanjavur district, Tamil Nadu, India.

Preparation of plant extract:

2 gram of the powder of *Ficus benghalensis* bark were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *Ficus benghalensis* bark were shake it well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrate used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1984) and Harborne (1973 and 1984).

Quantitative determination of the chemical constituency

Total phenols estimated by the method of Edeoga *et al.*, (2005). Flavonoid determine by the method of Boham and Kocipai-Abyazan (1994). Saponin determine by the method of Obadoni and Ochuko (2001). Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956).

Histochemical tests

A small quantity of dried and finely powdered leaves sample was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive test for histochemicals was indicated by the appearance of the appropriate colour change after application of the reagent.

***In-vitro* anti-arthritis activity**

In vitro anti-arthritis activity was carried out by the method of Sangita Chandra *et al.* (2012).

RESULTS AND DISCUSSION

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. (Sofowara, 1993).

Qualitative and quantitative analysis

In the present study was carried out on the *Ficus benghalensis* bark revealed the presence of medicinally active constituents. The phytochemical characters of the *Ficus benghalensis* bark investigated and summarized in Table-1. The phytochemical screening *Ficus benghalensis* bark showed that the presence of saponins, terpenoids, flavonoids, triterpenoids, polyphenol and glycosides whereas tannin, steroids, alkaloids and anthroquinone were absent in ethanol and aqueous extracts was present only ethanol extract. Coumarins was present only aqueous extract.

Table 1: Qualitative analysis of Phytochemicals in *Ficus benghalensis* bark

| S. No | Phytochemicals | Extracts | |
|-------|----------------|----------|---------|
| | | Ethanol | Aqueous |
| 1 | Tannin | - | - |
| 2 | Saponin | + | + |
| 3 | Flavonoids | + | + |
| 4 | Steroids | - | - |
| 5 | Terpenoids | + | + |
| 6 | Triterpenoids | + | + |
| 7 | Alkaloids | - | - |
| 8 | Antroquinone | - | - |
| 9 | Polyphenol | + | + |
| 10 | Glycoside | + | + |
| 11 | Coumarins | - | + |

(-) Indicates Absence; (+) Indicates Presence

Hassain *et al.* (2011) screened phytochemical constituents from methanol leaf extract of *Bombax malabaricum*. Various organic 11 solvent extracts of *Pedaliium murex* were subjected to preliminary phytochemical screenings by Thamizh mozhi *et al.* (2011). Selected 53 traditionally used medicinal plants from western region of India for their qualitative phytochemical screenings, total phenol and flavonoids contents.

Kumar *et al.*, (2013) investigated the preliminary phytochemical screening of the leaves of the plant *Lasia spinosa* (Lour) Thwaites. The phytochemical screening showed that the methanol and aqueous extracts contained alkaloid, the carbohydrates and the phenolic compounds were

present in all of the solvent extract except petroleum ether extract. The chloroform, ethyl acetate and the aqueous extract contained glycosides whereas the saponins present in methanol and aqueous extract. The ethyl acetate extract contain only the flavonoids.

Quantitative analysis

Quantitative analysis revealed that the *Ficus benghalensis* bark has flavonoids, saponin, phenol and terpenoids. Significant amount of terpenoids (28mg/gm) saponin (26mg/gm), flavonoids (97mg/gm) and phenol (110mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

Table 2: Quantitative phytochemical analysis of *Ficus benghalensis* bark extract

| S. No | Phytochemicals | Results (mg/gm) |
|-------|----------------|-----------------|
| 1 | Terpenoids | 28.00 ± 0.20 |
| 2 | Saponin | 26.00 ± 0.17 |
| 3 | Flavonoids | 97.00 ± 5.10 |
| 4 | Phenol | 110.00 ± 9.40 |

Values are expressed as mean ± SD for triplicates

Leo Stanley *et al.* (2011) reported that leaves of *C. pedata* showed the presence of alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids. Dinesh kumar *et al.* (2011) has been reported to terpenoids, flavonoids and tannin are present in *C. trifolia*. Rajmohanan *et al.* (2014) investigated the preliminary phytochemical analysis of various extracts of leaves of *C. pedata* and showed the presence of carbohydrates, flavonoids, tannins and phenolic compounds and terpenes.

Histochemical analysis of powder in *Ficus benghalensis* bark

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of

substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major phytocompounds (Krishnan *et al.*, 2001). In the present study, *Ficus benghalensis* bark were treated with specific chemicals and reagents. The *Ficus benghalensis* bark powder treated with diluted ammonia and H₂SO₄ gave yellow colour indicates Flavonoids, treated with FeCL₃ reagent gave dark blue to black colour indicates Tannin, treated with Few drops toluidine blue reagent gave Blue green / red colour indicates Polyphenol, treated with Few drops Con. H₂SO₄ reagent gave Yellow colour indicates Saponins. (Table 3). This results further confirmed the presence of phytochemicals.

Table 3: Histochemical analysis of powder in *Ficus benghalensis*

| S. No | Phytochemicals | Colour Observation | Results |
|-------|----------------|--------------------|---------|
| 1 | Terpenoids | Orange | ++ |
| 2 | Saponin | Yellow | + |
| 3 | Flavonoids | Yellow | + |
| 4 | Polyphenol | Blue and green | ++ |

Note: (+) Presence; (++) present with high intensity of the colour

John Peter Paul, (2014) attempt was taken for histochemical and fluorescence analysis of *Turbinaria ornata* (Turner). Histochemical analyses of the plant were carried out using light microscopy and fluorescence study was analyzed by UV lamp. Results of histochemical tests showed positive reaction to phenol compounds, polyphenol and tannin in the thallus. Fine powder and different

solvent extracts of *Turbinaria ornata* obtained using petroleum ether, benzene, chloroform, acetone, ethanol and aqueous were examined under visible and UV light.

In vitro Anti-arthritic activity of *Ficus benghalensis* bark

There are certain problems in using animals in experimental pharmacological research,

such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-arthritic property *Ficus benghalensis* bark. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in arthritic diseases may be due to *in vivo* denaturation of proteins. The mechanism of denaturation probably involves

alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Grant *et al.*, 1970). Agents that can prevent protein denaturation therefore, would be worthwhile for anti- arthritic drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein (Egg & bovine albumin) denaturation by and reference diclofenac sodium. *Ficus benghalensis* bark exhibited anti- arthritic activities in dose dependent manner (Table 4 and 5, fig 1).

Table 4: *In vitro* anti- arthritic activity of *Ficus benghalensis* (Egg albumin)

| S.No | Doses (µg/ml) | Plant extract | Standard (Diclofenac sodium) |
|------|---------------|---------------|------------------------------|
| 1 | 100 | 23.56±1.64 | 36.45±2.37 |
| 2 | 200 | 40.03±2.80 | 55.94±3.47 |
| 3 | 300 | 68.12±4.76 | 79.45±4.65 |
| 4 | 400 | 83.80±5.16 | 91.45±6.84 |

Values are expressed as Mean ± SD for triplicates

Table 5: *In vitro* anti- arthritic activity of *Ficus benghalensis* (Bovine albumin)

| S.No | Doses (µg/ml) | Plant extract | Standard (Diclofenac sodium) |
|------|---------------|---------------|------------------------------|
| 1 | 100 | 33.75±2.36 | 32.75±2.14 |
| 2 | 200 | 46.56±3.25 | 51.25±2.95 |
| 3 | 300 | 64.23±4.49 | 75.42±4.44 |
| 4 | 400 | 75.77±5.30 | 90.68±6.11 |

Values are expressed as Mean ± SD for triplicates

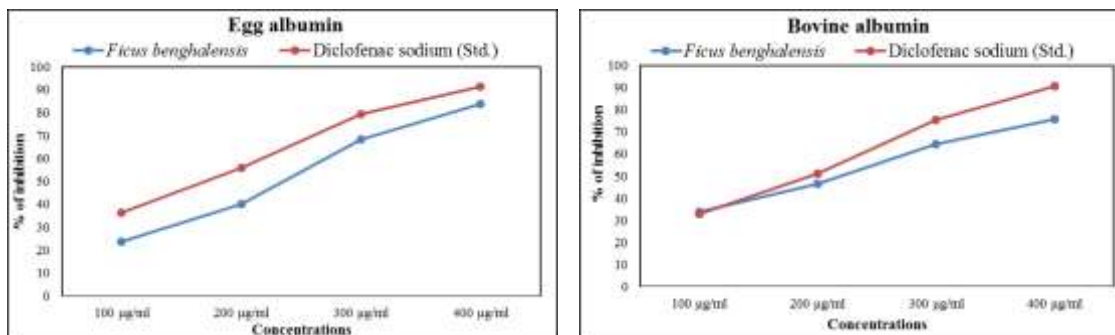


Fig 1: *In vitro* anti- arthritic activity of *Ficus benghalensis* (Egg albumin and Bovine albumin)

Sangita Chandra *et al.*, (2012) evaluated the *in vitro* anti- arthritic effect of aqueous extract of coffee (*Coffea arabica*) against the denaturation of protein. The extract at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity to assess the anti- arthritic property. Diclofenac sodium was used as the reference drug. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the coffee extract. The effect of diclofenac sodium was found to be less when compared with the test extract. He concluded that coffee possessed marked *in vitro*

anti- arthritic effect against the denaturation of protein

Divya Singh *et al* (2013) examined anti-arthritic Activity of seed extract of *Pongamia pinnata* (L.) Pierre by *in vitro* model. The anti-arthritic and anti-inflammatory activity of *P. pinnata* hydroalcoholic extract was done by Inhibition of protein denaturation and Human red blood cell membrane stabilization (HRBC) *in vitro* methods. The hydro alcoholic extract of *P. pinnata* was subjected to *in vitro* Inhibition of protein denaturation in various concentrations i.e. 10, 50, 100, 200, 400, 800, 1000 and 2000µg/ml. HRBC method was also used for the estimation of anti-

inflammatory activity from in various concentrations 100, 200, 400, 800 and 1600 µg/ml. *P. pinnata* hydroalcoholic extract exhibited a concentration dependent inhibition of protein (albumin) denaturation. The stabilization of HRBC membrane showed a concentration dependent anti-inflammatory activity, and the protection percent increased with increase in the concentration of the *P. pinnata* hydroalcoholic extract. The present study is support to the isolation and use of phytoconstituents from seed of *P. pinnata* in treatment of inflammation and arthritis.

Amar *et al* (2014) reported that the *in vitro* anti-arthritis activity of *Cassia tora* Linn. Leaves using effect of membrane stabilization and protein denaturation using different concentration. The results are compared with standard drug. The aqueous extract of the selected medicinal plant showed significant activity. Anti- arthritic effect of *Cassia tora* Linn. Leaves were studied by testing various in vitro studies. The effect of the selected plant on inhibition of protein denaturation and effect of membrane stabilization was 87.22 % and 87.25% respectively for the aqueous extract of the selected plant leaves. He concluded that *Cassia tora* possessed marked in vitro anti-inflammatory effect against the denaturation of protein

Susmitha Sudevan *et al.*, (2015) investigation exposed that the extracts of *Acmella Oleracea* have potent phytochemical and anti-arthritis activity which explains its use in traditional system of medicines. The qualitative analysis of the extracts from the leaf sample of *Acmella Oleracea* showed the presence of phytochemical constituents such as tannins, saponin, flavonoids, steroid, lipids, amino acids and terpenoids. Hence, *Acmella Oleracea* can source of natural anti-arthritis that can serve as a substitute to conventional medicines.

CONCLUSION

Overall, it can be concluded from the present study that *Ficus benghalensis* bark contains rich source of phytochemicals. This study is the first scientific report that provides convincing phytochemicals and anti- anti-arthritis activity evidence for the relevance of *Ficus benghalensis* bark thus providing scientific validity to its traditional consumption by the local population of south India.

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