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Evaluation of anti arthritic activity of *Diplazium esculentum* Leaf extract: An *In vitro* study

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Abstract

Arthritic is joint disorder characterized by pain, stiffness, tenderness etc that might make it difficult for a person to move around and/or exercise. Recently herbal medicine has become the main scientifically based system for the treatment of many disorders. The present study is aimed to evaluate the in-vitro anti-arthritic activity of methanolic leaf extract of *Diplazium esculentum* using inhibition of protein denaturation method. The results revealed that the inhibition of protein denaturation by the extract is moderate with respect to the standard group.

Keywords: Anti-arthritic, *Diplazium esculentum*, protein denaturation

Introduction

Rheumatoid arthritis is a common autoimmune disease that is characterized by pain, stiffness, tenderness etc that might make it difficult for a person to move around and/or exercise. Herbal medicines are the plant or plant part that gain lots of importance and has become the main scientifically based system for the treatment of many disorders. Traditionally people use herbal medicines to try to maintain or improve their health. *Diplazium esculentum*, the vegetable fern, is an edible fern. The extract of this plant showed analgesic activity ^[1], hepatoprotective ^[2], anti microbial ^[3], antioxidant ^[4], cytotoxic activity ^[5] etc.

Aim and objectives

The aim of the study is to evaluate the anti arthritic activity potential of the methanolic extract of the leaf of *Diplazium esculentum* using inhibition of protein denaturation method.

Materials and Methods

Collection of plant part

The fresh leaves of *Diplazium esculentum* were collected from the Mohanpur village, Hailakandi dist, Assam.

Drying, extraction and fractionation

The fresh leaves of *Diplazium esculentum* were cleaned, dried and then it was grinded to obtain coarse powder of standard size suitable for extraction.

Evaporation of the Solvent

The extract was obtained by evaporating the filtrate (methanolic) under ceiling. Water bath was used to concentrate the resultant filtrate in the semisolid form. This gummy concentrate was designated as crude extract or methanoli extract and stored in refrigerator until further investigation. The thick concentrate was obtained and stored in a desiccator.

Methods for evaluation of *In-vitro* anti-arthritic activity

The evaluation of *in-vitro* anti-arthritic activity was carried out according to Vollala V. Rajesham *et al.* ^[6] method.

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Diplazium esculentum* extracts (various dilutions). pH was adjusted at 6.3 using a small amount of 1 N HCl. 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.

Turbidity was measured spectrophotometrically at 660 nm. For control tests 0.05 ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The test control represents 100% protein denaturation. The results were compared with the various dilutions diclofenac treated samples. The percentage inhibition of protein denaturation was calculated as follows.

$$\% \text{ of Inhibition} = 100 \times [V_t / V_c - 1]$$

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

Results and Discussion

Anti-arthritis effect of methanolic leaf extract of *Diplazium esculentum* was studied significantly by using in-vitro inhibition of protein denaturation method.

Denaturation of the protein involves the disruption 3D structure of secondary, tertiary and quaternary molecules and finally leads to cell death, it may occurs due to high level of salt, high temperature and high level of acidity.

Many investigators have reported that denaturation of the protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins [7]. The mechanism of denaturation probably involves alteration in electrostatic,

hydrogen, hydrophobic and disulphide bonding [8]. Various anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation [9].

The present study was aimed to evaluate anti arthritic potential of *Diplazium esculentum* leaf on inhibition of protein denaturation. The results revealed that the methanolic extracts of leaves of *Diplazium esculentum* has not that much significant inhibition of protein denaturation in extract treated group and % inhibition of protein denaturation produced by extract at concentrations 500µg/ml is 47.33±1.9% where as standard drug (diclofenac sodium) at concentrations 500µg/ml shown 79.50±1.44. Therefore, it can be proposed that the moderate anti-arthritis activity of methanolic extract of the plant could be due to combined effect of flavonoids, saponins, steroids, tri terpenoids and alkaloids, which are present in the aerial parts of the plant

Table 1: Percentage inhibition of tests and standard

Concentration	% Inhibition	
	BSA + Extract	BSA + Diclofenac
100µg/ml	13.10±0.95	20.25±0.88
200µg/ml	23.23±0.65	28.23±1.78
300µg/ml	37.8±1.07	45.17±0.45
400µg/ml	41.43±0.5	55.36±0.70
500µg/ml	47.33±1.9	79.50±1.44

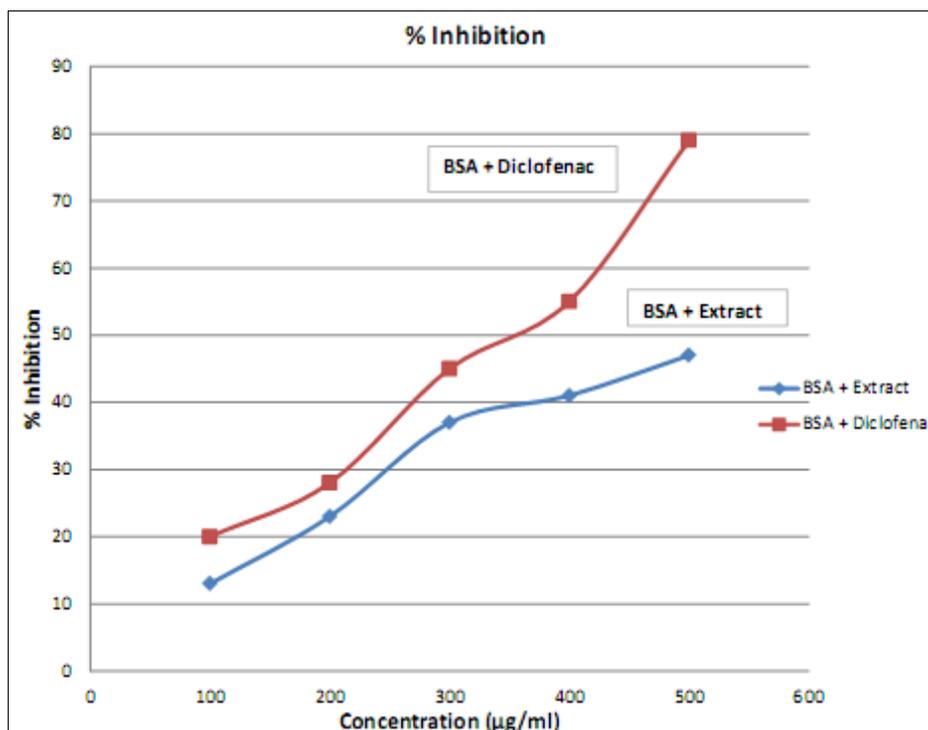


Fig 1: Graph showing percentage inhibition of tests and standard

From the results of present study it can be stated that methanolic extracts of leaves of *Diplazium esculentum* is having moderate antiarthritic activity and it can probably be capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

Conclusion

From the results it is revealed that methanolic extract of *Diplazium esculentum* leaves has moderate anti arthritic activity as compared to standard group. This is only a preliminary study and to make final comment the extract

should be thoroughly investigated phyto chemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

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