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IN SILICO AND *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC FRUIT EXTRACT OF *FLACOURTIA JANGOMAS*

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ABSTRACT

The present study aimed to evaluate the anti-inflammatory potential of ethanolic extracts of *Flacourtia jangomas* fruit using *in silico* and *in vitro* methods. *In silico* anti-inflammatory activity was studied using molecular docking method. *In vitro* anti-inflammatory activity of the ethanolic extract of *Flacourtia jangomas* was performed by using albumin denaturation inhibition assay, cyclooxygenase inhibition assay, lipoxygenase inhibition assay and cellular nitrite inhibitory assays. The preliminary phytochemical analysis indicated the presence of flavonoids, phenolic compounds, tannins, terpenoids, saponins. In docking studies, ligand molecule quercetin shows binding affinity with NLRC4 and P2X7 receptors respectively. The extract inhibits protein denaturation, cyclooxygenase, lipoxygenase and cellular nitrite. In this study, the results demonstrated that the ethanolic extract of *Flacourtia jangomas* fruit possess moderate anti-inflammatory activity.

KEYWORDS: Anti-inflammatory activity, *Flacourtia jangomas*, docking studies, *in vitro* studies

INTRODUCTION

The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. In India, traditional medicinal system such as ayurveda, siddha, unani system of healthcare are largely plant based and still play a major role in curing diseases [1], [2]. For centuries, many kinds of natural plants and their products have been used for the treatment of variety of pharmacological processes including inflammation. Medicinal plants are a rich source of active compounds known to modulate inflammation, bringing about wellbeing and stability to living systems [3].

Inflammation is a part of the complex biological response of body tissues to harmful stimuli such as pathogens, damaged cells or irritants. Infective agents like bacteria, virus, fungi; Immunological agents like cell

mediated and antigen- antibody reaction; Physical agent like heat, cold, radiation; Chemical agents like organic and inorganic poison; Inert material such as foreign bodies can act as cause of inflammation. At the beginning of inflammation, the cells release inflammatory mediators. These mediators contain histamine, serotonin, prostaglandins, leukotrienes, cytokine, nitric oxide and some coagulating system and the kinin system [4], [5].

Flacourtia jangomas is a commonly known as Indian coffee plum, Indian sour cherry or lovlolika belongs to the family Salicaceae. It is a small deciduous tree growing up to 6- 10m but occasionally reach up to 14m height. It has been used as a traditional medicine for the treatment of different diseases in India, Malay Peninsula, Bangladesh and Myanmar [6]. *Flacourtia jangomas* is an important fruit tree having immense nutritional and medicinal significance. The plant is not only use for its medicinal properties but also cultivated for its edible fruits and lumber. The fruit extracts, the plant leaves and roots have medicinal characteristics. *Flacourtia jangomas* fruits are fleshy ellipsoid berries, sub globose, 1.5-2.5 cm in diameter that enclosing 4 or 5(occasionally up to 10) flat seeds. Fruit turns green to dull-brownish red or dark purple in colour when they mature and they may eventually become blackish when fully mature [7], [8].

In silico studies have an ever-increasing role in drug discovery that are critical in the cost-effective identification of promising drug candidates. These computational methods are important in reducing the usage of animal models in pharmacological research. It facilitate the logical creation of novel and secure medication candidates. Molecular docking is the most popular technique that has been utilised extensively for structure-based drug design. Molecular docking tries to predict the molecular interaction between ligand and target macromolecules. In molecular docking, Small ligands with computer-generated 3D structures can be positioned in a receptor structure in a variety of orientations, conformations, and positions [9], [10]. pyRx 0.8 tools was used to carry out the docking-based virtual screening approach and PyMOL was used to visualise the results. PyRx is software used to screen libraries of compounds against potential drug targets. Pymol has been widely used for 3D visualization of macromolecules and it becomes one of the most popular tools for preparing high- resolution images of macromolecules [11], [12].

Denaturation of protein result in the production of auto-antigens in inflammatory disorders such as rheumatic arthritis. Hence, by inhibition of protein denaturation, inflammatory activity can also be inhibited. Any alteration to a protein's distinctive 3D shape without triggering concurrent breaking of the peptide bonds is referred to as denaturation of protein. It results in a short-term or long-term lack of activity and cause damage to secondary, tertiary, and quaternary structures. Change in pH, organic compound, heavy metal etc cause protein denaturation [13], [14]. Inflammation is a defence mechanism that is initiated either after injury, physical and chemical damage, or infection by microorganisms, but persistent inflammation may cause chronic diseases. Prostaglandins (PGs) and leukotrienes (LTs), which are hormone-like chemicals secreted by the body cause inflammation. These inflammatory inducing agents are produced from arachidonic acid (AA) by the enzymes cyclooxygenase (COX) and lipoxygenase (LOX). COX is the first enzyme in the pathway for producing PG and thromboxane (Tx) from arachidonic acid [15], [16]. Nitric oxide (NO) is recognized as a mediator and regulator in pathological reactions, especially in acute inflammatory responses. Nitric oxide is derived from the oxidation of L- arginine through three isoforms of nitric oxide synthase (NOS), namely neuronal(nNOS), endothelial(eNOS)

and inducible(INOS). iNOS is mostly found in macrophages and is induced by tumour necrosis factor and endotoxins. Pro- inflammatory agents, such as LPS, can significantly increase nitric oxide production in macrophages through activation of iNOS [17].

Fig No.1: Flacourtia jangomas fruit

MATERIALS AND METHODS

Collection of plant material

The fruits were collected from certain areas of Kanyakumari district, Tamil Nadu, India in the month of March. The botanical material was identified and authenticated from Department of Botany, Nesamony Memorial Christian College, Marthandam.

Preparation of plant material

The sample is thoroughly washed with fresh water. Fruit is cut into small pieces and were dried in shade for 10 days. The cutting pieces were pulverised to get coarse power and stored in an airtight container.

Preparation of plant extract

400gm of dried powdered of fruit of *Flacourtia jangomas* has been extracted with 2000ml ethanol by using Soxhlet extraction process for 48 hrs at 50^0c. After extraction process resultant solvent is removed by distillation. Filter the solvent using Whatman filter paper and the extract was remained on the filter paper. The extract was concentrated to dryness under reduced pressure and controlled temperature. After drying process, extract was weighed and calculate the percentage yield [18], [19].

The percentage yield of extract was determined by using following formula,

$$\textbf{Percentage yield} = \frac{W2}{W1} \textbf{ X } 100$$

Where,

W1– Weight in grams of dried plant material

W2- Weight in grams of extracts obtained

Phytochemical screening

Qualitative phytochemical investigation was carried out for flavonoids, phenolic compounds, tannins, terpenoids and saponins using standard procedures [20], [21].

In silico anti- inflammatory activity assay

Docking method

In the docking study of lead compounds, the following steps were taken:

Step 1: Ligand preparation

The chemical structure of the ligand Quercetin was obtained from open chemistry database Pubchem. The chemical composition of the compound were downloaded in the Standard Data Format(SDF) which is converted to Protein Data Bank(PDB) format.

Step 2: Protein preparation

The target macromolecules can be determined from literature survey and their respective PDB files ID- NLRC4, P2X7 were downloaded using Protein Data Bank. The protein is downloaded in their crystal structure and after protein processing it was converted to the input format.

Step 3: Protein processing

Protein structure processing / refinement of protein includes removal of hydrogens, water molecules, heteroatoms and bound ligands if any.

Step 4: Docking analysis

Docking analysis was done by Auto dock Via Program in PyRx software. For this installed Auto dock tools (Python 2.7.1, MGL tools 1.5.4 and Open babel) and PyRx. Installed Auto dock tools (Python 2.7.1, MGL tools 1.5.4 and Open babel) and PyRx

Step 5: Molecular visualization

PyMOL is software used for the protein preparation and molecular visualization. It produces high quality 3D images of protein [22], [23].

In vitro anti- inflammatory activity assay

➢ Cell lines

RAW 264.7 cell line was purchased from NCCS, Pune and was maintained in Dulbecco's modified eagles media (Himedia, India) supplemented with 10% fetal bovine serum (Himedia, India) and grown to confluence at 37ºC at 5% CO_2 in a CO_2 incubator.

➢ Pre-processing

RAW 264.7 cells were grown to 60% confluence followed by activation with 1 ml lipopolysaccharide (LPS) (1 µg/ml). LPS stimulated RAW cells were exposed with different concentration of test sample solution and the standard provided. The plates were incubated for 24 hours. After incubation the anti-inflammatory assays were performed using the cell lysate.

➢ Cell lysate preparation

- Trypsinize and harvest RAW cells (activated by LPS and treated with both standard diclofenac along with the test compound at different concentrations) and centrifuge 3000 rpm for 2 min.
- Resuspend the pellet in Tris HCL buffer Ph8.0
- Freeze the cells at -20ºC for 20 minutes
- Keep the cells at 65ºC for 5 minutes
- Immediately transfer to -20ºC for 10 minutes
- Cool to room temperature
- Centrifuge at 1200 rpm for 2 minutes
- Take supernatant which is the cell lysate for further analysis.

I. Protein Denaturation Inhibition Assay

The reaction mixture (0.5 ml) consisted of 0.4 ml bovine serum albumin (3% aqueous solution) and varying concentrations of test sample. The samples were incubated at 37ºC for 20 min and 2.5 ml phosphate buffered saline (Ph 6.3) was added to each tube and then heated at 80ºC for 10 min. The absorbance was measured using spectrophotometer at 660nm [24], [25].

The following formula was used for calculating the percentage inhibition of protein denaturation:

$$\textbf{Percentage of inhibition} = \frac{\textbf{absorbance of control} - \textbf{absorbance of test}}{\textbf{absorbance of control}} \textbf{ X 100}$$

All the data were analysed statistically by unpaired student ‘t’ test followed by One way ANOVA for multiple comparison test, they were taken as mean ± SD.

II. Cyclooxygenase (COX) Assay

The COX activity was assayed by the method of Walker and Gierse with slight modifications. The cell lysate in Tris-HCl buffer (pH 8) was incubated with glutathione 5 Mm/L, and haemoglobin 20 µg/L for 1 minute at 25°C. The reaction was initiated by the incorporation of arachidonic acid 200 Mm/L and terminated after 20 minutes of incubation at 37°C, by the introduction of 10% trichloroacetic acid in 1 N hydrochloric acid. After the centrifugal separation and the addition of 1% thiobarbiturate, COX activity was determined by reading absorbance at 632 nm [26].

Percentage inhibition of the enzyme was calculated as,

$$\textbf{Percentage of inhibition} = \frac{\textbf{absorbance of control} - \textbf{absorbance of test}}{\textbf{absorbance of control}} \textbf{ X 100}$$

***NB:** Haemoglobin stock solution should be prepared in 0.06 M HCl. Haemoglobin is soluble at a range of 20 mg/ml in 0.06 M HCl.

All the data were analysed statistically by unpaired student 't' test followed by One way ANOVA for multiple comparison test, they were taken as mean ± SD

III. Lipoxygenase (LOX) Assay

RAW 264.7 cell line was used for the study purpose. The determination of 5-LOX activity was as per Axelrod *et al.*(1981). Briefly, the reaction mixture (2 Ml final volume) contained Tris-HCl buffer (Ph 7.4), 50 Ml of cell lysate, and sodium linoleate (200 Ml; 10mg/ml). The LOX activity was monitored as difference in absorbance at 234 nm, which reflects the formation of 5-hydroxyeicosatetraenoic acid from linoleate [27].

Percentage inhibition of the enzyme will be calculated using the formula:

$$\textbf{Percentage of inhibition} = \frac{\textbf{absorbance of control} - \textbf{absorbance of test}}{\textbf{absorbance of control}} \textbf{ X 100}$$

All the data were analysed statistically by unpaired student 't' test followed by One way ANOVA for multiple comparison test, they were taken as mean ± SD

IV. Cellular nitrite inhibition assay

RAW 264.7 cell lines are used for the study. Method of Lepoivre et al., (1990) was used to estimate the level of nitrite. 0.1 ml of sulphosalicylic acid was added to 0.5 ml of cell lysate and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. Nitrite level was estimated using the protein-free supernatant. Added 30 ml of 10% sodium hydroxide to 200 ml of the supernatant followed by 300 ml of Tris-HCl buffer and mixed well. Added 530 ml of Griess reagent to this mixture and incubated in the dark for 10–15 minutes. The absorbance was measured at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained [28].

All the data were analysed statistically by unpaired student 't' test followed by One way ANOVA for multiple comparison test, they were taken as mean ± SD.

RESULT

Percentage yield

The percentage yield of the *Flacourtia jangomas* fruit extract using ethanol is shown in Table No.1

Table No. 1- Percentage yield of *Flacourtia jangomas* fruit extract

SL. NO	SOLVENT USED FOR EXTRACTION	PERCENTAGE YIELD (%w/w)
1	Ethanol	20.0% w/w

Phytochemical analysis

Qualitative phytochemical analysis results revealed that the ethanol extract contain flavonoids, saponins, terpenoids, tannins. Summary of preliminary phytochemical screening of *Flacourtia jangomas* ethanolic fruit extract is shown in table no. 2

Table no. 2- phytochemical constituents in the *Flacourtia jangomas* ethanolic fruit extract

SL. NO.	NAME OF THE CONSTITUENTS	EEFFJ
1	Flavonoids	+
2	Phenolic compounds	+
3	Tannins	+
4	Terpenoids	+
5	Saponins	+
6	Alkaloids	-

EEFJ Ethanolic Extract of Fruit of Flacourtia jangomas

→ + Positive → Negative

In silico anti- inflammatory assay

Docking studies are utilised to simulate the atomic-level interaction between small molecules and a protein, which allows to characterise how small molecules behave in target proteins in the binding sites. From review of literature, found that *Flacourtia jangomas* fruit contain flavonoids which is equivalent to Quercetin which has ability to produce anti-inflammatory activities. Quercetin shows binding affinity with NLRC4, P2X7 receptor. Quercetin show more binding affinity with P2X7 receptor. The docking study indicated that the quercetin has the anti- inflammatory activity. Fig 2 and 3 shows the docking image of quercetin with receptor NLRC4, P2X7 respectively and table 3 Shows the hydrogen bond interaction and binding affinity of quercetin with receptors.

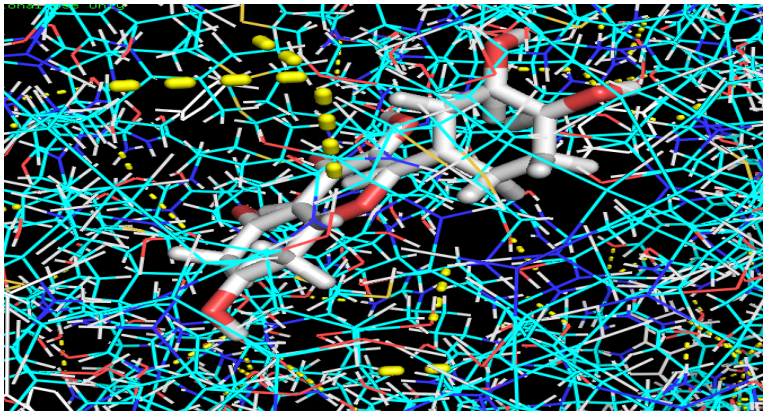


Figure 2: Docking image of quercetin with receptor NLRC4

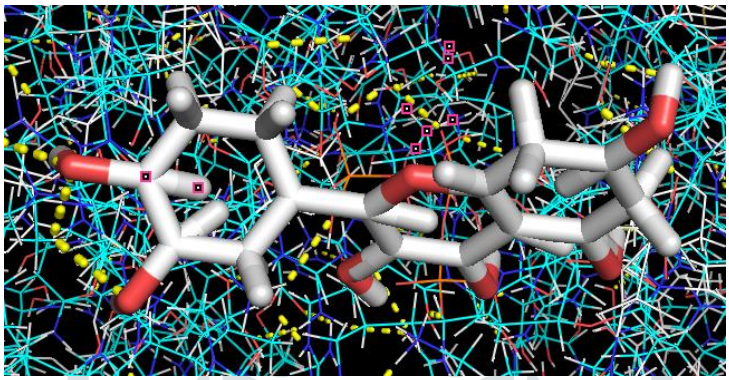


Figure 3: Docking images of quercetin with receptor P2X7

Table No. 3- Hydrogen bond interaction and binding affinity of Quercetin with receptors

RECEPTOR	NUMBER OF HYDROGEN BOND INTERACTIONS	BINDING AFFINITY (KCAL/MOL)
NLRC4	1	-7.4
P2X7	4	-7.5

In vitro anti- inflammatory assay

I. Protein Denaturation Inhibition Assay

The inhibitory rate of denaturation of proteins for ethanolic fruit extract of *Flacourtia jangomas* increased progressively as concentration increased. In this study ethanolic fruit extract of *Flacourtia jangomas* showed maximum inhibition, 65.75% at 200 µg/ml. Standard anti-inflammatory drug diclofenac demonstrated its greatest degree of inhibition, 84.12% at 200 g/ml concentration. The percentage inhibition value at different concentration are given in table no. 4

Table No 4- Effect of *Flacourtia jangomas* ethanolic fruit extract in Protein Denaturation Inhibition Assay

TREATMENT	CONCENTRATION (µg/ ml)	ABSORBANCE	PERCENTAGE INHIBITION
CONTROL	-	0.862	-
EEFFJ	6.25	0.7556±0.0001	12.34±0.01
	12.5	0.6957±0.0003	19.29±0.03
	25	0.6063±0.0001	29.66±0.01
	50	0.5012±0.0002	41.86±0.025
	100	0.3891±0.0002	54.86±0.025
	200	0.2952±0.0001	65.75±0.01
DICLOFENAC	6.25	0.725±0.001	7.88±0.13
	12.5	0.661±0.002	16.01±0.255
	25	0.545±0.0025	30.75±0.32
	50	0.368±0.002	53.24±0.26
	100	0.125±0.0025	84.12±0.32

EEFFJ - Ethanolic Extract of Fruit of Flacourtia jangomas

The values are expressed as mean± SD. Statistical test done by unpaired student 't' test followed by One way ANOVA for multiple comparison test($P < 0.05$).

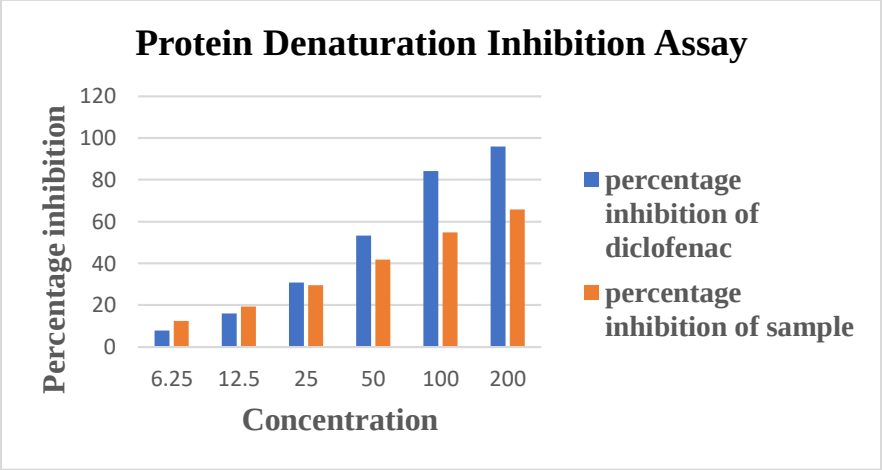


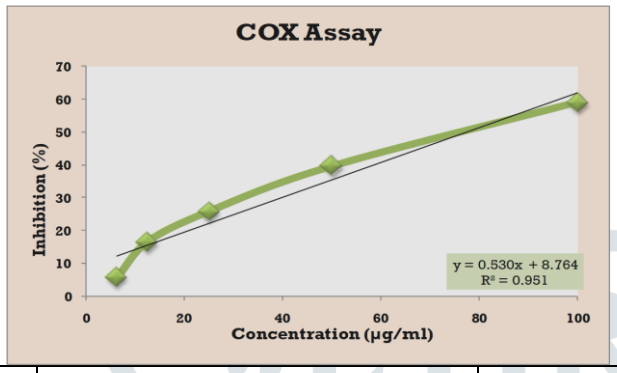
Fig 4- Effect of *Flacourtia jangomas* ethanolic fruit extract in Protein Denaturation Inhibition Assay

II. Cyclooxygenase (COX) Assay

At increasing concentrations, ethanolic extract of fruit of *Flacourtia jangomas* efficiently inhibited the COX enzymes. Thus the sample studied exhibited cyclooxygenase (COX) activity i.e. the sample has anti-inflammatory property. This is an indication that the test sample has the potential to be developed as anti-inflammatory drugs. The result shows COX have maximum efficacy at the concentration 100 µg/ml. The IC

50 value of COX was found to be 77.80 µg/ml. The percentage inhibition value at different concentration are given in table no. 5

Table 5- Effect of *Flacourtia jangomas* ethanolic fruit extract in Cyclooxygenase (COX) Assay

TREATMENT	CONCENTRATION (µg/ ml)	ABSORBANCE	PERCENTAGE INHIBITION
			
CONTROL	-	0.725	-
DICLOFENAC	6.25	0.636±0.1226	12.32
	12.5	0.556±0.2331	23.35
	25	0.486±0.3301	33.04
	50	0.364±0.4984	49.86
	100	0.224±0.6910	69.12
EEFFJ	6.25	0.683±0.05744	5.70
	12.5	0.605±0.1650	16.47
	25	0.538±0.2584	25.80
	50	0.437±0.3972	39.70
	100	0.298±0.5894	58.92

EEFFJ - Ethanolic Extract of Fruit of Flacourtia jangomas

The values are expressed as mean± SD. Statistical test done by unpaired student 't' test followed by One way ANOVA for multiple comparison test($P < 0.05$).

Fig 5 - Effect of *Flacourtia jangomas* ethanolic fruit extract in Cyclooxygenase (COX) Assay

Lipoxygenase (LOX) Assay

At increasing concentrations, ethanolic extract of fruit of Flacourtia jangomas efficiently inhibited the LOX enzymes. Thus the sample studied exhibited lipoxygenase inhibitory activity i.e. the sample has anti-inflammatory property. This is an indication that the test sample has the potential to be developed as anti-inflammatory drugs. The result shows LOX have maximum efficacy at the concentration 100 µg/ml. The IC 50 value of LOX was found to be 74.96 µg/ml. The percentage inhibition value at different concentration are given in table no 6.6

Table no 6- Effect of *Flacourtia jangomas* ethanolic fruit extract in Lipoxygenase (LOX) Assay

TREATMENT	CONCENTRATION (µg/ ml)	ABSORBANCE	PERCENTAGE INHIBITION
CONTROL	-	0.646	-
DICLOFENAC	6.25	0.585±0.0944	13.25
	12.5	0.516±0.2012	23.48
	25	0.414±0.4275	38.66
	50	0.325±0.4974	51.85
	100	0.192±0.7027	71.48
EEFFJ	6.25	0.595±0.0789	7.85
	12.5	0.525±0.1878	18.74
	25	0.474±0.2663	26.59
	50	0.355±0.4505	45.02
	100	0.236±0.6342	65.40

EEFFJ - Ethanolic Extract of Fruit of Flacourtia jangomas

The values are expressed as mean± SD. Statistical test done by unpaired student 't' test followed by One way ANOVA for multiple comparison test($P< 0.05$).

Fig 6- Effect of *Flacourtia jangomas* ethanolic fruit extract in Lipoxygenase (LOX) Assay

6.4.4 Cellular nitrite inhibition assay

Concentration dependent enhancement in cellular nitrite inhibition was elicited by the sample. The maximum inhibition was elicited by the concentration 100µg/ml. This indicates the capability of the sample in inhibiting oxidative stress as well as associated inflammatory responses. The percentage inhibition value at different concentration are given in table no 6.7

Table no 7- Effect of ethanolic fruit extract of *Flacourtia jangomas* in Cellular nitrite assay

TREATMENT	CONCENTRATION (µg/ ml)	ABSORBANCE	NITRILE CONCENTRATION FROM STANDARD GRAPH
CONTROL	-	1.528	6.587
DICLOFENAC	6.25	1.466	6.314
	12.5	1.42	6.074
	25	1.082	4.615
	50	0.491	2.001
	100	0.383	1.524
EEFFJ	6.25	1.511	6.512
	12.5	1.456	6.270
	25	1.374	5.906
	50	1.052	4.484
	100	0.770	3.236

EEFFJ - Ethanolic Extract of Fruit of Flacourtia jangomas

The values are expressed as mean. Statistical test done by unpaired student 't' test followed by One way ANOVA for multiple comparison test($P < 0.05$).

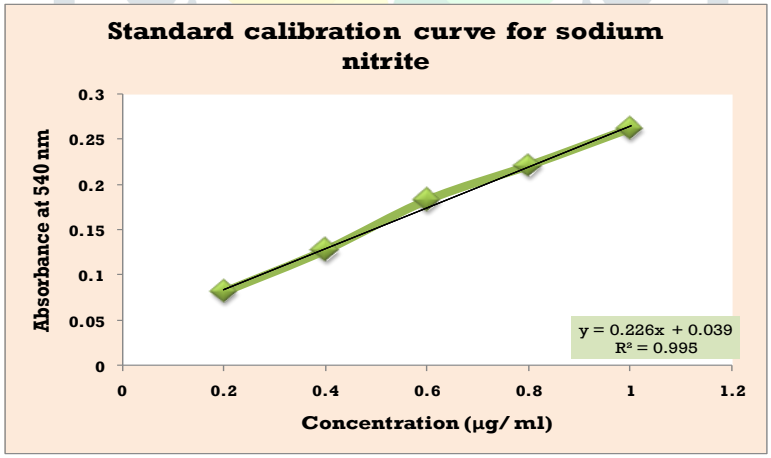


Fig 7- Effect of *Flacourtia jangomas* ethanolic fruit extract in Cellular nitrite assay

DISCUSSION

Medicinal plants are rich source of active compounds known to modulate a variety of pharmacological processes including inflammation**.** Recently, many researchers conduct studies in natural products obtained from natural sources due to its less adverse effect than the synthetic products [1]. Most of the research supported the utilisation of *Flacourtia jangomas* as a medication. Study was conducted in *Flacourtia jangomas* ethanolic fruit

extract on the basis of *Flacourtia jangomas* leaf extract have anti- inflammatory action due to the existence of flavonoids [18]. The percentage yield of an ethanolic extract of fruit was 20.0% w/w.

Phytochemical analysis result showed the presence of flavonoids, phenolic compounds, tannins, terpenoids, saponins. From the most of the studies, it is indicated that the flavonoids have several medicinal benefits including anti- oxidant, anti- inflammatory, analgesic, anti- cancer and anti- viral properties [29].

In silico molecular docking methods are used. The docking is an economical, reliable and time saving method for screening large set of lead molecule. In this study, docking studies of the lead molecules were implemented using Autodock via program in PyRx software and visualisation was done by using pyMOL software. From the review of literature, revealed that flavonoid quercetin has anti- inflammatory activity. *Flacourtia jangomas* fruit contain quercetin flavonoid [30]. So the quercetin was taken as the ligand and docking was performed with many receptors which have significant role in inflammation. The quercetin expresses the strong binging affinity with the selected receptor such as NLRC4, P2X7. Quercetin show more binding affinity with P2X7 receptor. The docking study indicated that the quercetin has the anti- inflammatory activity.

Inflammation occurs due to tissue injury and the denaturation of intracellular chemical substances and protein components within cells. Therefore, it seems that a substance's potential for anti-inflammatory effect is determined by its capacity to suppress protein denaturation. As a part of the current investigation the ability of ethanolic fruit extracts of *Flacourtia jangomas* to inhibits protein denaturation was studied at different concentration 6.25, 12.5, 25, 50, 100, 200 µg/ml. The results revealed that extracts produce concentration dependent activity. According to reports, NSAIDS like diclofenac, which are used to treat inflammation, inhibit protein denaturation. The extract shown moderate reducing protein denaturation when compared to standard diclofenac in protein denaturation inhibition assay. The secondary metabolites such as tannins, phenolics and flavonoids in the extracts might be accountable for the observed activity. [29] The flavonoid rich extract shows effective albumin denaturation inhibition.

Two enzymes implicated in the arachidonic pathway that causes inflammation are COX and LOX. The COX enzyme is crucial to the cyclooxygenase process of inflammation. It is a membrane-bound glycoprotein that is present in cells that generate prostanoids. It manifests as COX-1 and COX-2, two identical COX forms that have distinct intracellular sites and substrate and inhibitor selectivity. Nonsteroidal anti-inflammatory medicines (NSAIDs) are COX inhibitors, but they also inhibit COX-1, which is problematic because COX-1 is required for a number of physiological processes. Therefore, there is a particular need for substances that selectively inhibit COX-2 without harming COX-1. LOX enzymes have significant roles to play key role in asthma, inflammation, and angiogenesis. In the present investigation, various concentrations of *Flacourtia jangomas* ethanolic fruit extract were analysed for cyclooxygenase and 5- lipoxygenase inhibitory activity using *in vitro* assay models. The results revealed that the extract show inhibition of cyclooxygenase and 5-lipoxygenase in a dose dependent manner as compared with standard diclofenac and this inhibition might be attributed to the anti-inflammatory activity of the extracts. Various investigation proven that certain polyphenols and flavonoids regulate the activity of the COX and LOX enzymes. The synthesis of metabolites of COX and

LOX that have the potential to exacerbate the oxidative lesion in tissues. Certain flavonoids and polyphenols have the ability to reduce COX and LOX activity. [31].

When nitric oxide synthetase (NOS) oxidises the nitrogen in L-arginine, it releases nitric oxide (NO) in the forms of free radical and citrulline. Although sustained amounts of NO production cause direct cellular level toxicity, septic shock, and anti-inflammatory circumstances. The ability to block nitric oxide synthase and hence inhibit the generation of nitric oxide is indicated by the drop in cellular nitrite levels. In the present investigation, a concentration dependent and gradual increase in the percentage inhibition was observed in ethanolic fruit extract of *Flacourtia jangomas*. Sodium nitrite solution was used as the standard. The maximum inhibition was elicited by the concentration 100 µg/ml. the result indicated that the plant extract caused a concentration dependent suppression of cellular nitrite. Studies demonstrate that polyphenol modulated NO generation by interacting with nitric oxide synthase activity. Inflammatory cytokines and bacterial endotoxin can excite macrophages which resulting increased iNOS expression and NO generation and leading to oxidative damage. Polyphenols may reduce the expression of the iNOS gene and thus resulting in decreased oxidative damage [31].

CONCLUSION

Recent studies have been shown that many flavonoids and phenolic compounds contribute significantly to the antioxidant and anti-inflammatory activity of many medicinal plants. Hence the presence of these phytochemical compounds in the *Flacourtia jangomas* ethanolic fruit extract may contribute to its antioxidant and anti-inflammatory properties. The present study explores the phytoconstituents of *Flacourtia jangomas* ethanolic fruit extracts and evaluation of its anti- inflammatory activity based on the evidences from the ethnomedicinal studies of the plant. From the *in vitro* and *in silico* screening it is conveying that ethanolic *Flacourtia jangomas* fruit extract have moderate anti- inflammatory activity due to the existence of significant phytoconstituents.

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