



## Research Journal of Pharmaceutical, Biological and Chemical Sciences

### Investigation of *In-vitro* anti-Inflammatory, anti-platelet and anti-arthritic activities in the leaves of *Anisomeles malabarica* Linn

R Lavanya\*, S Uma Maheshwari, G Harish, J Bharath Raj, S Kamali, D Hemamalani,  
J Bharath Varma, C Umamaheswara Reddy

Department of Pharmacology, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai 600116, Tamil Nadu, India.

#### ABSTRACT

Previous phytochemical analysis of methanolic extract of *A. malabarica L.* has indicated the presence of steroid, flavonoid and terpenoid types of compounds. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check *A. malabarica L* for possible anti-inflammatory activity by HRBC (Human Red Blood Cell) membrane stabilization method, anti-arthritic activity by the inhibition of protein denaturation method and anti-platelet activity. The methanolic extracts of the plant exhibited notable anti-inflammatory activity and remarkable anti-arthritic, anti-platelet action. The maximum membrane stabilization of *A. malabarica L* was found to be 98.34% at a dose of 1000mcg/0.5ml and that of protein denaturation was found to be 97.47% at a dose of 250mcg/ml. Hence, the methanolic extracts of *A. malabarica L* demonstrated the anti-inflammatory, anti-platelet and anti-arthritic activities. Therefore, our studies support the isolation and the use of active constituents from *A. malabarica L* in treating inflammations and rheumatism.

**Keywords:** *A. malabarica L*, Rheumatism, Anti-arthritic Activity, Anti-inflammatory, anti-platelet, Membrane Stabilization.

**\*Corresponding author**

Email: lavansaro@yahoo.com

## INTRODUCTION

The uses of traditional medicine wide spread medicine and plants still represent a large source of natural anti-oxidants that might serve as leads for the development of the novel drugs. Several anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant or radical scavenging mechanism as part of their activity [1]. The mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen species from activated neutrophil and macrophages. This over production leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as interleukine- 1, tumor necrosis factor- $\alpha$ , and interferon- $\gamma$ , which stimulate recruitment of additional neutrophil and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation [2-4]. Most clinically important medicine belongs to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation related diseases. Though these have potent activity and long term administration is required for treatments of chronic diseases. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally originated agents with very little side effects are desirable to substitute chemical therapeutics [5]. Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease predominantly affecting the joints and peri-articular tissues. RA still remains a formidable disease, being capable of producing severe crippling deformities and functional disabilities. RA is classified as an inflammatory arthritis, the disease comprises of 3 basic inter-related processes like inflammation, synovial proliferation and joint tissue destruction. RA factor containing immune complexes found in the joints activate the pathological process. Tumour necrosis factor alpha (TNF-alpha) is the product of macrophages has been demonstrated to play an important role in the pathogenesis of RA. *A. malabarica* L is a shrubby, erect, densely tomentose or thickly woolly; stems slightly branched, obtusely quadrangular, clothed with soft woolly hairs [6-9]. Leaves are very thick, oblong-lanceolate, acute, pale above, white below, crenate-serrate, base rounded or shortly cuneate; petioles long, stout, softly woolly<sup>[10]</sup>. In the present study, we investigated whether these plants have anti-arthritis, anti-platelet and anti-inflammatory activities.

## MATERIALS AND METHODS

### Collection and extraction of medicinal plant material

The raw material of medicinal plant *Coldenia procumbens* was collected from different regions around Chennai and authenticated by Dr.P.Jayaraman (Botanist), Director PARC, West Tambaram, Chennai. Voucher specimen [No: PARC/2008/186], deposited in our college herbarium for future reference. The dried powdered leaves of the plant materials were extracted separately with methanol using soxhlet apparatus for 48 hrs. The solvent was distilled at lower temperature under reduced pressure and concentrated on water bath to get the crude extract which is stored in desiccator for future use.

### **Invitro anti-inflammatory activity by HRBC membrane stabilization method**

The principle involved here is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.5 ml of plant extracts of various concentrations (31.25, 62.5, 125, 250, 500, 1000, 2000 µg/0.5ml), standard drug diclofenac sodium (250, 500 1000, 2000 µg/0.5ml) and control [distilled water instead of hypo saline to produce 100 % hemolysis] were incubated at 37° C for 30 min and centrifuged respectively [10]. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm. The percentage hemolysis produced in the presence of distilled water was taken as 100 %. The results were tabulated in Table: 1 & Fig: 1). Percentage of HRBC membrane stabilization or protection was calculated using the formula,

$$\text{PERCENTAGE STABILIZATION} = 100 - \left[ \frac{\text{(OPTICAL DENSITY OF DRUG)}}{\text{(OPTICAL DENSITY OF CONTROL)}} \times 100 \right]$$

### **Invitro anti-arthritic activity by inhibition of protein denaturation method**

1. The Test solution (0.5ml) consist of 0.45ml of Bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of test solution (250mcg/ml).
2. Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of distilled water.
3. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution (250mcg/ml).
4. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml Of Diclofenac sodium (250mcg/ml).

All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°c for 20minutes and the temperature was increased to keep the samples at 57°c for 3minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416nm. [11-12]  
The percentage inhibition of protein denaturation can be calculated as,

$$\text{PERCENTAGE INHIBITION} = \left[ \frac{100 - (\text{OPTICAL DENSITY OF TEST SOLUTION} - \text{OPTICAL DENSITY OF PRODUCT CONTROL})}{\text{OPTICAL DENSITY OF TEST CONTROL}} \right] \times 100.$$

The control represents 100% protein denaturation .The results were compared with Diclofenac sodium (250mcg/ml. The percentage inhibition of protein denaturation of different concentration was tabulated in Table.3 & Fig: 2.

### **Invitro Anti-platelet activity**

The platelet rich plasma  $0.13 \times 10^7$  for each assay was re-suspended in pH 7.4 Tyrode buffer. The platelet aggregation was recorded as transmittance values of spectrophotometer measurement. To determine the invitro inhibition of platelet aggregation different concentrations of methanolic extract of *A. malabarica L* like 31.2, 62.5, 125, 250, 500, 1000, 2000 $\mu\text{g/ml}$  in DMSO (Dimethyl sulphoxide) were used. The platelet aggregation was induced with ADP at a concentration of  $5\mu\text{M}$  which is used as control [13-15]. The Aspirin 100 $\mu\text{g/ml}$  is used as a standard. The transmittance is recorded at interval of 1min for every 5min. The results were tabulated in Table.3 & Fig: 3.

## **RESULTS AND DISCUSSION**

### **Anti-inflammatory activity**

The investigation is based on the need for newer anti-inflammatory agents from natural source with potent activity and lesser side effects as substitutes for chemical therapeutics. The percentage protection of methanolic extracts was 98.34% at 1000 $\mu\text{g/ml}$ . It possesses significant activity comparable with that of the standard Diclofenac sodium [16].

*A. malabarica L* has significant anti-inflammatory activity which may be due to presence of chemical profile such as Flavones, Tri-Terpenoids, Flavonones and Phenols.

### **Anti-arthritic activity**

The methanolic extract fabricates significant activity at 97.47% at 250 $\mu\text{g/ml}$  by inhibition of protein denaturation and its effect was compared with the standard drug Diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein [17-19]. From the results of present study it can be stated that methanolic extract is capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

### **Anti-Platelet**

Platelets play an important role in the process of atherombosis by adhering to the damaged regions (caused by reactive oxygen species) of the endothelial surface. The activated platelets to platelets bond, binds also to leucocytes bringing them in to a complex process of plague formation and growth. The anti- platelet therapy constitutes the best available tool for ameliorating the mechanism related to atherogenesis and have interestingly inhibited platelet aggregation. Platelets stick to the damaged vessel wall, they stick to each other (aggregate) and release ADP, thromboxane  $A_2$  ( $\text{TXA}_2$ ) which promotes further aggregation, and thus a platelet plug is formed. In the veins, due to sluggish blood flow, the fibrinous tail is formed which traps

RBC's 'The red tail' [20]. In arteries platelet mass are the main constituents of the thrombus. Anti-platelet drugs are more useful in arterial thrombosis, while anti-coagulant are more effective in venous thrombosis [21-24]. The methanolic extract of *A. malabarica L* showed significant anti-platelet aggregation at 125mcg/ml.

### CONCLUSION

*In vitro* studies on *A. malabarica L* demonstrate suppression of both inflammation and arthritis. The methanolic extracts of the leaves of *A. malabarica L* must contain some principles, which possess anti-inflammatory, anti-platelet and anti-arthritic activities. From the preliminary screening study, it showed the presence of Flavonones, Flavones, Tri-Terpenoids and Phenolics. Hence proper isolation of the active principles might help in the findings of new lead compounds in the fields of anti-arthritic, anti-platelet and anti-inflammatory drug research. Studies related to active constituents on lipid derived eicosanoids, enzyme expression (COX2, lipoyxygenase) and cytokines are necessary to understand the mechanism of action in relation to the observed anti-inflammatory activity. Hence it can be used as a potent agent against it.

**Table: I Effect of *Anisomeles malabarica* on HRBC membrane stabilization**

Concentration (mcg/0.5ml)	Methanolic extract of <i>Anisomeles malabarica</i>	Diclofenac Sodium
31.25	97.33	-
62.5	97.45	-
125	97.28	-
250	95.86	82.74
500	96.77	88.39
1000	98.34	90.10
2000	97.20	99.94

**Table 2: Effect of *Anisomeles malabarica* on anti-arthritic activity**

Concentration(mcg/ml)	Anisomeles malabarica	Diclofenac sodium
250	97.47	94.22

**Table 3: Percentage transmittance values for the methanolic extract of *Anisomeles Malabarica***

Concentration(mcg/ml)	0 Min	1 Min	2 Min	3 Min	4 Min	5 Min
ADP 5μM	27.596	30.271	31.202	29.492	27.299	25.311
31.25	46.116	34.981	37.322	40.792	34.979	33.611
62.5	59.756	45.581	44.112	39.222	33.209	31.211
125	98.606	84.371	81.742	81.572	76.479	72.551
250	59.236	45.801	43.592	36.942	33.099	33.601
500	71.166	56.661	54.372	51.222	44.929	41.431
1000	94.116	84.331	71.642	68.372	66.891	63.231
2000	63.426	53.061	52.832	50.652	51.349	50.331
ASPIRIN(100mcg/ml)	93.536	87.321	85.962	81.802	78.749	76.391

**Fig:1 Effect of *Anisomeles malabarica* on HRBC membrane stabilization**

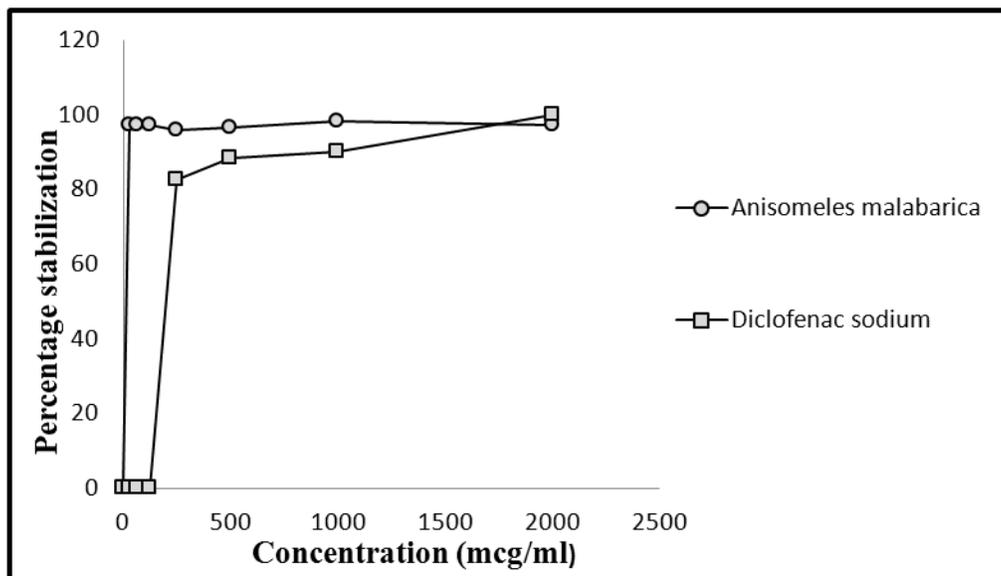


Fig: II Effect of *Anisomeles malabarica* on anti-arthritic activity

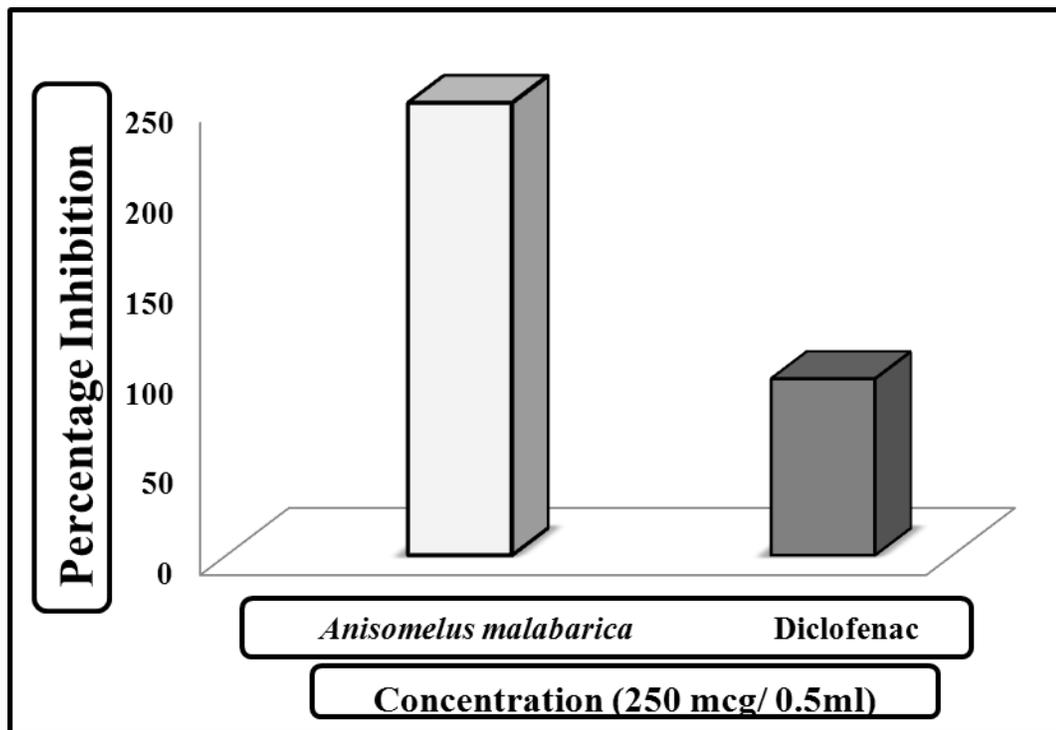
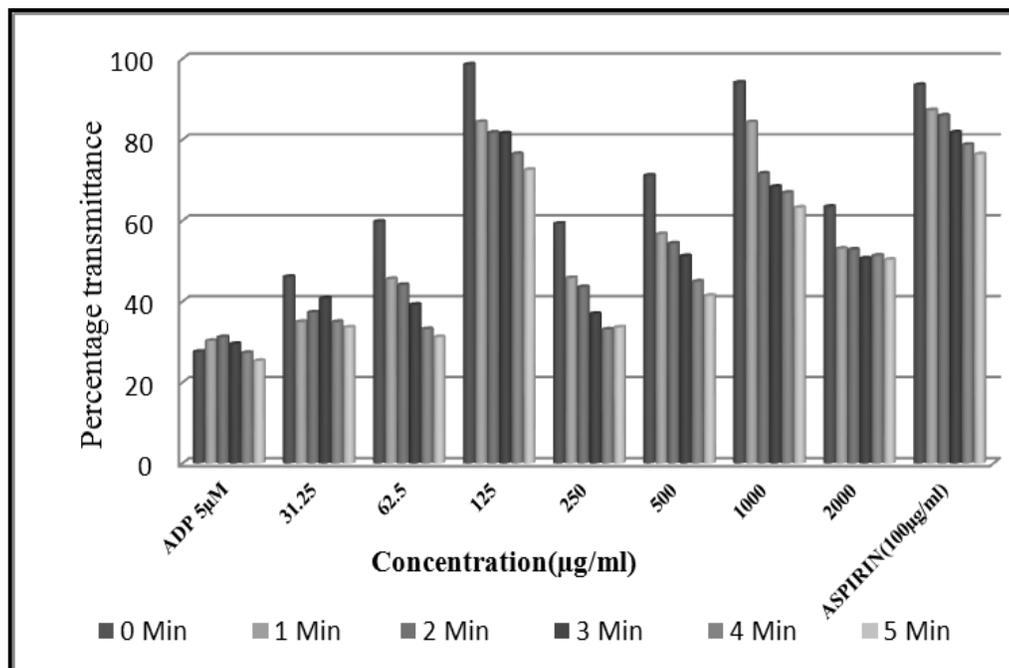


Fig: III Effect of *Anisomeles malabarica* on anti-platelet activity



## ACKNOWLEDGEMENT

I thank the management of Faculty of pharmacy, Sri Ramachandra University for providing necessary facilities and encouragement.

## REFERENCES

- [1] Kiritikar KR & Basu BD. Indian Medicinal Plants. Vol-3, 2<sup>nd</sup> Edition.
- [2] Indian Medicinal Plants, A compendium of 500 species, Vol-1, by Vaidyaratnam P S Varier's, Arya Vaidya Sala, Kottakkal, Coll.No. AVS 1481
- [3] DR KM Nadkarni's Indian Materia Medica, Vol-1, Edited by DR. K.R.Nadkarni, P.NO.114
- [4] Chatterjee A. The Treatise of Indian Medicinal Plants, National Institute of Science and Communication CSIR 1997, New Delhi. Vol.IV:212-217p.
- [5] Koleva II, Van Beek TA, Linssen JP, de Groot A, Evstapieva LN. Phytochem Anal 2002; 13; 8-17.
- [6] Nakayama, J, Yamada M. Biochem. Pharmacol 1995; 45; 265-267.
- [7] Polterait O. Org Chem 1997; 1: 415-440.
- [8] Yildirim A, Mavi A, Kara A. J Agric Food Chem 2001; 49: 4083-4089.
- [9] Amin I, Zamaliah M M, Chin W F. Food Chemistry 2004; 87: 581-586.
- [10] Sadique J, AL- Rqobahs WA, Bughait MF and El Gindi AR. Fitoterpia 1989; 60:525-532.
- [11] Mizushima Y and Kobayashi M. J Pharm Pharmacol 1968; 20:69-73.
- [12] Aruoma O I. Journal of the American Oil Chemists' Society 1998; 75: 199-212.
- [13] Chen P N, Chu S C, Chiou H L, Kuo W H, Chiang C L. Cancer letters 2006; 28: 248 -259.
- [14] Delaporte R H, Sanchez G M, Cuellar A C. J Ethnopharmacol 2002; 82: 127-130.
- [15] De las heras Et al. J Ethnopharmacol 1998; 61: 161-166.
- [16] Geronikaki AA, Gavalas AM. Comb Chem High Throughput Screening 2006; 9:425-442.
- [17] Gutteridge JM. Clinical Chem 1995; 41:1819-1828.
- [18] KahkonenM.P et al. J Agric Food Chem 1999; 47, 3954-3962.
- [19] Kris-Etherton PM, Lefevre M, Peecher GR, Gross MD. Annual Rev Nut 2004; 24:511-538.
- [20] Lee H J, Hyun E A, Yoon W J et al. J Ethanopharmacol 1996;103:208-216.
- [21] Lin C C, Huamg P C. Phytother Res 2002; 14:489-494.
- [22] Perry EK, Pickering AB, Wang WW et al. J Pharm Pharmacol 1999; 51:527-534.