Research Article

Antioxidant and anti-inflammatory activities of leaf extracts of *Flacourtia jangomas* (Lour.) Raeusch: An *in vitro* study

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Abstract

Objective: The present study aimed to evaluate the antioxidant and anti-inflammatory potential of ethanol and aqueous extract of *F. jangomas* using *in vitro* methods. **Materials and Methods:** Shade dried, pulverized leaves of *F. jangomas* was extracted with ethanol and water separately and subjected to qualitative and quantitative phytochemical analysis using standard procedures. The antioxidant potential of the plant extracts was assessed using ABTS, DPPH, hydroxyl radical, superoxide radical and hydrogen peroxide radical scavenging assay. The anti-inflammatory activity was assessed by nitric oxide inhibitory assay, *5-lipoxygenase* inhibitory assay, *cyclooxygenase* inhibitory assay, HRBC membrane stability assay, Inhibition of protein denaturation and *Proteinase* inhibitory assay. **Results:** Qualitative phytochemical analysis indicated the presence of glycosides, alkaloids, phytosterols, tannins, flavonoids and phenolic compounds. The total phenol content of the ethanol and aqueous extracts was found to be 86.12 ± 2.41 & 74.38 ± 1.28mg of gallic acid equivalents per gram of dry extract, respectively. Total flavonoids content of ethanol extract was 168.14 ± 3.68 and in aqueous extract it was 145.42 ± 2.24mg of rutin equivalent per gram of dry extract. Ethanol and aqueous extracts of *F. jangomas* potentially scavenge the free radicals in ABTS and DPPH radical scavenging assay methods. They exhibit moderate activity in hydroxyl, superoxide and hydrogen peroxide radical scavenging assays. The results obtained are comparable with ascorbic acid and rutin. In the *in vitro* anti-inflammatory assay, the extracts showed potent inhibition on the tested methods. Moreover, the extracts selectively block the action of cyclooxygenase-2 enzyme. **Conclusion:** These results demonstrated that the ethanol and aqueous extracts of *F. jangomas* possess antioxidant and anti-inflammatory effects and provide support of the traditional use of this plant in the treatment of inflammatory conditions. Further studies could reveal metabolites of the extracts responsible for the observed effects.

Keywords: *Flacourtial jangomas*, Free radical scavenging, Antioxidant, Anti-inflammatory, Cyclooxygenase, 5-lipoxygenase

Introduction

Inflammation is a defensive response of the body towards various injurious stimuli like infections and trauma. At the same time, it is accompanied by pain, redness, swelling and malfunctioning of the affected part of the body (Kumar et al., 2016). Progression of inflammation involves the release of a variety of chemical mediators that are accountable for signs and symptoms linked with such conditions. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses implicated in inflammation. The biosynthesis of prostaglandins has been concerned in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's disease. To alleviate the pain and other related symptoms various anti-inflammatory drugs are used, most of which are synthetic drugs, associated with an array of side effects such as peptic ulcer, bleeding etc. Ethno-pharmacological

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reviews revealed that many medicinal plants have attracted considerable interest, particularly in the treatment of several disease conditions including chronic inflammatory diseases (Moro et al., 2012). The research into medicinal plants with assumed folkloric use as analgesics, anti-inflammatory agents, should be viewed as a fertile and rational research strategy in the search for new analgesic and anti-inflammatory agents.

Flacourtia jangomas (Lour.) Raeusch. an Indian medicinal plant commonly known as Indian coffee plum or Indian sour cherry belongs to the family Flacourtiaceae. Fruits of *F. jangomas* is traditionally used in biliousness, fever and digestive disorders (Kirtikar and Basu, 1993). Leaves and barks are used in the treatment of diarrhoea, inflammation, bleeding gums, tooth pain, piles and weakness of limbs (Yusuf et al., 2007). Literature review reveals that the leaves and stem of *F. jangomas* have potential antidiabetic activity in streptozotocin and alloxan-induced diabetic rats (Singh et al., 2010 Singh and Singh, 2010). The plant extract also possess antibacterial, antifungal and cytotoxic activities (Srivastava et al., 2009; Sarkar et al., 2011; Pravin et al., 2011; Srivastava et al., 2012; Das et al., 2017; Geroge et al., 2017). Phytochemicals such as limolin, jangomolide, corymbulosine, tremulacin, hydrnocarpic acid and chaulmoogric acid have been reported in *F. jangomas* (Ahmad et al., 1984; Pandey and Dubey, 2014). Based on the literature survey and ethnopharmacological data, the present study aimed to evaluate the antioxidant and anti-inflammatory activities of ethanol and aqueous extracts of leaves of *F. jangomas* using various *in vitro* models.

Materials and methods

Chemicals

2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), Kappa carrageenan type III, acetylsalicylic acid, linoleic acid, *lipoxygenase* (*lipoxidase* from soyabean), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, naphthylethylenediamine dihydrochloride, LPS were purchased from Sigma Aldrich Co., St. Louis, USA. Rutin and p–nitroso dimethyl aniline (p–NDA) were obtained from Acros Organics, New Jersy, USA. Ascorbic acid and nitro blue tetrazolium (NBT) was obtained from S.D. Fine Chem, Ltd., Biosar, India. 2-Deoxy–Dribose was from Hi–Media Laboratories Ltd., Mumbai. Aceclofenac was obtained from Micro Labs (Hosur, India). All other chemical used were of analytical grade. Murine monocytic macrophage cell line RAW 264.7 was procured from National Centre of Cell Science (Pune, India).

Plant collection and extraction

The fresh leaves of *F. jangomas* was collected from the foothills of Kolli hills, Namakkal District, Tamilnadu, India and the plant material was identified and authenticated by the botanist, Botanical Survey of India, Coimbatore, Tamilnadu, India where a voucher specimen was preserved. The leaves were shade dried and pulverized to get coarse plant material. About 500g of the powdered plant material was extracted separately in a soxhlet apparatus for 8h at 50˚C using ethanol (EEFJ) and water (AEFJ) separately. The extracts were filtered and concentrated to dryness under reduced pressure and controlled temperature (40ºC to 50ºC) in a rotary evaporator and stored at 4˚C until further use.

Qualitative phytochemical analysis

Prepared plant extracts of *F. jangomas* were analyzed for the presence of various phytochemical constituents employing standard procedures (Wagner et al., 1984). Conventional protocol for detecting the presence of steroids, alkaloids, tannins, flavonoids, glycosides, etc., was used.

Total phenol content assay

Total phenol content in the plant extracts was measured by the Folin-Ciocalteu method according to a previously published method using gallic acid standard curve. The total phenol content was expressed as gallic acid equivalent in mg/g of extract (Kumar et al., 2012).

Total flavonoids content assay

Total flavonoids content in the plant extracts was estimated according to a previously described method using rutin standard curve. The total flavonoids content was expressed as rutin equivalent in mg/g of extract (Kumar et al., 2012)

In vitro antioxidant activity

The ethanol and aqueous extracts of *F. jangomas* was tested for it's in vitro antioxidant activity using various methods as described by Kumar et al. (2012). In all these methods, a particular concentration of the extracts or standard solution was used which gave a final concentration of 1000-15.625 µg/ml after all the reagents were added. Absorbance was measured against a blank solution containing the extract or standards, but without the reagents. A control test was performed without the extract or standards. The percentage inhibition was calculated using the following formula:

Radical scavenging activity (%)

$$= [OD_{Contol} - OD_{Sample}/OD_{Control}]x100.$$

ABTS Radical Scavenging Assay

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS; 54.8mg, 2mM) was dissolved in 50 ml of distilled water followed by potassium persulphate (0.3ml, 17mM).

The reaction mixture was left to stand at room temperature overnight in darkness before use. To 0.2ml of various concentrations of the samples/standard, 1.0ml of freshly distilled DMSO and 0.16ml of ABTS solution were added to make a final volume of 1.36ml. After 20min, absorbance was measured at 734nm.

DPPH Assay

The assay was carried out in a 96 well microtitre plates. To 200µl of 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) solution, 10µl of each of the samples or standard solution was added separately in wells in the microtitre plate. The plates were incubated at 37°C for 30min and the absorbance of each solution was measured at 490 nm.

Hydroxyl Radical Scavenging Assay Using p-NDA

To a reaction mixture containing ferric chloride (0.5ml, 0.1mM), EDTA (0.5ml, 0.1mM), ascorbic acid (0.5ml, 0.1mM), hydrogen peroxide (0.5ml, 2mM) and p-nitrosodimethylaniline (p–NDA, 0.5ml, 0.01mM) in phosphate buffer (pH 7.4, 20mM), various concentrations of samples or standard (0.5ml) was added to make a final volume of 3ml. Sample blank was prepared by adding 0.5ml of sample and 2.5ml of phosphate buffer. Absorbance of these solutions was measured at 440nm.

Superoxide Radical Scavenging Assay Using Alkaline DMSO

To the reaction mixture containing 1ml of alkaline DMSO (1ml DMSO containing 5mM NaOH in 0.1ml water) and 0.3ml of the sample in freshly distilled DMSO at various concentrations, 0.1ml of nitroblue tetrazolium (NBT; 1mg/ml) was added to give a final volume of 1.4ml. The absorbance was measured at 560nm.

Hydrogen Peroxide Radical Scavenging Assay

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (pH 7.4). Various concentrations of 1ml of the samples or standard were added to 2ml of hydrogen peroxide solution in PBS. After 10 min, the absorbance was measured at 230nm.

***In vitro* anti-inflammatory activities**

Cell culture and stimulation

The murine monocytic macrophage cells RAW 264.7 was grown in a plastic culture flask in Dulbecco's Modified Eagle's Medium (DMEM) with L-glutamine supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution under 5% CO_2 at 37°C. After 4-5 days, cells were detached from the culture flask and centrifuged for 10min at 1500rpm. The medium was removed and the cells re-suspended with fresh DMEM. The cell concentration was adjusted to $1x10^6$ cells/ml in the same medium. 100µl of the above concentration were cultured in a 96-well plate for one day to become nearly confluent. Concentrations ranging from 10-100µg/ml of the extract or standard (Aceclofenac) were prepared in DMEM to give a volume of 100µl in each well of a microtitre plate. Then wells were cultured with the vehicle, test extract and standard in the presence of 1µg/ml of LPS for 24 h.

Nitric oxide inhibitory assay

The nitrite concentration in the culture medium was measured as an indicator of NO production based on the Griess reaction (Kumar et al., 2016). Briefly, 100µl of each supernatant was mixed with the same volume of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochlorie in water) and the remaining mixture was then incubated at room temperature for 10min. The absorbance at 540nm was measured using a mictrotitre plate reader. Nitrite concentration in the supernatant was determined by comparison with a sodium nitrite standard curve.

5-Lipoxygenase Inhibitory assay

The assay was performed using linoleic acid as substrate and *5-lipoxygenase* as enzyme. Total volume of 200µl assay mixture containing 160µl sodium phosphate buffer (100mM, pH8.0), 10µl test extracts (10-100µg in 100mM Tris buffer, pH 7.4) and 20µl of *lipoxygenase* enzyme was used. The contents were incubated at 25°C for 10min. The reaction was then initiated by the addition of 10µl linoleic acid solution. The change in absorbance was observed after 6min at 234nm (Kumar et al., 2016). All reactions were performed in triplicates in 96-well microplates. Aceclofenac was used as reference standard. The percentage inhibition was calculated by the following formula:

$$\text{Inhibition (\%)} = [A_{Control} - A_{Test}/A_{Control}] \times 100$$

Cyclooxygenase inhibitory assay

In vitro COX-2 inhibitory activities of EEFJ and AEFJ have been evaluated using a commercial kit according to the manufacturer's instruction (Cayman Chemical Company, Ann Arbor, MI) with 96-well plates. All the reagents added were prepared just before use. COX-1 and COX-2 initial activity tubes were prepared taking 950µl of reaction buffer, 10µl of heme and 10µl of COX-1 and COX-2 enzymes in respective tubes. Similarly, COX-1 and COX-2 inhibitor tubes were prepared by adding 20µl of the extract at various concentrations (10-100µg/ml) in each tube in addition to the above ingredients. The background tubes corresponding to inactivated COX-1 and COX-2 enzymes obtained after

keeping the tubes containing enzymes in boiling water for 3min along with vehicle control. Reactions were initiated by adding 10µl of arachidonic acid in each tube and quenched with 50µl of 1M HCl then 100µl of $SnCl_2$ was added. The prostaglandin produced in each well was quantified using prostaglandin specific antiserum that binds with major prostaglandins and reading the plate at 405nm (Kumar et al., 2016).

HRBC membrane stability assay

The human red blood cells (HRBC) membrane stabilization assay was performed as per the method described by Gandhidasan et al. (1991). Briefly blood was collected from healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the study and mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride). The blood was centrifuged at 3000rpm and packed cells were washed with isosaline (0.9%w/v NaCl) and a 10% suspension was made with the same. Various concentrations of the extracts were prepared (250, 500 and 1000µg/ml) using distilled water and to each concentration 1ml phosphate buffer, 2ml hyposaline, and 0.5ml HRBC suspension was added. These were incubated at 37°C for 30min and centrifuged at 3000rpm for 20min. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. Aceclofenac (100 & 200µg/ml) was used as the reference standard and a control was prepared by omitting the extracts. The percentage hemolysis was calculated by assuming the hemolysis produced by the control group as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the formula: % protection = 100 x $[(OD_1\text{-}OD_2)/OD_1]$ where OD_1 is optical density of drug treated sample, OD_2 is optical density of control.

Protein denaturation Inhibitory assay

About 0.2ml of egg albumin (obtained from fresh hen egg), 2.8ml of phosphate buffer saline (PBS, pH 6.4) and 2.0 mlof different concentrations of EEFG and AEFG were taken in test tubes. Final concentration of each extract in 5.0ml of reaction mixture was 250, 500, 1000µg/ml. Distilled water (5.0ml) served as control. Test tubes were incubated at 37ºC in a BOD incubator for 15min and then heated at 70ºC for 5min. Test tubes were cooled and the absorbance was measured at 660nm using UV Spectrophotometer (Sen et al., 2015). Aceclofenac (100 & 200µg/ml) was used as the standard drug and treated similarly to determine the absorbance. Percentage inhibition of protein denaturation was calculated by using the following formula: % inhibition = $100 \times [V_t / V_c - 1]$, where V_t is absorbance of test and V_c is absorbance of control.

Proteinase Inhibitory assay

The reaction mixture (2ml) was containing 0.06mg trypsin, 1ml of 20mM Tris HCl buffer (pH 7.4) and 1ml test sample of different concentrations. The reaction mixture was incubated at 37°C for 5min and then 1ml of 0.8% w/v casein was added. The mixture was incubated for an additional 20min, 2ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was measured at 210nm against buffer as blank. Aceclofenac at the concentration of 100 & 200µg/ml was used as standard. The percentage of inhibition of proteinase inhibitory activity was calculated (Oyedapo and Famurewa, 1995).

Results and discussion

Inflammation is a reaction occurring in several types of tissue injuries, infections and immunological stimulation as a defense mechanism against foreign or endogenous substances. Several medicinal plants and plant products exhibit potent anti-inflammatory activity in both *in vitro* and *in vivo* models. Hence the present work is aimed to study the anti-inflammatory potential of ethanol and aqueous leaf extract of *F. jangomas*. The yield of ethanol extract was found to be 10.42% w/w, whereas for aqueous extract the yield was found to be 12.33% w/w. Qualitative phytochemical analysis results revealed that the ethanol extract contains several phytochemicals such as glycosides, phytosterols, alkaloids, glycosides, polyphenolics, flavonoids, proteins and amino acids. Except phytosterols and alkaloids, remaining compounds were found to be

Table 1. Qualitative phytochemical studies of ethanol and aqueous leaf extract of *F. jangomas*

Phytochemical constituents	EEFJ	AEFJ
Carbohydrates	Present	Present
Phytosterols	Present	Absent
Alkaloids	Present	Absent
Glycosides	Present	Present
Terpenoids	Absent	Absent
Proteins & aminoacids	Present	Present
Saponins	Present	Present
Tannins	Present	Present
Phenolic compounds	Present	Present
Flavonoids	Present	Present
Fixed oils & Fats	Absent	Absent
Gums & Mucilages	Absent	Absent

Table 2. *In vitro* antioxidant activity of ethanol and aqueous extract of *F. jangomas*

Drug/Standard	IC_{50} (µg/ml)*				
	ABTS radical scavenging assay	DPPH radical scavenging assay	Hydroxyl radical scavenging assay by p-NDA method	Superoxide radical scavenging assay by alkaline DMSO method	Hydrogen peroxide radical scavenging assay
EEFJ	68.42 ± 0.48	34.32 ± 0.81	117.41 ± 1.32	148.42 ± 5.83	106.17 ± 2.94
AEFJ	72.31 ± 0.56	37.42 ± 0.37	140.34 ± 3.24	176.82 ± 6.71	112.14 ± 3.16
Ascorbic acid	21.32 ± 1.12	11.42 ± 0.08	192.41 ± 4.18	214.31 ± 5.68	35.87 ± 2.96
Rutin	16.87 ± 0.48	12.32 ± 0.23	230.12 ± 5.41	284.17 ± 6.11	109.42 ± 4.21

Data are expressed as Mean ± SEM; *Average of three independent determination.

Table 3. Nitric oxide and *5-lipoxigenase* inhibitory activities of ethanol and aqueous extract of *F. jangomas*

Drug/ Standard	IC_{50} (µg/ml)*	
	Nitric oxide inhibitory assay	*5-lipoxygenase* inhibitory assay
EEFJ	114.32 ± 3.41	78.42 ± 2.59
AEFJ	148.54 ± 5.12	86.11 ± 4.13
Aceclofenac	89.94 ± 2.98	52.94 ± 2.74

Data are expressed as Mean ± SEM; *Average of three independent determination.

present in aqueous extract. Terpenoids, gums and mucilages, fixed oils and fats were found to be absent in both ethanol and aqueous extract. Qualitative phytochemical analysis results are displayed in table 1.

The phenolics compounds present in the medicinal plants are known to possess the ability to reduce the oxidative damage and act as antioxidants. They can trap free radicals directly and/or scavenge them through a series of coupled reactions with antioxidant enzymes. In addition, it was reported that the phenolics substances were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation. This activity is mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. The total phenol content of the ethanol and aqueous extracts was found to be 86.12 ± 2.41 and 74.38 ± 1.28mg of gallic acid equivalents per gram of dry extract, respectively. Total flavonoids content of ethanol extract was found to be 168.14 ± 3.68, whereas it was 145.42 ± 2.24mg of rutin equivalent per gram of dry extract in aqueous extract.

In order to assess the free radical scavenging and antioxidant potential of the prepared extracts, they were tested against several *in vitro* antioxidant and free radical scavenging assay models and the results are presented in table 2. Among the tested models, EEFJ and AEFJ showed potent activity in ABTS radical scavenging assay and DPPH radical scavenging assay models. The activities were compared with standard compounds like ascorbic acid and rutin. In hydroxyl radical scavenging assay and superoxide radical scavenging assay model, both the extracts showed potent activity than the standards used. The extracts potentially scavenge the hydrogen peroxide radicals and the activity is equipotent to the standard rutin. The antioxidant and free radical scavenging activity of these extracts may be due to the presence of high amount of phenolic compounds and flavonoids.

Nitric oxide is a short lived free radical produced from L-arginine by *nitric oxide synthase* that mediate diverse functions by activating on various cells through interactions with different molecular targets. Excess production of nitric oxide by inducible *nitric oxide synthase* is involved in various types of inflammation and carcinogenesis in inflammatory sites (Kumar et al., 2016). In the present investigation, a concentration dependent and gradual increase in the percentage inhibition was observed with both EEFJ and AEFJ. The inhibitory concentration value was found to be 114.32 ± 3.41µg/ml for EEFJ and 148.54 ± 5.12µg/ml for AEFJ. Like the plant extracts, a gradual and dose dependent increase in the percentage of inhibition was observed in the standard drug aceclofenac also. The IC_{50} value of aceclofenac against nitric oxide inhibitory assay was found to be 89.94 ± 2.98 µg/ml.

Lipoxygenases (LOX) are members of a class of iron containing dioxygenases that catalyze the addition of molecular oxygen to fatty acids containing a 1,4-pentadiene system to give an unsaturated fatty acid hydroperoxides. *Lipoxygenases* play a key role in a variety of disorders like asthma, inflammation and angiogenesis. Various concentrations of EEFJ and AEFJ were analysed for *5-lipoxygenase* inhibitory activity. The extracts potentially block the activity of the enzyme and the IC_{50} value was found to be 78.42 ± 2.59µg/ml for EEFJ and 86.11 ±

4.13µg/ml for AEFJ and the results are comparable with the standard drug aceclofenac with the IC_{50} of 52.94 ± 2.74µg/ml. The extract and standard aceclofenac inhibits *5-lipoxygenase* in a dose dependent manner and this inhibition might be attributed to the anti-inflammatory potential of the extracts. The results are presented in table 3.

The enzyme *cyclooxygenase* (COX) plays an important role in the inflammatory process. It is a membrane bound glycoprotein found in prostanoid forming cells. It occurs as COX-1 and COX-2 which are two identical forms of COX but have differences in substrate and inhibitor selectivity and in their intracellular locations. Among these two forms, COX-2 involved in the inflammatory process and is inducible and up-regulated by a variety of stimuli like mitogens, oncogenes and cytokines. Non-steroidal anti-inflammatory drugs are COX blockers but they also block COX-1 and this is problem causing because COX-1 is necessary for several physiological functions. Hence there is a special need for the agents which inhibit COX-2 selectively without affecting COX-1. In the present investigation, EEFJ and AEFJ were screened for their inhibitory efficacy against COX-1 and COX-2 using *in vitro* assay models. The results revealed that both the extract selectively block the COX-2 than COX-1. The selectivity was compared with the aceclofenac, a non-selective COX inhibitor and celecoxib, a selective COX-2 inhibitor. If the COX-1/COX-2 selectivity is more than 1, then the drug is considered as selective COX-2 inhibitor. In our study, the COX-2 selectivity ratio for the tested extracts is more than 1 and it was higher than the standard aceclofenac which indicate that the extracts are having selective COX-2 inhibition. The results are presented in table 4.

Stabilization of human RBC membrane was studied to establish the mechanism of anti-inflammatory activity of EEFJ and AEFJ and the result obtained are presented in table 5. The results obtained revealed that both the extracts were effectively inhibiting the hemolysis at different concentrations. EEFJ showed the maximum inhibition of 87.69% at 1000µg/ml concentrations and the IC_{50} value

Table 4. *Cyclooxygenase* inhibitory activities of ethanol and aqueous extract of *F. jangomas*

Drug/ Standard	IC_{50} (µg/ml)*		
	COX-1	COX-2	COX-2 Selectivity (COX-1/COX-2)#
EEFJ	94.32 ± 3.16	13.62 ± 0.98	6.93
AEFJ	128.41 ± 5.42	24.31 ± 1.18	5.28
Celecoxib	18.42 ± 0.92	0.38 ± 0.002	48.47
Aceclofenac	0.06 ± 0.001	0.23 ± 0.001	0.261

Data are expressed as Mean ± SEM; *Average of three independent determination; #Ratio of the IC_{50} values for *COX-1* and *COX-2* can be used as an indicator of the *COX-2* selectivity of inhibition. A *COX-1/COX-2* ratio is greater than 1 indicates preferential *COX-2* selectivity.

Table 5. HRBC membrane stability, Inhibition of protein denaturation and *Proteinase* inhibitory activities of ethanol and aqueous extract of *F. jangomas*

Drug/Standard	Conc. (µg/ml)	% Inhibition		
		HRBC membrane stabilization	Protein denaturation	*Proteinase* inhibition
EEFJ	250	44.23 ± 2.14	43.29 ± 2.41	27.32 ± 1.12
	500	50.12 ± 1.18	63.35 ± 2.62	59.42 ± 1.47
	1000	87.69 ± 2.44	97.22 ± 3.74	93.86 ± 2.32
IC_{50} (µg/ml)		409.33	333.12	469.42
AEFJ	250	31.53 ± 0.98	34.43 ± 3.22	21.23 ± 1.41
	500	58.46 ± 1.13	59.47 ± 3.87	54.31 ± 2.26
	1000	86.15 ± 3.12	85.72 ± 4.21	81.42 ± 2.94
IC_{50} (µg/ml)		461.71	434.85	556.91
Aceclofenac	100	58.31 ± 3.12	53.28 ± 1.82	48.42 ± 2.17
	200	82.47 ± 2.44	81.01 ± 2.11	83.16 ± 3.42
IC_{50} (µg/ml)		46.79	41.12	33.78

Data are expressed as Mean ± SEM; *Average of three independent determination.

was observed at 409.33µg/ml. In case of AEFJ, the maximum inhibition was observed at 1000µg/ml and the IC_{50} was found to be 461.71µg/ml. Aceclofenac, the standard drug showed maximum inhibition of 82.47% at 200µg/ml concentration. These provide evidence for membrane stabilization as a mechanism of anti-inflammatory activity. The extracts might be possibly preventing the release of lysosome constituents including enzymes and protease which upon release cause further tissue inflammation and damage. Thus a possible mechanism for anti-inflammatory activity of EEFJ and AEFJ may be due to the inhibitory activity on release of inflammatory mediators responsible for inflammation through membrane stabilizing effect.

Denaturation of proteins is a well known cause of inflammation. As a part of the present investigation on the mechanism of anti-inflammatory activity, the ability of extracts inhibits protein denaturation was studied and the results obtained are presented in table 5. The results revealed that extracts produce concentration dependent activity. The IC_{50} value of EEFJ was found to be 333.12µg/ml where as it was found to be 434.85µg/ml for AEFJ. Denaturation of proteins express antigens related to type-III hypersensitive reaction. These denatured proteins are equally potent as native proteins and play a key role to provoke delayed hypersensitivity. Auto-antigen production in inflammatory diseases may be due to the Denaturation of protein in *in vivo* condition. A number of NSAIDs like diclofenac, aceclofenac,indomethacin and phenylbutazone prevent the denaturation of proteins along with the inhibition of the COX enzyme. The result of the present study clearly shown that *F. jangomas* extracts are able to modify the synthesis of auto-antigen and inhibit the protein denaturation. The secondary metabolites such as alkaloids, tannins, phenolics and flavonoids in the extracts might be responsible for the observed activity.

Proteinases have been implicated in inflammatory responses. Neutrophils are known to be a rich source of *proteinase* which carries in their lysosome granules with *serine proteinases*. It was reported that leukocytes *proteinase* play an important role in the development tissue damage during the inflammatory process and significant level of protection was given by *proteinase* inhibitors. Both EEFJ and AEFJ exhibited pronounced antiproteinase activity at different concentrations as showed in table 5.

Recent studies have been shown that many flavonoids and phenolics compounds contribute significantly to the antioxidant and anti-inflammatory activity of many medicinal plants. Hence the presence of these phytochemical compounds in the ethanol and aqueous extract of *F. jangomas* may contribute to its antioxidant and anti-inflammatory activities.

Conclusion

In conclusion, the present study revealed the *in vitro* antioxidant and anti-inflammatory activities of ethanol and aqueous extract of *F. jangomas*. The presence of flavonoids and other polyphenolic compounds present in the extract may be responsible for the observed activities. Further studies are warranted to find the active constituents of the extracts and to confirm the mechanism of action.

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Conflict of interest

The authors declare that no potential conflict of interest.

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