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Research Article

***In vitro* Anti-inflammatory and Antiarthritic activity
of ethanolic leaf extract of *Alternanthera sessilis* (L.)**

**R.BR. ex DC and *Alternanthera philoxeroides*
(Mart.) Griseb**

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ABSTRACT

Ethanolic leaf extract of *Alternanthera sessilis* and *Alternanthera philoxeroides* (Amaranthaceae) were assessed for their anti-inflammatory activity and anti-arthritis activity by *in vitro* methods. *In vitro* anti-inflammatory activity and anti-arthritis activity were evaluated using HRBC (Human Red Blood Cell) membrane stabilization method and inhibition of bovine serum albumin denaturation method and egg albumin denaturation method respectively. The present findings exhibited concentration dependent HRBC membrane stabilization and inhibition of protein denaturation activity of both the plant extracts and they were compared with the standard drug diclofenac sodium.

Key words: Arthritis, Inflammation, *Alternanthera sessilis*, *Alternanthera philoxeroides* and Diclofenac sodium.

INTRODUCTION

Arthritis is a chronic auto immune disorder principally attacks the joints and characterized by pain, swelling, inflexibility and stiffness of the involved joints¹. Its etiology is still obscure². Rheumatoid arthritis may rapidly progress into a multisystem inflammation with irreversible joint destruction and increase the risk of mortality. Rheumatoid arthritis affects about 1% of the general population and is two to three times more common in women than in men¹. Its prevalence depends upon age. Arthritis can affect at any age but more common in the age range of 25-50 years³.

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury and pain. Even

though most of the synthetic anti-inflammatory drugs are available in the market, due to their well-known side effects, toxic effects and production cost, presently people are searching for natural anti-inflammatory drugs without any adverse effects⁴. A systemic study of anti-inflammatory effects of Indian medicinal plants began in 1956 and they screened a number of plants for their Anti-inflammatory effects. Subsequently, various workers from different laboratories in India have made significant contributions⁵.

Green leafy vegetables (GLVs) are rich sources of many nutrients and form major category of vegetable group that have been designated as "nature's anti-aging wonders". The genus *Alternanthera*, a medicinally important member of family Amaranthaceae is reported to contain volatile constituents, essential amino acids, flavonoids,

glycosides and steroids. *A. Sessilis* is an annual or perennial prostate herb with several spreading branches, found throughout the hotter parts of India, ascending to an altitude of 1200m and even cultivated as a pot herb⁶. The speciality begins with its tamil name which indicates 'Ponnankanni - Pon aagum kaan nee' (Literally meaning – Your body will get golden luster). *A. philoxeroides* is a herbaceous perennial plant, is considered a vigorous invader in many regions of the world due to its ability to adapt to different ecosystems. Its tamil name is Vella Ponnankanni. Ponnankanni and Vella Ponnankanni are cooked as keerai masiyal, poriyal, kootu and sambar in South Indian traditional cooking methods.

MATERIALS AND METHODS

Plant material:

Fresh plants of *Alternanthera sessilis* and *Alternanthera philoxeroides* were harvested from Coimbatore district, the Western Ghats and were identified by Botanist, Arignar Anna Government Arts College, Mussiri. The leaves were cleaned, washed with copious amounts of distilled water, shade dried, coarsely powdered and stored in air tight container for extraction.

Preparation of crude plant extracts:

For assessment of *in vitro* activities, the coarsely powdered plant samples were subjected to successive solvent extraction. 10g of air dried powder of *Alternanthera sessilis* and *Alternanthera philoxeroides* were taken in 100mL of ethanol. Plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24hours. After 24hours the supernatant was collected and the solvent was evaporated to make the final volume, one-fourth of the original volume and stored at 4°C in air tight container.

Assessment of *in vitro* anti-inflammatory activity:

Membrane stabilization:

Preparation of Red Blood cells (RBCs) suspension^{7,8}:

The blood was collected from healthy human volunteer who has not taken any Non Steroidal Anti-Inflammatory Drugs for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000

rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared viz., 100, 200,300,400 and 500 mcg/ ml using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It is incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. The haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm.

$$\% \text{ inhibition of haemolysis} = 100 \times (\text{OD1} - \text{OD2}/\text{OD1})$$

where,

OD1 = Optical density of hypotonic-buffered saline solution alone

OD2 = Optical density of test sample in hypotonic solution.

In-vitro Anti-arthritis Activity:

The *in-vitro* anti-arthritis activity was studied using bovine serum protein denaturation method⁹ and Egg Albumin denaturation method¹⁰.

Bovine Serum Protein Denaturation Method:

Preparation of Reagents

0.5% Bovine Serum Albumin (BSA): Dissolved 500mg of BSA in 100 ml of water.

Phosphate Buffer Saline pH 6.3: Dissolved 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na_2HPO_4), 0.24 g of potassium dihydrogen phosphate (KH_2PO_4) in 800 ml distilled water. The pH was adjusted to 6.3 using 1N hydrochloric acid (HCl) and made the volume to 1000 ml with distilled water.

Method:

- Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5% W/V aqueous solution) and 0.05ml of test solution of various concentrations.
- Test control solution (0.5ml) consists of 0.45ml of bovine serum albumin (0.5% W/V aqueous solution) and 0.05ml of distilled water.
- Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution.
- Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5% w/v aqueous solution) and 0.05ml of Diclofenac sodium of various concentrations.

Procedure:

0.05 ml various concentrations (100,200,300,400 and 500 µg/ml) of test drugs and standard drug diclofenac sodium (100,200,300,400 and 500 µg/ml) were taken respectively and 0.45 ml (0.5% w/V BSA) mixed. The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 255 nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. The percentage inhibition of protein denaturation can be calculated as.

Percentage Inhibition = $100 - \left[\frac{\text{optical density of test solution} - \text{optical Density of control}}{\text{optical density of test}} \right] \times 100$

Egg Albumin Denaturation Method:

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations (100,200,300,400 and 500 µg/ml) of drug. A similar volume of double-distilled water served as the control. Next, the mixtures were incubated at $37 \pm 2^\circ\text{C}$ in a BOD incubator for 15 minutes and then heated at 70°C for five minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Diclofenac sodium in the concentrations of 100,200,300,400 and 500 µg/ml was used as the reference drug and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times \left[\frac{V_t}{V_c} - 1 \right]$$

where,

V_t = absorbance of the test sample, V_c = absorbance of control.

Each experiment was done in triplicate and the average was taken.

RESULT***In vitro* anti-inflammatory activity:**

The inhibition of hypotonicity induced HRBC membrane lysis *i.e.*, stabilization of HRBC membrane was taken as a measure of the *in vitro* anti-inflammatory activity. The percentage of membrane stabilization for ethanolic leaf extracts of *A.sessilis*, *A.philoxeroides* and Diclofenac sodium done at 100, 200, 300, 400 and 500 µg/ml. Ethanolic leaf extracts of *A.sessilis* and *A.philoxeroides* were effective in inhibiting the heat induced hemolysis of HRBC at

different concentrations (100-500µg/ml) as shown in Table 1.

A.sessilis showed the maximum inhibition 73.8% at 500µg/ml where as *A.philoxeroides* showed the maximum inhibition 62.2% at 500µg/ml. With the increasing concentration, the membrane haemolysis is decreased and membrane stabilization/protection is increased as shown in figure 1 and figure 2 respectively. Hence anti inflammatory activity of the extracts were concentration dependent.

In vitro* Anti arthritic activity:*Bovine Serum denaturation method:**

In vitro anti-arthritic activity by Bovine Serum denaturation method at concentration of 500 µg/ml *A.sessilis* showed 75.43% and in *A.philoxeroides* it was 64.92% whereas, standard diclofenac it was 100% (Table 2 and figure 3).

Egg albumin denaturation method:

In vitro anti-arthritic activity by Egg Albumin denaturation method at concentration of 500 µg/ml *A.sessilis* showed 84.34% and in *A.philoxeroides* it was 64.73 % whereas in standard diclofenac it was 97.51% (Table 3 and figure 4).

DISCUSSION

In the present study, HRBC membrane stabilization method, absorbance of haemoglobin was taken. The haemoglobin was released as a result of lysis of RBC membrane. Due to stabilization of membrane less absorbance was noted in spectrometer results. The ethanolic leaf extract of *A.sessilis* and *A.philoxeroides* exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extracts may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituent of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage¹¹.

Both the extracts have shown significant membrane stabilizing property, which suggested that the anti-inflammatory activity observed in this study may be related to the inhibition of the release of phospholipases that trigger the formation of inflammatory mediators.

Rajani *et al.* revealed the anti-inflammatory activity of *A.sessilis* on acute phase of inflammation induced by carrageenan¹². The process takes place in three phases of chemical mediator which will be released in an orderly sequence. An initial phase takes place

with the release of histamine and serotonin for the first 1.5 hours and second phase is facilitated by bradykinin for the consecutive 1.5-2.5 hours. The third and final phase occurs between 2.5 and 6 hours and is presumably mediated by prostaglandins (PGs)¹³. Besides, in the carrageenan-induced rat paw edema model, the production of prostanooids has been through the serum expression of COX-2 by a positive feedback mechanism. Therefore, it is suggested that the mechanism of action of *A.sessilis* may be related to the prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of Diclofenac sodium in the inhibition of the inflammatory process induced by carrageenan¹⁴.

Saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects^{15,16}. The phytochemical study revealed the presence of saponins, terpenoids, flavonoids, steroids and alkaloids in ethanolic extract of *A.philoxeroides* and *A.sessilis*¹⁷. It may also be the reason for anti-inflammatory activity. From the above study it was concluded that the ethanolic extract of *A.sessilis* has significant membrane stabilization property compared to the ethanolic extract of *A.philoxeroides* and it was comparable to the standard drug Diclofenac Sodium.

In vitro anti arthritic activity was done for the first time in *A.sessilis* and *A.philoxeroides*. Bovine serum denaturation method and egg albumin denaturation method were studied. Protein denaturation is a process in which proteins lose their tertiary structure

and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured¹⁸. Sangita chandra *et al.* reported that denaturation of protein is one of the cause of rheumatoid arthritis⁹. Production of auto-antigens in certain rheumatic diseases may be due to *in vitro* denaturation of proteins. An alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding are the mechanism of denaturation. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation¹⁹. In the present study, ethanolic leaf extract of *A.sessilis* and *A.philoxeroides* inhibited heat induced protein denaturation and may be one of the reason of possessing anti-inflammatory and anti-arthritic activity.

CONCLUSION

This is the first comparative *in vitro* study on anti-inflammatory and anti-arthritic activity of *A.sessilis* and *A.philoxeroides*. These leafy vegetables might be a potential alternative agent for anti-inflammatory and antiarthritic activity. Hence, it was anticipated that *A.sessilis* and *A.philoxeroides* would be a useful pharmaceutical material to treat inflammation and arthritis. Anti-inflammatory activity may be due to the presence of many phytochemicals present in the extract. However, further studies are required to identify the lead molecule in the extract and to study the action of mechanism.

Table 1
***In Vitro* anti-inflammatory activity**

Conc µg/ml	% Membrane stabilization			% Haemolysis		
	<i>A.sessilis</i>	<i>A.philoxeroides</i>	Diclofenac sodium	<i>A.sessilis</i>	<i>A.philoxeroides</i>	Diclofenac sodium
100	31.09	27.15	68.09	68.91	72.85	31.91
200	37.89	33.43	73.31	62.11	66.57	26.69
300	46.07	37.87	85.09	53.93	62.13	14.91
400	58.53	48.5	93.15	41.47	51.5	6.85
500	73.8	62.2	97.05	26.2	37.8	2.95

Table 2
***In Vitro* anti-arthritic activity**
Bovine Serum protein denaturation method

Concentration $\mu\text{g/ml}$	% Membrane stabilization		
	<i>Alternanthera sessilis</i>	<i>Alternanthera philoxeroides</i>	Diclofenac sodium
100	45.7	35.13	73.23
200	55.02	40.81	78.54
300	71.2	44.56	81.56
400	73.8	58.56	93.67
500	75.43	64.92	100

Table 3
Egg albumin denaturation method

Concentration $\mu\text{g/ml}$	% Membrane stabilization		
	<i>Alternanthera Sessilis</i>	<i>Alternanthera philoxeroides</i>	Diclofenac sodium
100	48.75	43.18	61.23
200	53.14	51.35	76.65
300	68.65	59.73	86.22
400	76.97	64.73	95.43
500	84.34	71.38	97.51

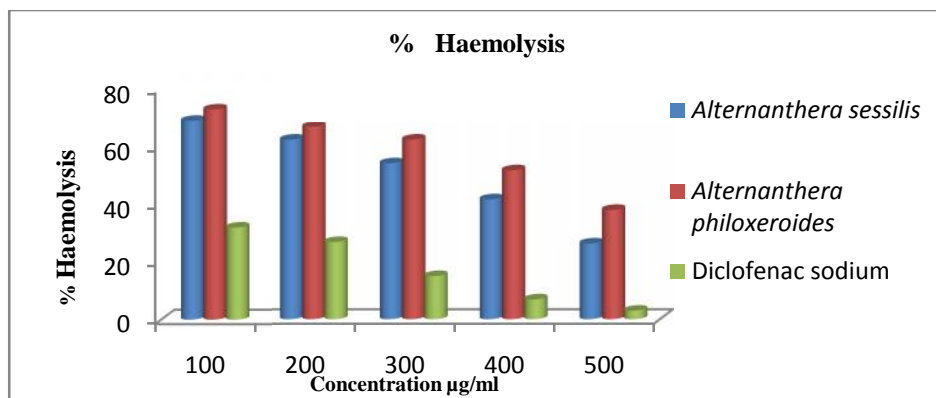


Figure 1
***In vitro* anti-inflammatory activity**

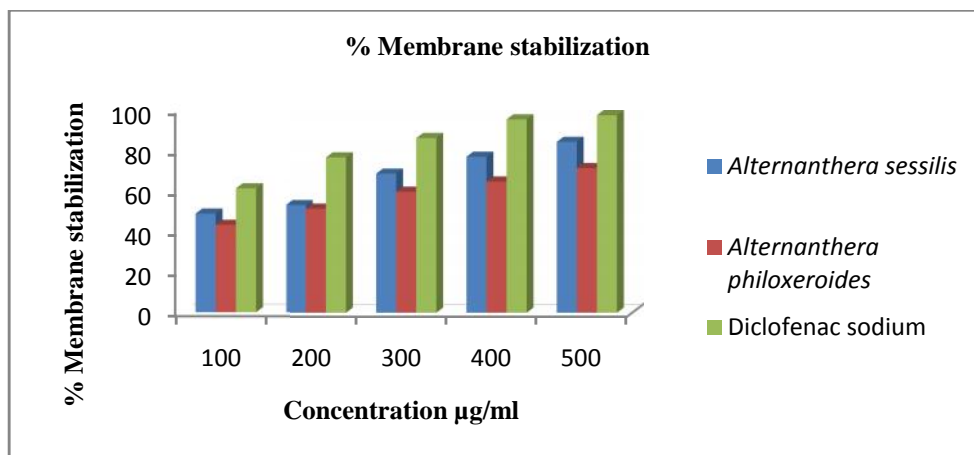


Figure 2
In vitro anti-inflammatory activity

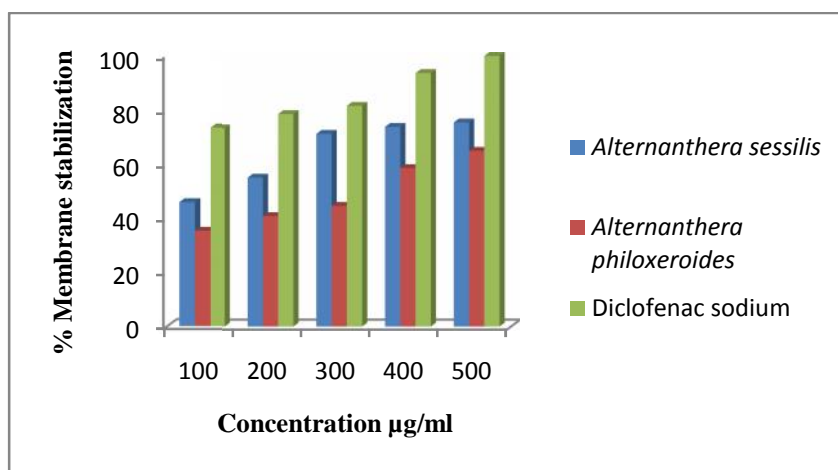


Figure 3
In vitro anti-arthritic activity (Bovine serum albumin denaturation method)

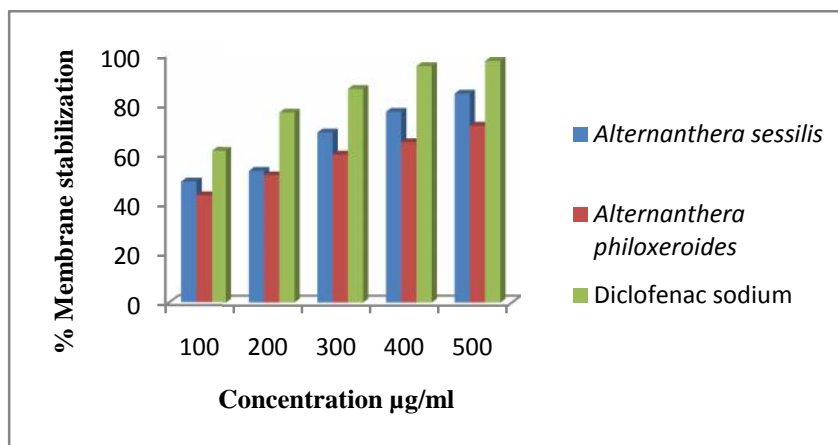


Figure 4
In vitro anti-arthritic activity (Egg albumin denaturation method)

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