

## Anti-inflammatory (*invitro*) activity of the Leaves of *Ficus gibbosa* Blume.by HRBC Membrane stabilisation

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**Abstract:** Plants have been used as alternative remedy for the treatment of various ailments since ancient times. Phytochemical analysis, and *in vitro* anti-inflammatory activity of leaf extract of *Ficus gibbosa* were studied. The methanolic leaf extract was tested for the presence of phytochemicals. Phytochemical screening reveals the presence of saponins, tannins, glycosides, alkaloids and flavonoids. Since many flavonoids have remarkable anti inflammatory activity the present work aims at evaluating the anti inflammatory activity of *Ficus gibbosa* by HRBC membrane stabilization.

**Key Words:** Anti-inflammatory, *Ficus gibbosa*, HRBC Membrane stabilisation.

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### I. Introduction

Inflammation was considered as a single disease caused by disturbances of body fluids. The modern concept of inflammation is based on the theory of John Hunter who considered inflammation to be salutary operation, resulting from some disease. Inflammation can be defined as the reaction to injury of the living microcirculation and related tissues. Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, cell migration, tissue breakdown and repair (Vane *et al.*, 1995). It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy *et al.*, 2010).

*Ficus gibbosa* is a small, at time large tree often epiphytic or climbing, enclosing the trunks of trees in a perfect network of branches or creeping a long walls and on the sides of wells. The decoction of the root act as a powerful aperient.

### II. Materials And Methods

#### Plant material

The Fresh leaves of *Ficus gibbosa* were collected from the Medicinal garden of Academy of Pharmaceutical Sciences, Pariyaram, Kannur, Kerala in January 2013. The plant material was identified and a voucher specimen (FGBL) was deposited in the herbarium of the Department of Pharmacognosy of Academy of Pharmaceutical Sciences.

#### Preparation of extracts

The leaves were dried under shade and powdered. The powder was transferred to Soxhlet extractor and subjected to extraction with ethanol. After extraction, the solvent was distilled off and the extract was concentrated on water bath to a dry residue and kept in a desiccator.

#### Anti-Inflammatory Activity

The HRBC membrane stabilization has been used as a method to study the anti inflammatory activity. (Gandidasan.R, 1991) Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of sterilized Alsever solution. The blood was centrifuged at 4000 rpm and packed cells were washed with isosaline and a 10 % v/v suspension was made with isosaline. The assay mixture contains the drug at various concentration, 1 ml phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Indomethacine was used as the reference drug. Instead of hyposaline 2 ml of distilled water was used in the

control. All the assay mixtures were incubated at 37°C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100 %. The percentage of HRBC membrane stabilization or protection was calculated by using the formula,

$$\text{Percentage Protection} = 100 - (\text{OD sample} / \text{OD Control}) \times 100$$

\*OD=Optical Density

### III. Results

Table 1 Phytochemical screening of ethanolic extract of *Ficus gibbosa*

Sl No	Phytoconstituents	Ethanolic Extract of the Leaves
1	Flavonoids	+
2	Carbohydrates	+
3	Tannins	+
4	Alkaloids	+
5	Glycosides	+
6	Saponins	+
+ = Positive (Present)		

Table 2 Anti inflammatory activity of *Ficus gibbosa* at various concentrations

Sl No	Concentration mg/ml	Anti-inflammatory activity(%Protection)	
		Ethanolic Extract of <i>Ficus gibbosa</i>	Standard Indomethacin
1	Control	-----	-----
2	100	57.4± 0.03	74.93± 0.02
3	200	60.2 ± 0.04	84.86 ± 0.04
4	300	63.3 ± 0.06	89.20 ± 0.09
5	400	71.2 ± 0.02	92.35 ± 0.01
6	500	77.3± 0.05	94.10± 0.04

(Values are expressed as SEM of 3 readings)

### IV. Discussions

The alcoholic extract of *Ficus gibbosa* subjected to erythrocyte (RBC) membrane stabilization induced haemolysis by hypotonic solution. The erythrocyte membrane resembles to lysosomal membrane and as such the erythrocyte could be extrapolated to the stabilization of lysosomal membrane (Omale et al., 2008). The extra cellular activity of the lysosomal enzymes is said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. (Rajendran Vadivu, 2008). It was observed from the table 2 that the ethanolic extract shows significant anti inflammatory activity at the concentration of 500 mg/ml which is comparable to the standard drug Indomethacin. The anti inflammatory activity of the extract was concentration dependent, with the increasing concentration the activity was also increased. The phytochemical screening of the extract revealed the presence of flavonoids, resins, tannins etc. The anti-inflammatory effect of the ethanolic extract may be due to the presence of flavonoids and saponins. Flavonoids and steroids show remarkable anti-inflammatory activity by inhibiting the cox and lox systems [Robert et al., 2001; Tapas et al., 2008].

### V. Conclusion

This study reveals that, the ethanol fraction prepared from *Ficus gibbosa* leaves contains high amounts of total phenolics and total flavonoids and flavonoids are well documented to be having strong antioxidant activity and antiinflammatory activity. Thus, ethanol seems to be most promising solvent for extraction and isolation of natural antioxidative compounds from *Ficus gibbosa* leaves. Further studies in isolation of individual phenolic compounds particularly flavonoids in this fraction and its effect on antioxidant in animal models are needed to evaluate their potential benefits.

The ethanolic extract of the leaves exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane.

From the above study it was concluded that the ethanolic extract of *Ficus gibbosa* has significant membrane stabilization property and it was comparable to the standard drug Indomethacin.

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