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Antioxidant [*In vitro*] and analgesic activity [*In vivo*] of tannin fraction of stem bark of *Ficus racemosa* Linn

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ABSTRACT

The purpose of the present study was to investigate the antioxidant and analgesic effect of the tannin fraction of stem bark of *Ficus racemosa* Linn. The antioxidant potential of tannin fraction was tested in DPPH, FRAP assay in vitro model and the analgesic activity was done by using Eddy's hot plate method. In antioxidant assays the tannin fraction shows the very good free radical scavenging capacity and In eddy's hot plate method the tannin fraction showed significant analgesic activity at the doses of 20 mg/kg [p<0.01] and 40 mg/kg [p<0.001] and 60 mg/kg [p<0.001] as compared to control group, when analyzed statistically by Dunnet's multiple comparison Test. The result obtained show that the tannin fraction of *Ficus racemosa* Linn. Possesses significant antioxidant potential & analgesic activity which confirms the traditional claims of the plant mentioned in Ayurveda.

Keywords: *Ficus racemosa*, tannin fraction, analgesic, 2,2-diphenyl-1-picrylhydrazyl, Eddy's hot plate.

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INTRODUCTION

Ficus racemosa Linn is a common tree distributed all over India, found throughout the year, grows in evergreen forests most localities along the sides of ravines, streams and banks [1]. In former phytochemical study with the fruit and bark of this species were reported the isolation and identification of several compounds like α -amyrin [2], terpenoids [3]. The various pharmacological actions like anti-diabetic [4], anti-diuretic [5], antioxidant [6] and hepatoprotective activity [7-9] analgesic activity [10] of bark, stem, fruit and root parts of the plant was reported. In view of the various medical properties and growing interest in the development of ecofriendly, biodegradable and safer analgesic herbal preparations the plant was screened for analgesic property. The genus of *Ficus racemosa* cited in Indian system of medicine Ayurvedha for the treatment of diabetes, hyperlipidemia and various diseases like pyrexia, dysentery, fatigue, piles, cough, pain etc. There is no scientific evidence for the separation and evaluation of analgesic activity in tannin fraction of the *Ficus* species. In view of the various medical properties and growing interest in the development of ecofriendly, biodegradable and safer analgesic herbal preparations the plant was screened for analgesic property.

Collection of plant material and authentication

Ficus racemosa bark was collected from Walajabad, Chennai, Tamil Nadu. They were identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre [PARC], Tambaram, Chennai, Tamil Nadu, the voucher specimen no: Parc/2008/229 has been deposited at the herbarium unit of the Department of Pharmacognosy, School of Pharmaceutical Sciences, Vel's University, Pallavaram, Chennai.

Separation of tannins from *Ficus racemosa* Linn

The barks of *Ficus racemosa* were shade dried and coarsely powdered. Total tannins were separated from crude acetone [70% v/v] extract as described by McCallum et al., [11]. Briefly, the powdered material [1kg] was extracted with acetone in water [70% v/v] [2500ml] by cold maceration. The acetone extract was filtered and saturated with sodium chloride [saturated NaCl] to salt out acetone and the upper solvent phase was removed. This acetone phase was then extracted with three successive 250ml portions of the deionized water containing 0.1% ascorbic acid to prevent auto-oxidation. Excess of acetone in the aqueous portion is removed in vacuo at 25°C. To the aqueous portion was treated with an equal volume of water then extracted with three successive portions of petroleum ether [40-60°C] to remove any lipid material and ethyl acetate. Finally the remaining aqueous phase containing crude tannins was collected and freeze dried. The yield of the crude tannin fraction [TF] was found to be 15% w/w. The tannin fraction was subjected to qualitative chemical test showed positive test for tannins.

Chemicals and drugs

Ferrous sulphate, Ascorbic acid, Deoxyribose, were obtained from Sisco Research Laboratory [SRL], Mumbai, India 1, 1-diphenyl-2-picrylhydrazyl [DPPH] was procured from Sigma-Aldrich Co MO, USA. Other chemicals used were of analytical grade. The tannin fraction from *Ficus racemosa* .

Assay for DPPH radical scavenging capacity

The effect of extract and fraction on DPPH radical was estimated by Lim et al., 2003 [12] with minor modification. In brief, 2ml of DPPH in methanol [3.6×10^{-5} M] were added to 50 μ L of various concentrations of extracts, and fractions [0.025Mm – 1mM]. The mixture was vortexed for 15 sec and left to stand at 37°C for 30min. The decrease in the absorbance at 515 nm was continuously recorded in a spectrophotometer for 15 min at room temperature. All determination was performed in triplicate. The DPPH scavenging activity [decrease of absorbance at 515 nm] of extract and fraction were plotted against time and the [%] percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 15 min duration as follows:

$$\% \text{ Inhibition} = [\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}] / \text{Abs}_{\text{Control}} \times 100$$

Where $\text{Abs}_{\text{Control}}$ is absorbance of control at time = 0 and $\text{Abs}_{\text{Sample}}$ is absorbance of test sample at time = 15 m

FRAP method: [Ferric reducing property]

This method can be determined by taking 5 of different dilutions of standard solution of gallic acid [10-100 μ g/ml] and aqueous tannin fraction that has adjusted to come under the linearity range[500 μ g/ml] was taken in 10ml volumetric flasks and mixed with 2.5ml of potassium buffer [0.2M pH6.6] and 25ml of 1% potassium ferric cyanide . the mixture was incubated at 50⁰ for 20min. then 2.5ml of 10% trichloacetic acid was added to the mixture to stop the reaction . to the 2.5ml of the above solution 2.5ml of distilled water is added and the 0.5ml of 0.1% fecl₃ was added and allow to stand for 30 min before measuring the absorbance at 593nm.[13]

Experimental animals

Swiss albino mice weighing 18-25 g of either sex were used for the study. The animals were procured and housed in the animal house maintained under standard hygienic conditions, at 20 \pm 2o C, humidity [60 \pm 10%] with 12 hour day and night cycle, with food and water ad libitum. Experimentation on animals is approved by Institutional Animals Ethics Committee [IAEC], School of Pharmaceutical Sciences, Vels University.

Acute toxicity studies

The acute oral toxicity studies were performed to study the acute toxic effects and to determine minimum lethal dose of the drug extracts. Swiss albino mice of either sex weighing 18-25 g were used for the study. The tannin were administered orally to different groups of overnight fasted mice at the doses of 500, 1000 mg/kg body weight. After administration of the extracts, animals were observed continuously for the first three hours for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 hrs. Further the animals were under investigation up to a period of one week [14].

Eddy’s hot plate method

The animals were divided into five groups of 6 animals each. Group I served as normal control. Group II and IV were treated orally with aqueous extract of 20, 40 and 60 mg/kg body weight respectively. Group V served as standard and were injected Diclofenac sodium [9 mg/kg] intraperitoneally. The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds.[14]

Statistical analysis

All the values were statistically analyzed by one-way analysis of variance [ANOVA] followed by Dunnet’s test. Comparison between control and drug treated groups were considered to be significant. All values are expressed as mean ± SEM.

RESULTS

Effect of tannin fraction [TF] from *Ficus racemosa* L in DPPH radical scavenging assay

Figure 1: Effect of TF from the bark of *Ficus racemosa* in DPPH method

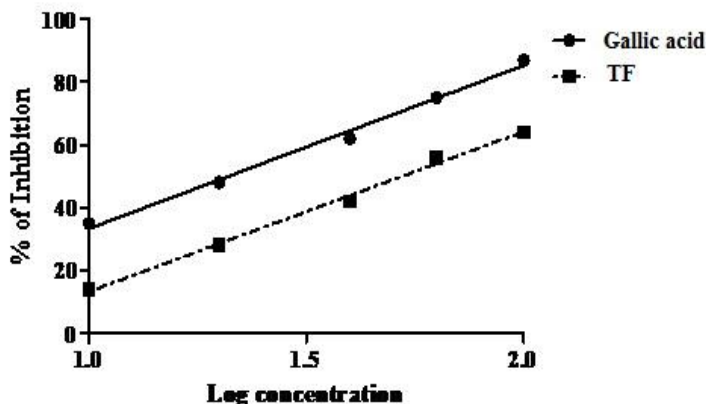


Fig 1, depicts the free radical scavenging capacity of TF fraction using DPPH generated radical in in-vitro. It was observed that increase in % inhibition of free radicals has observed in increasing concentration of TF. The IC_{50} values of TF was found to be 53 $\mu\text{g/ml}$ and the R^2 -linear regression value was found to be 0.9951. The fractions was compared with the standard Gallic acid IC_{50} -26.93 $\mu\text{g/ml}$; R^2 -0.9987.

Effect of tannin fraction [TF] from *Ficus racemosa* L in FRAP method : [Ferric reducing property]

Effects of tannin fractions from *Ficus racemosa* Linn in FRAP method

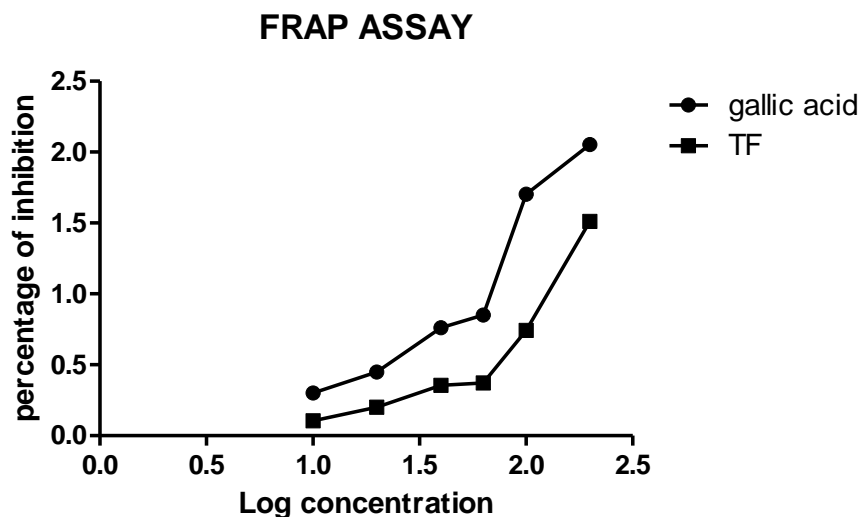


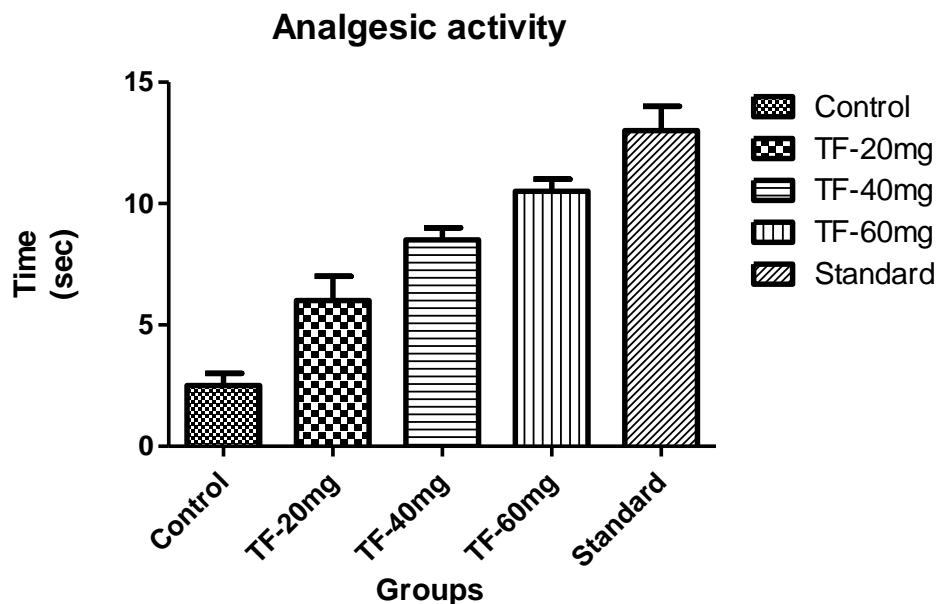
Fig 2 depicts the 40 $\mu\text{g/ml}$ of tannin fraction containing 35 $\mu\text{g/ml}$ equivalent to standard gallic acid

Effect of tannin fraction [TF] from *Ficus racemosa* L in Acute Toxicity studies

Acute toxicity studies show that drug is safe up to the dose of 1000 mg/kg with tannin fraction. In future it will provide high margin of safety during formulation.

Effect of tannin fraction [TF] from *Ficus racemosa* L in Eddy's hot plate method

In analgesic studies, the tannin fraction showed significant analgesic activity at all tested dose levels. In Eddys hot plate method, the tannin fraction of *Ficus racemosa* stem bark at a dose of 20mg/kg,40mg/kg and 60mg/kg showed significant activity [6 \pm 1.000*] [8.5 \pm 0.5000**] and [10.5 \pm 0.5000***][*P < 0.05] showed significant activity after 15 minutes interval of experiment at all tested dose levels [Fig 3] the results were compared with the standard drug diclofenac sodium [9mg/kg] . The results clearly showing an increase in reaction time is generally considered an important parameter of analgesic activity.



One- way Analysis of Variance ANOVA: p value found to be 0.05 is considered extremely significant. The data were expressed as mean \pm S.E.M.; Dunnet 't' test compare all vs control: *P<0.05.

DISCUSSION

Medicinal plants have become the focus of intense study in terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore [15]. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid via cyclooxygenase [COX], and prostaglandin biosynthesis [16]. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability [17]. Preliminary qualitative phytochemical screening reveals that the fraction shows the presence tannin in *Ficus racemosa*.

Therefore, it is assumed that these tannins may be responsible for the observed analgesic activity. The antioxidant effect of tannin fraction was significant in the two methods used. The antioxidant power of tannin fraction was less than the antioxidant power of gallic acid. Thus, the antioxidant property of tannin fraction would produce an additional therapeutic benefit enhancing its analgesic activity. In analgesic studies, the tannin fraction showed significant analgesic activity at all tested dose levels. In Eddys hot plate method which is compared with the standard with the standard drug diclofenac sodium. The present study indicates that tannin fraction possesses significant analgesic and antioxidant effects.

CONCLUSION

From the above investigation, it is quite apparent that tannin fraction of *Ficus racemosa* L. stem bark possesses potent antioxidant [in vitro] & analgesic effect against heat stimuli. This

is evidenced by significant increase in the reaction time by stimuli in different experimental models.

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