



## IN-VITRO ANTI-ARTHRITIC ACTIVITY OF ISOLATED FRACTIONS FROM METHANOLIC EXTRACT OF *ASYSTASIA DALZELLIANA* LEAVES

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### ABSTRACT

Previous phytochemical analysis of methanolic extract of *Asystasia dalzelliana* has indicated the presence of steroid, alkaloid, tannins and flavonoid types of compounds. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to evaluate *Asystasia dalzelliana* for its possible anti-arthritic activity by HRBC membrane stabilization and inhibition of protein denaturation method. Methanolic extract upon the column chromatography yielded five fractions named (AD-01, AD-02, AD-03, AD-04, AD-05) and were screened for their anti-arthritic activity. Among the five fractions tested, AD-3 and AD-4 shown good anti-arthritic activity when compared with standard Diclofenac sodium. The maximum membrane stabilization of AD-3 and AD-4 fraction was found to be at 71.64% and 94.68% (average) respectively. The protein denaturation inhibition of AD-3 and AD-4 fraction was found to be 52.84% and 64.56% respectively. Therefore, our studies support the use of active constituents from *Asystasia dalzelliana* leaves in treating rheumatoid arthritis.

**Key words:** *Asystasia dalzelliana*, Anti-arthritic, HRBC membrane Stabilization, protein denaturation.

### INTRODUCTION

Rheumatoid arthritis is highly inflammatory poly arthritis, often leading to joint destruction, deformity and loss of function which has a worldwide distribution with an estimated prevalence of 1 to 2%. Prevalence increases with age, approaching 5% in women over age 55. The average annual incidence in the United States is about 70 per 100,000. Both incidence and prevalence of rheumatoid arthritis are 2-3 times greater in women than in men<sup>1</sup>.

The management of rheumatoid arthritis (RA) rests on several principles such as drug treatment, which comprises disease-modifying anti rheumatic drugs (DMARDs) and also non-steroidal anti-inflammatory drugs and gluco corticoids (GCs), as well as non-pharmacological measures, such as physical, occupational and psychological therapeutic approaches, together may lead to therapeutic success.

However, the mainstay of RA treatment is the application of DMARDs. It is DMARD treatment, especially, which has undergone dramatic changes during the past decade, providing previously unforeseen therapeutic dimensions. New and highly effective DMARDs have continued to emerge until the most recent years<sup>2</sup>. With the growing interest in herbal therapies among the persons with rheumatoid arthritis, there exists a need for investigation into their safety and efficacy of newer drugs.

*Asystasia dalzelliana* commonly known as violet *Asystasia* (Marathi: Neelkanth) belongs to family acanthaceae. It is a perennial branched herb, about 60-100m length. Quadrangular stem and swollen at nodes. Leaves are opposite, elliptic-oblong acute apex truncate at base; petiole 2cm long<sup>3</sup>.

The whole plant is used in Indian folk medicine as antioxidant, anti-inflammatory, anti venom a novel whose pharmacology yet to be proved<sup>4</sup>. In-vitro methods play an important role to go further for the preclinical studies for any activity, which makes support to the in-vivo studies. The present work was planned to evaluate the effect of isolated fractions on inflammation events such as membrane stabilization, protein denaturation using In-vitro pharmacological models.

### MATERIAL AND METHODS:

#### Plant material

The fresh leaves were collected from Bidar (District of Karnataka state, India) and authenticated by Dr. Shiddamallay Regional Research Institute, Bangalore. The voucher specimen (RRR/BNG/SMP/2009-10/717) was deposited in the same institute.

Prior to use, it was ensured that the leaves were free from contamination, sand and no microbial growth.

#### PREPARATION OF METHANOLIC EXTRACT

Dried leaves of *Asystasia dalzelliana* macerated with methanol (1x4) for 24 hrs at room temperature and filtered. Filtrates were combined, concentrated under vacuum at temperature not more than 70 °C and dried in vacuum drier.

#### Fractionation Isolation and separation

50g of methanolic extract was chromatographed on a silica gel (60-120 mesh) column using gradient elution starting with n-hexane and ending with methanol to obtain 5 fractions. Fractions were concentrated to 1/10th of their volume and kept for crystallization at room temperature for 7 day.

Three out of five fractions showed crystal formation and named as AD-01 (90% hexane in ethyl acetate), AD-02 (60:40 methanol and ethyl acetate), AD-03 ( 50:50 methanol and ethyl acetate) AD-04 (75:25 methanol and ethyl acetate), AD-05 (100% methanol). Further purification was done by re-crystallization. The compounds were confirmed for the there single constituent by running TLC, different solvent methods were used and finally the spots were separated as single in ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.6).

#### Inhibition of Protein Denaturation<sup>5,6</sup>

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml isolated fraction (250 µg/ml of final volume), pH was adjusted to 6.3 using small amount of 1N hydrochloric acid. The samples were incubated at 37 °C for 20 min and then heated at 57° C for 3min.

After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. The absorbances were measured using spectrophotometer at 416 nm. For control tests 0.05 ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated

$$\frac{100 - (\text{Absorbance of test solution} - \text{Absorbance of product control})}{(\text{Absorbance of test control})} \times 100$$

The control represents 100% protein denaturation. The results were compared with Diclofenac sodium (250µg/ml). The percentage inhibition of protein denaturation of different concentration was tabulated in Table.1 & Fig: 1.

### Effect on Membrane Stabilization <sup>7-10</sup>

The principle involved membrane stabilization is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.5 ml of plant extracts of various concentrations (250, 500, 750, 1000 µg/0.5ml), standard drug diclofenac sodium (250, 500, 750, 1000 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. 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-2 & Fig-2. Percentage of HRBC membrane stabilization or protection was calculated using the formula,

$$\text{Percentage Stabilization} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Optical density of Control}} \times 100$$

### RESULTS:

Methanolic extract upon the column chromatography yielded five fractions named (AD-01, AD-02, AD-03, AD-04, AD-05) and were screened for their anti-arthritis activity. Among the five fractions tested AD-3 and AD-4 shown good anti-arthritis activity when compared with standard Diclofenac sodium. The crude methanolic extract of *Asystasia* shown good activity in HRBC membrane stabilization and inhibition of protein denaturation method, which leads to the isolation of compounds which are responsible for the activity. We found the AD-4 fraction (99.04%) was more active than the standard diclofenac sodium (93.71%). AD-3 also showed activity but less than AD-4 and standard. In protein denaturation method the extract, AD-3, AD-4 and standard has shown 69%, 52.84%, 64.56% and 94% respectively at 250 µg concentration. The above results have showed that in both the activities, AD-4 shown good activity than the crude extract, AD-3 and standard.

**Table 1: Effect of extract and isolated compounds *Asystasia dalzelliana* on HRBC membrane stabilization**

Concentration (µg/0.5ml)	Methanolic extract of <i>Asystasia dalzelliana</i>	AD-3 Fraction	AD-4 Fraction	Diclofenac Sodium
250	75%	68.70%	91.71%	83.41%
500	79.64%	72.61%	94.26%	85.42%
750	82.16%	66.29%	98.09%	88.69%
1000	82.19%	78.98%	99.04%	93.71%

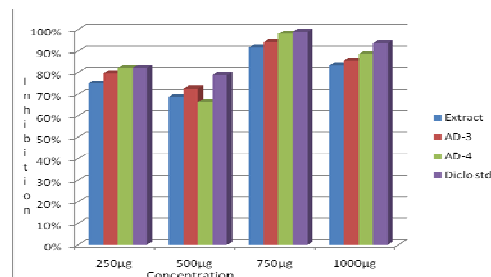
**Table 2: Effect of extract and isolated compounds of *Asystasia dalzelliana* on inhibition of protein denaturation**

Concentration (µg/0.5ml)	Methanolic extract of <i>Asystasia dalzelliana</i>	AD-3 Fraction	AD-4 Fraction	Diclofenac Sodium
250	69%	52.84%	64.56%	94%

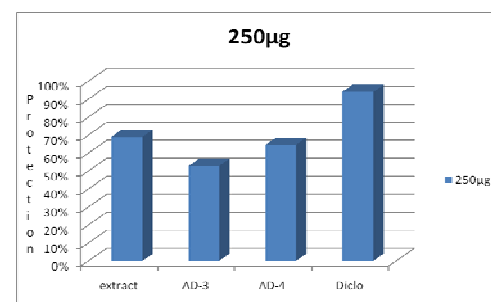
### DISCUSSION AND CONCLUSION

The isolated fractions named AD-01, AD-02 and AD-05 are neglected in result part due to their less activity or no activity. In vitro studies on *Asystasia dalzelliana* demonstrate suppression of both inflammation and arthritis. The methanolic extract of the leaves of *Asystasia dalzelliana* and isolated AD-3, AD-4 fractions must contain some principles, which possess anti-arthritis activities. From the preliminary screening study, it has shown the presence of steroids, alkaloids, tannins and flavanoid type of compounds. Hence attempt was made to compare the methanolic extract and its fraction for

anti-arthritis activities. The present study concludes that AD-4 fraction shows more anti-arthritis activity compared to standard Diclofenac sodium. This made proper attempt to isolate the active principles from *Asystasia dalzelliana* leaves which might help in the findings of new lead compounds in the fields of anti-arthritis drug research. Studies related to active constituents on lipid derived eicosanoids, enzyme expression (COX2, lipoxygenase) and cytokines are necessary to understand the mechanism of action in relation to the observed anti-arthritis activities and characterization of isolated compounds.



**Fig. 1: Percentage inhibition of protein denaturation**



**Fig. 2: Percentage protection or stabilization of HRBC membrane**

### REFERENCE

- Kelly WN, Harris EDJ, Ruddy S, Sledge CB (eds): Textbook of Rheumatology, 5th ed. Philadelphia, WB Saunders, 1997.
- Harris ED Jr: Rheumatoid arthritis. Patho physiology and implications for therapy. N Engl J Med, 1990:322.
- Murthy KKK, Yoganarasimhan SN: flora of Coorg (kodagu) Karnataka India. Vismat Publishers Bagalore.1990:326-7.
- Jain SK: Dictionary of Indian folk medicine and ethnobotany, Deep publications, New Delhi, 1991:311.
- Mizushima Y, Kobayashi M: Interaction of anti-inflammatory drugs with serum proteins especially with some biologically active proteins. J Pharm Pharmacol 1968;20:169-73.
- Aruoma OI: Free radicals, oxidative stress and antioxidants in human health and disease. J. Am.Oil Chem. Soc. 1998;75:199-212.
- Sadique J, Al-Rqobahs WA, Bughaith MF, El-Gindi AR: The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterapia, 1989;60: 525-32.
- Oyedapo, Famurewa AJ: Anti-protease and membrane stabilizing activities of extracts of *Fagra zanthoxiloides*, *Ola subscorpioides* and *Tetrapleura tetraptera*. Int J Pharmacogn 1995;33: 65-9.
- Manivannana R, Sukumar D: The RBC membrane stabilisation in an in vitro method by the drug isolated from *Leucas aspera*. Int. J. Appl. Sci. Eng. 2007; 5(2):133-8.
- Shinde UA, Phadke AS, Nari AM, Mungantiwar AA, Dikshit VJ and Saraf MN: Membrane stabilization activity-a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil. Fitoterapia 1999;70:251-7.