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Spathulenol and Farnesol as Potential Anti-inflammatory Sesquiterpenoid Molecules from leaves of *Acronychia pedunculata*

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ABSTRACT: It is well known that various NSAID drugs reduces pain and inflammation through blockage of metabolism of arachidonic acid through lipoxygenase and cyclooxygenase, thereby reducing the production of prostaglandin and help in the treatment of inflammatory disorders. However, there are many side effects associated upon chronic administration of these NSAIDS. Therefore, medicinal plants provide an alternative to these as bioactive compounds present in them have excellent anti-inflammatory potential with minimal side effects. The aim of the present study was to evaluate the anti-inflammatory potential of sesquiterpenes namely spathulenol and farnesol from leaves of *Acronychia pedunculata*. The in vitro anti-inflammatory activity of essential oil and isolated sesquiterpenes was evaluated by hemolysis inhibition, proteinase inhibition, protein denaturation inhibition, cyclooxygenase and lipoxygenase inhibition. Both spathulenol and farnesol exhibited hemolysis inhibition of 17.11–29.92%, inhibition of protein denaturation of 46.2–71.4%, proteinase inhibition of 32.6–43.9%, cyclooxygenase inhibition of 29.6-43.4% and lipoxygenase inhibition of 26.3–41.2%. Lineweaver-Burk plots were established with the obtained data for the determination of V_{max} and K_m for lipoxygenase and cyclooxygenase enzymes and also to unravel the mechanism of inhibition. The kinetic parameters studies showed that both the sesquiterpene molecules lowered the V_{max} and K_m at all the tested concentrations. The results reveal that the isolated sesquiterpenes possess excellent anti-inflammatory activity and further metabolic profiling is needed for production of potential lead compounds for the treatment of inflammatory diseases.

Keywords: *Acronychia pedunculata*, sesquiterpenes, anti-inflammatory activity, LOX, COX-1 and COX-2 inhibition.

INTRODUCTION

Medicinal plants are well known as reservoirs of novel phytochemical compounds and has been used in Ayurveda and Siddha to treat various ailments and diseases since ancient times (Aye *et al.,* 2019). The bioactive phytochemicals have excellent properties such as antioxidant, anti-mutagenic, anti-cancer, anti-hypertensive and immuno-modulatory properties, making the medicinal plants as potent valuable sources in the treatment of diseases (Vujanovic *et al*., 2019; Navya *et al.,* 2023). There is a growing awareness in using medicinal plants extracts and biologically active phytochemicals for curing various diseases and home based remedies where, around 80% of the worlds' population are relying on plant-based bioactive components as they offer acceptable safety profile.

Inflammation is a well known physiological response that protects the body from any kind of tissue injury by rapid exudation of fluid and plasma proteins as in acute stage which lasts for few days and if it continues leads to chronic inflammation which lasts for several weeks to months manifesting symptoms such as pain, rheumatoid arthritis, asthma etc (Paramita *et al.,* 2018). In order to alleviate these symptoms, non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are being employed in the treatment as they inhibit cyclooxygenase (COX) and phospholipase A2 (PLA2) enzymes respectively (Gao *et al.,* 2021; Sinniah *et al.,* 2021). Despite their beneficial effects, these drugs show many side effects on long term usage including nausea, vomiting, peptic ulcers, and gastric bleeding (Paramita *et al.,* 2018). In this connection, conscientious efforts have been placed throughout the world on phytoconstituents derived from botanicals as an alternative to the synthetic drugs as they offer minimal side effects with excellent efficacy.

Acronychia pedunculata (*A. pedunculata*) belonging to the family Rutaceae is a large shrub or small evergreen tree grows up to 12 m tall. The plant commonly known as Indian Aspen is found in in dense evergreen forests of tropical Asia including India and Eastern Himalaya. Flowering can be observed in February to April and July to August. It has wide medicinal properties and found to be used against sores, scabies and intestinal infections, cough, asthma, relieves analgesic, anti-inflammatory and anti-nociceptive (Ratnayake *et al.,* 1992; Hapuarachchi *et al*., 2020). Therefore, an attempt was made in the present study to evaluate the

anti-inflammatory potential of sesquiterpenes from *A. pedunculata* and we herein, present the results our investigative study.

MATERIAL AND METHODS

Plant Material and Chemicals. Leaves of *A. pedunculata* were collected from the forest of Malnad region near Hassan, Karnataka, India and a voucher specimen was deposited at herbarium of Department of Botany, A.V.K. College for Women, Hassan, Karnataka, India (No. AVK BOT 4784, 4785 and 4786). Enzymes such as COX-1 (C0733), COX-2 (C0858), Phospholipase A2 (P8913), Lipoxygenase (437996), compounds namely Trypsin (T2600000), Diclofenac (SML3086) and Indomethacin (17378) were purchased from Sigma-Aldrich, St. Louis, MO, USA. All other chemicals and solvents used were of analytical grade and obtained from Sigma-Aldrich, St. Louis, MO, USA.

Isolation of Sesquiterpenes. Leaves (1 kg) of *A. pedunculata* were dried in shade (air dried) and essential oil was extracted in a Clevenger apparatus by hydro distillation for about 4 hr. The oil obtained after distillation was dried (using anhydrous sodium sulphate) and stored under nitrogen atmosphere for further use. The major sesquiterpenes were isolated by preparative GC.

Preparative GC. Sesquiterpenes, spathulenol and farnesol were isolated by preparative GC and was done on a HP (Hewlett Packard) GC fitted with a TCD detector. A packed stainless steel (5% OV 101 on Chromosorb W-HP 100/ 120 mesh) column was used for the analysis. The temperature of injector and detector were 250°C and 260°C respectively. The column temperature was 150°C (for isothermal process) and 200-240°C at 20°C/min (for the programmed process). Nitrogen was employed as a carrier gas at 100 ml/min flow rate.

Erythrocyte suspension preparation. The erythrocyte suspension was carried out as per the method of Shinde *et al.* (1999) with some modifications. The blood collected from the volunteer was subjected to centrifugation at 3500 rpm for about 5 min, washed thrice with saline (0.9% NaCl). The volume was then reconstituted in 10% v/v isotonic sodium phosphate buffer (10mM, pH 7.4) and used for further experimentation.

Heat Induced Hemolysis. Hemolysis was carried out by the method of Okoli *et al*. (2008) with slight modifications. To the blood cell suspension (50 µl), 50 µl of essential oil and sesquiterpenes were mixed thoroughly along with phosphate buffer (2.95ml, pH 7.4). After incubation at 54°C for 20 min, the mixture was subjected to centrifugation at 2500 rpm for 5 min. Subsequently the absorbance of the supernatant was recorded at 540 nm using a UV/VIS spectrometer. Diclofenac and Indomethacin served as a positive controls and Phosphate buffer as negative control in the analysis. All the experiments were performed in quintuplicate.

The percentage of inhibition was calculated as follows

$$\% \text{ inhibition of hemolysis} = 1 - \frac{A_2}{A_1} \times 100$$

Where, A_1 = Absorbance of control samples; A_2 = Absorbance of test samples

Effect on protein denaturation. Protein denaturation was carried out as per the method of Gunathilake *et al.* (2018) with slight modifications. The reaction mixture consisting of 200 µl of BSA (1%), 4.78 ml of PBS (phosphate buffered saline, pH 6.4), and 20 µl of essential oil and isolated sesquiterpenes was incubated at room temperature for 15 min. and subsequently heated at 70°C for 5 min. The absorbance of the turbidity after cooling was recorded at 660 nm using a UV-VIS spectrophotometer (Shimadzu). Diclofenac and Indomethacin served as a positive controls and Phosphate buffer as negative control in the analysis. All assays were carried out in quintuplicate. The inhibition percentage was determined by the formula

$$\% \text{ inhibition of protein denaturation} = 1 - \frac{A_2}{A_1} \times 100$$

Where, A_1 = Absorbance of control samples; A_2 = Absorbance of test samples

Proteinase inhibition. Proteinase inhibition of extracts and isolated compounds was performed as per the method of Sakat *et al*. (2010). The reaction mixture consisting of trypsin (60µg), Tris-HCl buffer (20 mM, pH 7.4, 1 ml), and 1 ml of essential oil and sesquiterpenes was incubated at room temperature for 5 min followed by addition of casein (0.8% w/v, 1 ml) and incubation at the same temperature for 20 min. The reaction was terminated by addition of perchloric acid (70%, 2 ml), centrifuged and subsequently the absorbance of the supernatant was recorded at 210 nm in UV-VIS spectrophotometer. Diclofenac and Indomethacin served as a positive controls and Phosphate buffer as negative control in the experimentation. All assays were performed in quintuplicate. The inhibition percentage was determined by using the formula

$$\% \text{ inhibition of protein denaturation} = 1 - \frac{A_2}{A_1} \times 100$$

Where, A_1 = Absorbance of control samples; A_2 = Absorbance of test samples

Lipoxygenase inhibition. Lipoxygenase inhibition potential of essential oil and isolated sesquiterpenes was performed as per the method of Wu (1996) with some modifications and in quintuplicate. In this assay, the reaction mixture was constituted using extracts or isolated compounds, sodium borate buffer (1 ml, 100 mM, pH 8.8) and lipoxygenase (10 µl, at a final concentration amounting to 8000 U/mL). Initiation of reaction was carried out by the addition of linoleic acid (10µl, 10mmol) as a substrate and then incubated at 30°C for 5 min. followed by measuring the absorbance at 234 nm using a UV-VIS spectrophotometer (Shimadzu). Phosphate buffer as a negative control and Diclofenac and Indomethacin as a positive controls was employed in the experiment. The percentage inhibitory activity of lipoxygenase was determined using the formula

% inhibition of lipoxygenase =

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Cyclooxygenase inhibition assays. The COX-1 and COX-2 inhibition assays were carried out as per the methods of Jager *et al.* (1996); Noreen *et al.* (1998) respectively with slight modifications. The two enzymes obtained from Sigma-Aldrich) were subjected to activation using co-factor solution i.e., 0.3 and 0.6 mg of adrenaline respectively for COX-1 and COX-2 and pre-incubated on ice for about 5 min. From this 60 µl was taken and added to 20 µl of sample solution consisting of 2.5 µl of oil and sesquiterpenes and 17.5 µl deionized water, incubated at room temperature for 5 min. followed by addition of 20 µl of ^{14}C labeled Arachidonic acid and further incubated for 10 min. Then the reaction was terminated by adding HCl (2N, 10 µl). Further, carrier solution (4 µl) consisting of unlabelled prostaglandins (0.2 mg/ml) were added and subsequently Prostaglandins from the un-metabolized arachidonic acid were separated by silica gel column chromatography. By loading samples on to separate Pasteur pipettes (packed with silica gel, particle size 0.063–0.200 mm). Then mobile phase consisting of hexane: dioxane: acetic acid in 3:1:0.05 v/v ratio was used for the elution of arachidonic acid followed by elution of prostaglandins by employing ethyl acetate: methanol in 6:1 v/v ratio and collected in scintillation vials. Subsequently, the radioactivity of the solutions containing prostaglandins was measured using a Perkin Elmer 4810 scintillation counter. All the experiments were performed in quintuplicate. Percentage inhibition of oil and sesquiterpenes as well as IC_{50} values were determined by comparing the amount of radioactivity measured in samples and blank.

Statistical Analysis. The data obtained was subjected to statistical analysis using Graph Pad Prism 9.0 (Graph pad software, Inc., La Jolla, CA, USA.) and sigma plot (enzyme module) statistical software.

RESULTS AND DISCUSSION

The essential oil and sesquiterpenes, spathulenol and farnesol from the leaves of *A. pedunculata* were studied for in vitro anti-inflammatory activity. In the hemolysis of HRBC assay, the isolated sesquiterpenes showed IC_{50} values of 72.63 µg/ml and 79.74 µg/ml respectively for spathulenol and farnesol with an inhibition of 17.11–29.92%, (Table 1). The results were compared with positive control samples, Indomethacin and Diclofenac where, they showed an IC_{50} of 39.34 and 35.28 µg/ml respectively. The essential oil extract, spathulenol, farnesol and standard samples inhibited heat induced hemolysis in a concentration-dependent manner (Fig. 1). With increase in concentration slight but noticeable increase in inhibition was observed. The essential oil extract and sesquiterpenes were also able to inhibit heat induced albumin protein denaturation process and the results were depicted in Table-2. The IC_{50} values obtained were 52.28, 58.91 and 101.89 µg/ml for spatulenol, farnesol and leaf oil respectively and compared favourably with the positive controls, indomethacin and diclofenac. As evident from Fig. 2, in a concentration dependent manner there was an increase in the inhibition of protein denaturation with increase in the concentrations of oils, sesquiterpenes and standard samples. The Proteinase inhibitory activity of oil and sesquiterpenes were evaluated and depicted in Table 3. Spathulenol and farnesol displayed excellent proteinase inhibition with IC_{50} of 59.35 µg/ml and 66.08 µg/ml respectively followed by the leaf oil (119.26 µg/ml). While the positive control samples, indomethacin and diclofenac showed IC_{50} of 39.64 µg/ml and 32.46 µg/ml respectively.

The results of the LOX, COX inhibition using leaf poil and isolated sesquiterpenes are summarized in (Tables 4-6). Overall, it was found that the spathulenol and farnesol demonstrated excellent inhibition of LOX enzyme with IC_{50} of 32.63 µg/ml and 37.74 µg/ml respectively followed by leaf oil (72.27 µg/ml). Similarly, the sesuqiterpenes were observed to be significant inhibitors of both COX-1 and COX-2 with more selectivity towards COX-1 inhibition. While the leaf oil showed moderate inhibition. The results were compared with indomethacin and diclofenac which served as positive control samples in the assays. The percentage inhibition of LOX, COX-1 and COX-2 at varying concentrations of oil and sesquiterpenes were recorded and presented in Fig. 3-5. The lineweaver-Burk plots were established for the determination of V_{max} and K_m and the mode of inhibition and the results were presented in Fig. 6-17. The V_{max} and K_m for the enzyme for positive control samples were found to be 0.1683 µmoles/ml/min & 12.321 µM and 0.2 µmoles/ml/min & 18.079 µM respectively for Diclofenac and Indomethacin. The isolated compounds, spathulenol and farnesol demonstrated a V_{max} and K_m of 0.1283 µmoles/ml/min & 24.172 µM and 0.1332 µmoles/ml/min & 33.726 µM respectively. The V_{max} was decreased and K_m value was increased with both the bioactive molecules which clearly signify that the affinity of the enzyme for the substrate is decreased resulting in the inhibition of enzyme. All the samples tested displayed mixed type of inhibition of lipoxygenase. Similarly, the line weaver-Burk plots were constructed for establishment of V_{max} and K_m and the mode of inhibition for COX-1 and COX-2 enzymes. The V_{max} for COX-1 was found to be 0.1835, 0.1604, 0.137 and 0.1026 µmoles/ml/min for Diclofenac, Indomethacin, spathulenol and farnesol respectively whereas, it was 0.1636, 0.1467, 0.1151 and 0.1096 µmoles/ml/min for COX-2 enzyme. Diclofenac and Indomethacin showed a K_m value of 24.2013 & 21.172 and 24.166 & 22.346 µM respectively, while the two bioactive compounds displayed the same K_m values of control samples. An unaltered K_m value with decrease in V_{max} was observed with both spathulenol and farnesol indicating that the compounds bind on the enzyme at a site other than active site and altering the activity. All the samples tested displayed non-competitive type of inhibition of both the enzymes.

The development and progression of many diseases can be related with an inflammatory process, which could affect different organs or tissues. Currently, many drugs

are used to treat inflammation. However, some of these compounds induce severe side effects. For this reason, the search of new therapeutic options for the treatment of inflammation is very desirable. Medicinal plants have been an interesting source for obtaining new active compounds, including several terpenes and terpenoids with anti-inflammatory activity. It is well known that leucocytes play a crucial role in cellular infiltration which forms an important aspect of an inflammatory response (Karrat *et al*., 2022). During inflammation, these leucocytes release the contents of lysosomes including proteases as a means of defense mechanisms and this in turn causes further tissue damage and subsequent inflammation (Akhtar, 2022). Due to this, the cell becomes more susceptible to secondary damage through lipid peroxidation (free radical mediated) (Karrat *et al*., 2022). As the membrane of RBC show similarity to that of lysosomal membrane, the RBC hemolysis inhibition might provide insights into the inflammatory process (Karrat *et al*., 2022). The cell membranes inhibits the lysis followed by the release of the cytoplasmic contents which, in turn, reduce the tissue damage and hence shows inflammatory response (Akhtar, 2022). Therefore, molecules that contribute significant protection of cell membrane are very important and aids in inhibition of inflammation progression. The sesquiterpenes exhibited membrane stabilization by inhibiting heat induced inhibition of RBC membrane and its stabilization implies that the molecules are involved in the stabilization of lysosomal membranes and this is in accordance with the published reports (Chou, 1997). Stabilization of liposomal membrane is very important in that it limits the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil namely proteases, which cause further tissue inflammation and damage by extracellular release. Spathulenol and Farnesol inhibit these processes, and clearly indicates that these molecules might stimulate the efflux of intracellular components.

In arthritis condition, due to inflammation, there occurs the denaturation of proteins (Karrat *et al*., 2022). Currently available NSAIDs extend protection through inhibition of protein denaturation (Ashagrie *et al*., 2023). In the present study, the isolated sesquiterpenes, spathulenol and farnesol displayed excellent inhibition of protein denaturation which is in accordance with those of published reports (Govindappa *et al.,* 2011; Kumar *et al.,* 2013). Proteinases are the enzymes which are associated with various inflammatory disorders. Proteinases of leukocytes are responsible for the development of tissue damage during inflammatory processes. Therefore, inhibitors of proteinase offer significant level of protection in these conditions. In the present study, the oil and sesquiterpenes significantly inhibited the proteinase enzyme indicating their possible role as anti-inflammatory agents.

Inflammation is a well known body's response to any kind of infection or injury and a key process for restoration and maintenance of homeostasis (Bian *et al*., 2021; Moudgil and Venkatesa 2022). Prostaglandin E2 (PGE-2) derived from arachidonic acid (AA), via up-regulation of cyclooxygenase-2 (COX-2), is a key mediator of inflammation which can be induced by several other factors such as stress, chromosomal aberration, nutritional and environmental factors and if not controlled on time leads to chronic inflammation and often leads to sepsis (Vyshnevska *et al*., 2022). In addition, lipoxygenase also forms an important modulators of inflammation. The resolution of uncontrolled inflammatory response requires anti-inflammatory agents which include both steroidal glucocorticoids and non-steroidal anti-inflammatory drugs that target the cyclooxygenase (COX) enzyme isoforms. But prolonged usage of NSAIDS poses side-effects includes gastric ulceration, renal toxicity, joint destruction and cardiovascular disorders (Miligya *et al*., 2022; Mukhopadhyay *et al*., 2023). The inhibition of any single pathway in the arachidonic acid metabolism leads to a shunting of the fatty acid to the lipoxygenase (LOX) pathway leading to the formation of harmful leukotrienes and the side effects. The isolated sesquiterepens, spathulenol and farnesol exhibited excellent inhibition of LOX, COX-1 and COX-2 comparable to the standards reference molecules. The anti-inflammatory activity and their mechanism of action of various sesquiterpenes and sesquiterpene lactones have been demonstrated and reported (Sa *et al*., 2015; Matos *et al*., 2021; Liu *et al*., 2022). The results obtained in the present study are in accordance with those of published reports.

Table 1: Percentage hemolysis of essential oil and sesquiterpenes from leaves of *A. pedunculata*

Compound	IC_{50} (µg/ml)	IC_{90} (µg/ml)
Leaf oil	145.67±3.48* (132.436 – 159.704)	231.08±3.84* (222.527 – 244.407)
Spathulenol	72.63±1.97** (66.231 – 79.813)	128.17±1.28** (118.216 – 138.098)
Farnesol	79.74±1.79** (69.674 – 86.104)	139.53±1.32** (127.492 – 150.037)
Indomethacin	39.34±1.46** (28.423 – 48.833)	69.66±0.81** (58.116 – 81.231)
Diclofenac	35.28±1.34** (29.208 – 42.175)	62.72±0.79* (53.712 – 72.629)

*Test samples were significant ($P < 0.05$); **Test samples were significant ($P < 0.01$)
Data expressed as Mean ± S.E.
The numbers in parenthesis represents 95% confidence limits

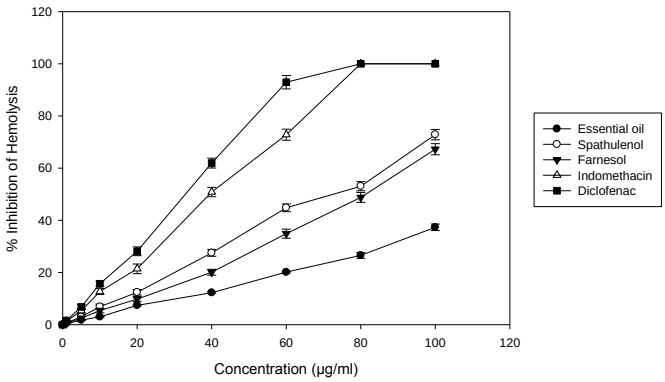
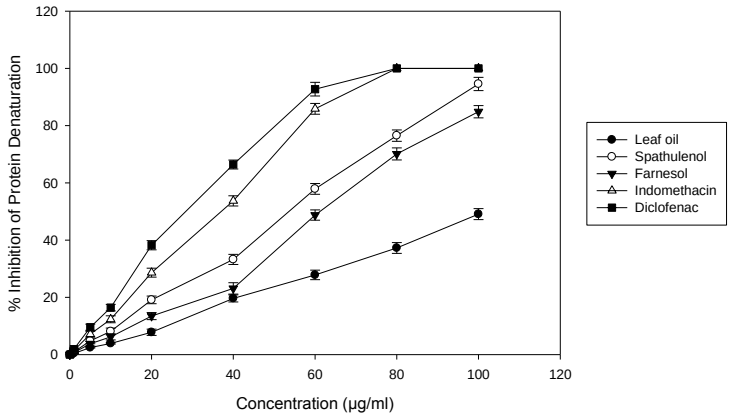


Fig. 1. Percentage inhibition of Hemolysis at varying concentrations of essential oil and isolated sesquiterpenes Data presented as Mean ± S.E.

Table 2: Effect of essential oil and sesquiterpenes from *A. pedunculata* on protein denaturation.

Compound	IC_{50} (µg/ml)	IC_{90} (µg/ml)
Leaf oil	101.89±3.48* (132.436 – 159.704)	192.77±3.84* (222.527 – 244.407)
Spathulenol	52.28±1.97** (66.231 – 79.813)	95.28±1.28** (118.216 – 138.098)
Farnesol	58.91±1.79** (69.674 – 86.104)	103.39±1.32** (127.492 – 150.037)
Indomethacin	34.94±1.46** (28.423 – 48.833)	56.58±0.81** (58.116 – 81.231)
Diclofenac	26.17±1.34** (29.208 – 42.175)	45.63±0.79* (53.712 – 72.629)

*Test samples were significant (P < 0.05); **Test samples were significant (P < 0.01)
Data expressed as Mean ± S.E.
The numbers in parenthesis represents 95% confidence limits



Data presented as Mean ± S.E

Fig. 2. Percentage inhibition of protein denaturation at varying concentrations of essential oil and isolated sesquiterpenes.

Table 3: Proteinase inhibitory activity of essential oil and sesquiterpenes from *A. pedunculata.*

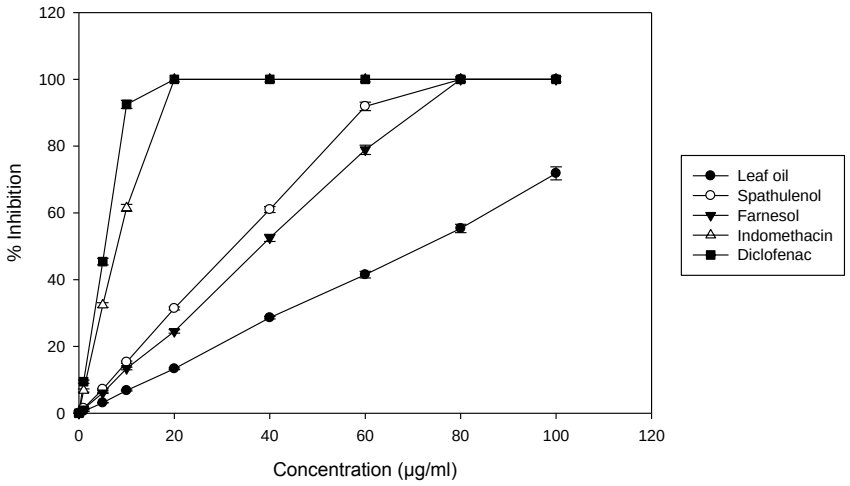
Compound	IC_{50} (µg/ml)	IC_{90} (µg/ml)
Leaf oil	119.26±3.27* (107.135 – 134.096)	192.77±3.84* (222.527 – 244.407)
Spathulenol	59.35±1.63** (47.331 – 71.172)	95.28±1.28** (118.216 – 138.098)
Farnesol	66.08±1.74** (54.097 – 79.782)	103.39±1.32** (127.492 – 150.037)
Indomethacin	39.64±1.41** (28.423 – 48.833)	56.58±0.81** (58.116 – 81.231)
Diclofenac	32.46±1.28** (29.208 – 42.175)	45.63±0.79* (53.712 – 72.629)

*Test samples were significant (P < 0.05); **Test samples were significant (P < 0.01)
Data expressed as Mean ± S.E.
The numbers in parenthesis represents 95% confidence limits

Table 4: Lipoxygenase inhibitory activity of essential oil and sesquiterpenes from *A. pedunculata.*

Compound	IC_{50} (µg/ml)	IC_{90} (µg/ml)
Leaf oil	72.27±1.12* (67.244 – 79.282)	134.83±1.43* (127.245 – 145.094)
Spathulenol	32.63±0.77* (28.627 – 37.608)	58.64±1.28* (49.712 – 67.107)
Farnesol	37.74±0.71* (32.228 – 43.417)	69.03±1.32* (58.332 – 78.308)
Indomethacin	7.34±0.31** (4.124 – 10.086)	12.33±0.81** (8.108 – 16.121)
Diclofenac	5.28±0.22** (3.102 – 8.311)	9.53±0.79* (6.809 – 13..243)

*Test samples were significant (P < 0.05); **Test samples were significant (P < 0.01)
Data expressed as Mean ± S.E.
The numbers in parenthesis represents 95% confidence limits



Data expressed as Mean ± S.E

Fig. 3. Percentage inhibition of Lipoxygenase activity at various concentrations of callus extract and isolated compounds.

Table 5: COX-1 inhibition potential of essential oil and sesquiterpenes from *A. pedunculata.*

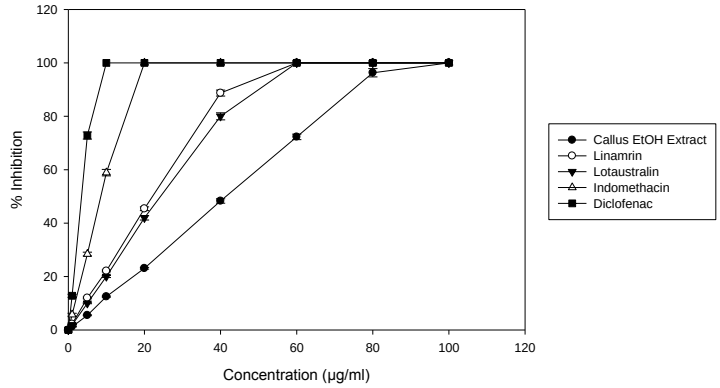
Compound	IC_{50} (µg/ml)	IC_{90} (µg/ml)
Leaf oil	41.46±1.38* (35.407 – 47.822)	74.33±1.49* (67.579 – 87.406)
Spathulenol	22.62±0.82* (16.119 – 28.701)	40.35±1.39* (33.291 – 48.704)
Farnesol	24.77±0.74* (19.711 – 29.082)	44.74±1.42* (36.198 – 51.726)
Indomethacin	8.49±0.51** (3.442 – 13.607)	15.73±0.88** (10.722 – 20.193)
Diclofenac	3.18±0.35** (1.026 – 7.194)	6.08±0.61* (3.092 – 8.414)

*Test samples were significant (P < 0.05); **Test samples were significant (P < 0.01)
Data expressed as Mean ± S.E.
The numbers in parenthesis represents 95% confidence limits

Table 6: COX-2 inhibition potential of essential oil and sesquiterpenes from *A. pedunculata.*

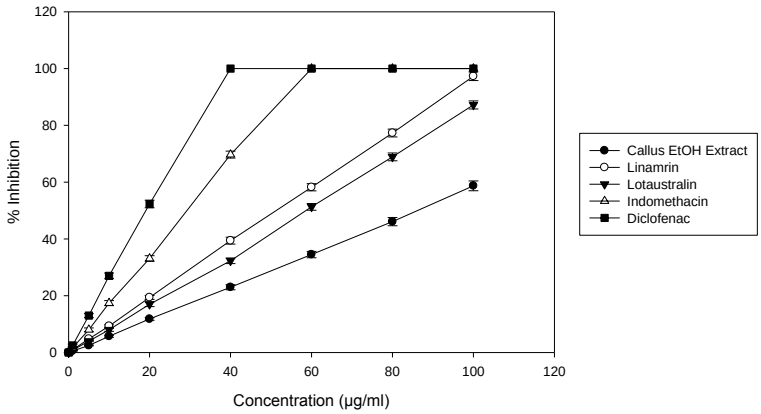
Compound	IC_{50} (µg/ml)	IC_{90} (µg/ml)
Leaf oil	86.78±1.98* (78.339 – 94.306)	156.16±3.27* (142.231 – 171.107)
Spathulenol	51.46±1.82* (42.186 – 61.164)	93.61±1.93* (85.712 – 105.405)
Farnesol	57.32±1.69* (46.133 – 67.017)	102.48±2.16* (89.227 – 115.707)
Indomethacin	28.66±0.98** (23.387 – 34.611)	50.56±1.22** (39.126 – 62.313)
Diclofenac	18.24±0.86** (14.213 –22.492)	31.29±0.99* (24.917 – 39.793)

*Test samples were significant (P < 0.05); **Test samples were significant (P < 0.01)
Data expressed as Mean ± S.E.
The numbers in parenthesis represents 95% confidence limits



Data expressed as Mean ± S.E

Fig. 4. Percentage inhibition of COX-1 activity with increase in concentration of leaf oil and isolated sesquiterpenes.



Data expressed as Mean ± S.E

Fig. 5. Percentage inhibition of COX-2 activity with increase in concentration of leaf oil and isolated sesquiterpenes.

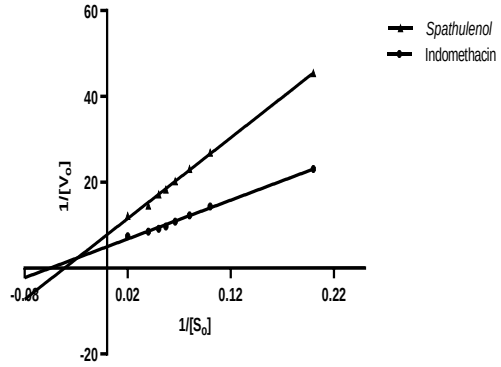


Fig. 6. Lineweaver-Burk plot to determine mode of inhibition lipoxygenase by Spathulenol.

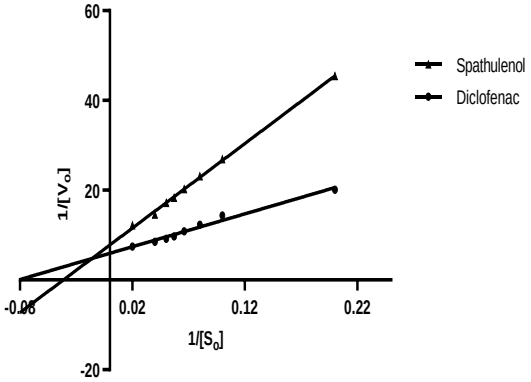


Fig. 7. Lineweaver-Burk plot to determine mode of inhibition lipoxygenase by Spathulenol.

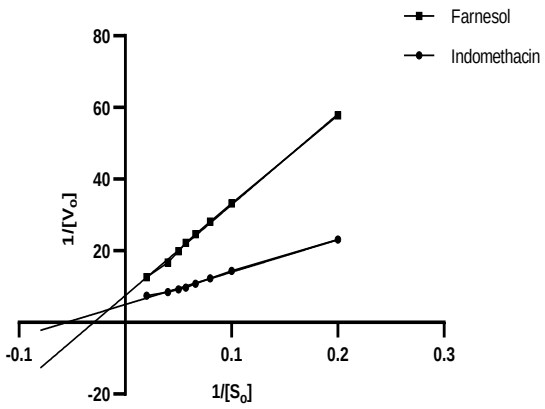


Fig. 8. Lineweaver-Burk plot to determine mode of inhibition lipoxygenase by Farnesol.

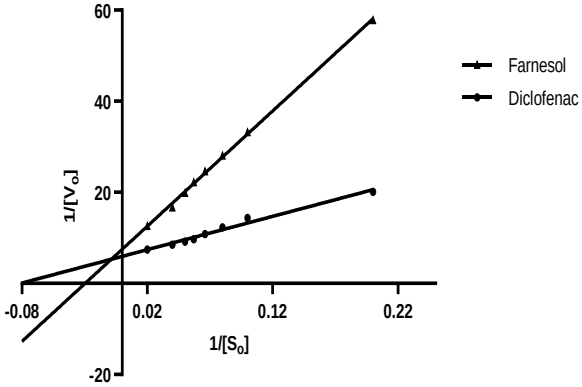


Fig. 9. Lineweaver-Burk plot to determine mode of inhibition lipoxygenase by Farnesol.

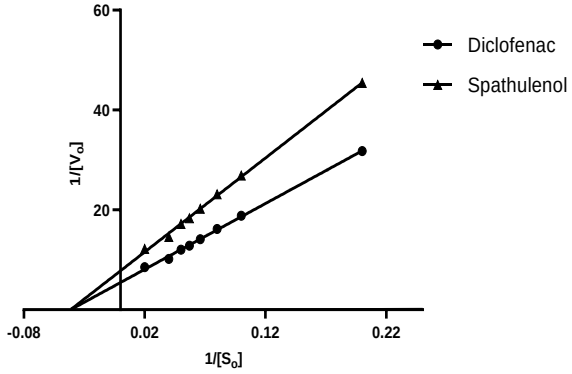


Fig. 10. Lineweaver-Burk plot to determine mode of inhibition COX-1 by Spathulenol.

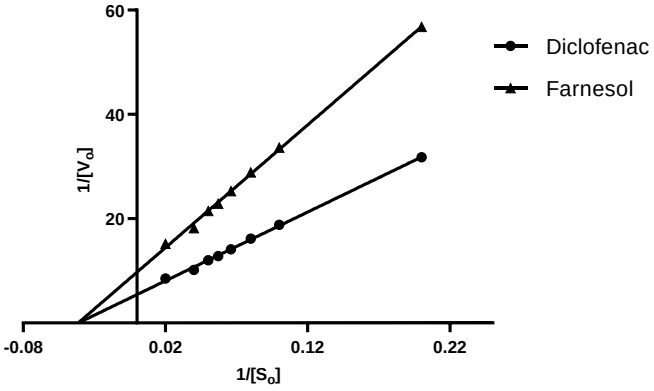


Fig. 11. Lineweaver-Burk plot to determine mode of inhibition COX-1 by Farnesol.

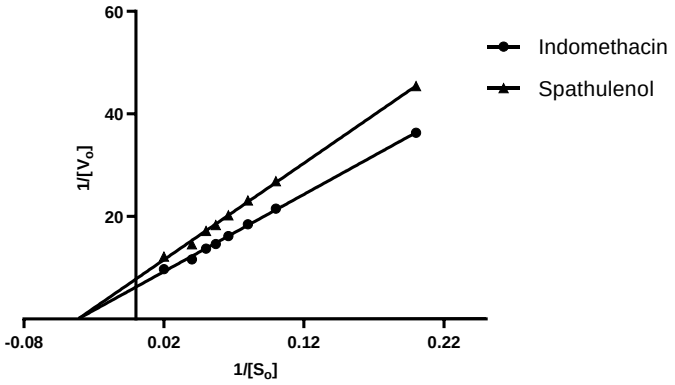


Fig. 12. Lineweaver-Burk plot to determine mode of inhibition COX-1 by Spathulenol.

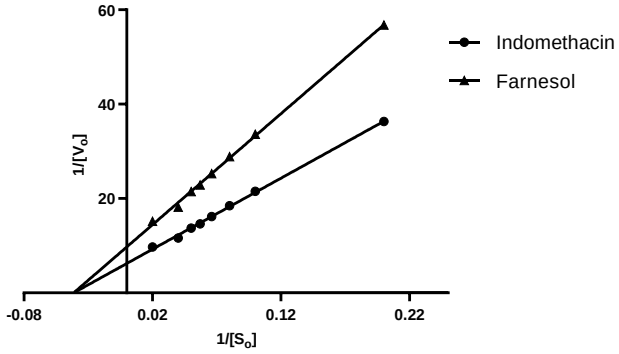


Fig. 13. Lineweaver-Burk plot to determine mode of inhibition COX-1 by Farnesol.

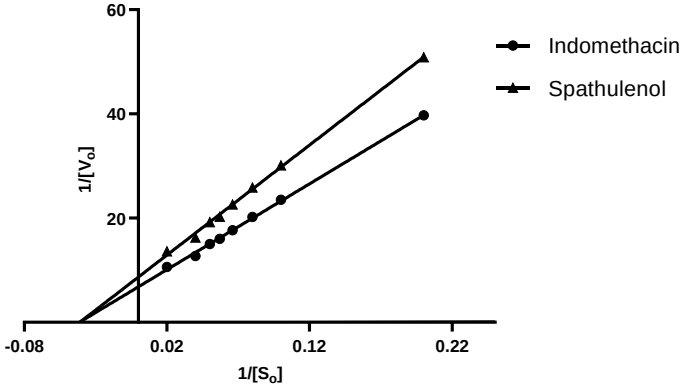


Fig. 14. Lineweaver-Burk plot to determine mode of inhibition COX-2 by Spathulenol.

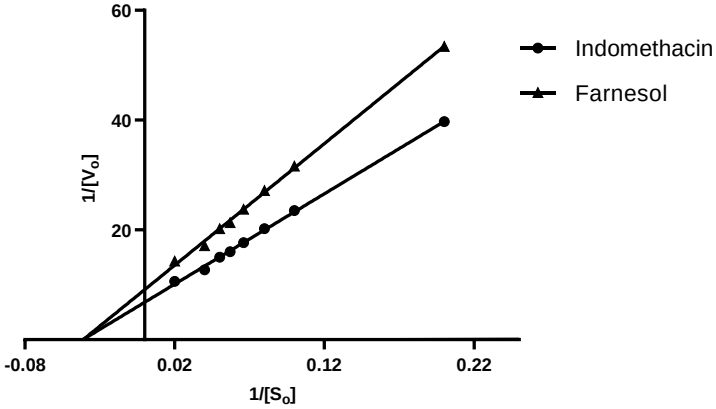


Fig. 15. Lineweaver-Burk plot to determine mode of inhibition COX-2 by Farnesol.

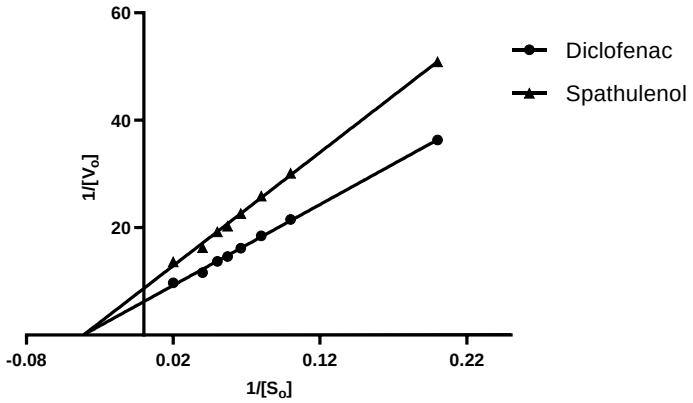


Fig. 16. Lineweaver-Burk plot to determine mode of inhibition COX-2 by Spathulenol.

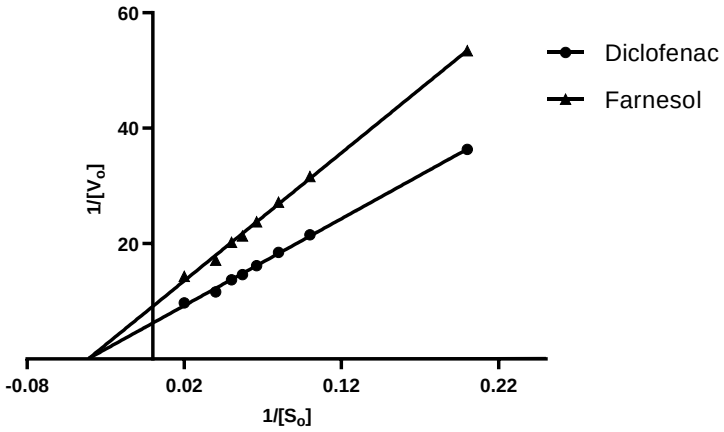


Fig. 17. Lineweaver-Burk plot to determine mode of inhibition COX-2 by Farnesol.

CONCLUSIONS

The results obtained indicates the anti-inflammatory potential of sesquiterpenes, spathulenol and farnesol by membrane stabilization and enxyme inhibition. This might be useful in the development of dual LOX/COX inhibitors with a higher safety profile as eco-friendly anti-inflammatory agents. Further work is underway to unravel the biochemical mechanism of action of these molecules where the data obtained might provide a promising approach against development of anti-inflammatory conditions and be considered for further validation to ensure the therapeutic efficacy and action.

FUTURE SCOPE

The pharmaceutical and agrochemical industries are currently engaged actively in Research and Development of biofriendly molecules of botanical origin as 80% of the world population are relying on products which are of plant origin. These attributes clearly indicate the demanding position of natural products in the present market. This research opens up new avenues in sesquiterpene research wherein, the sesquiterpene molecules can be developed as lead molecules for the treatment of inflammatory disorders. Further, there is a scope to elucidate the mechanism of action of sesquiterpenes which would definitely add a great contribution to the natural products research for human health and longevity.

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Conflict of Interest. None.

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