

Trypsin inhibiting activity of methanolic extract of aerial parts of *Mikania micrantha*

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ABSTRACT

Objective: To investigate trypsin inhibiting activity of methanolic extract of aerial parts of *Mikania micrantha*. **Methods:** Powdered plant material was extracted with methanol at room temperature and trypsin inhibiting activity of the extract was studied using bovine serum albumin (BSA) as the substrate. **Result:** Percentage inhibition of trypsin activity by the methanolic extract of aerial parts of *Mikania micrantha* were 27.33 ± 0.79 , 39.14 ± 1.02 , 48.48 ± 2.62 , 57.40 ± 3.50 and 70.12 ± 2.19 respectively for 200, 400, 600, 800 and 1000 mg of extract. **Conclusion:** Agents with trypsin inhibiting activity are emerging drugs to treat allergic and inflammatory diseases. Since *Mikania micrantha* exhibited excellent trypsin inhibiting activity the plant is a novel source for the development of newer drugs against allergic and inflammatory diseases in traditional as well as modern medicine.

Key words: *Mikania micrantha*, protease inhibiting activity, trypsin, allergy, inflammation

INTRODUCTION

Proteases catalyse hydrolysis of proteins into aminoacids. They are involved in the regulation of biological events both inside and outside the cell like elimination of damaged proteins, control of protein levels, precursor activation, antigen presentation etc. Thus proteases act as signalling molecules in many processes like embryonic development, cell differentiation, cell death, coagulation, immunity etc [1]. Based on the mechanism of catalysis proteinases can be classified into five groups- aspartate, cysteine, serine, threonine and metallo proteases. Certain proteinases signal to cells by the activation of a family of G protein coupled receptors known as Proteinase activated receptors (PARS) [2]. To date Four PARs have been identified- PAR-1, PAR-2, PAR-3, PAR-4. They are activated enzymatically by serine proteases through proteolysis. PAR-1, PAR-3 and PAR-4-by thrombin and PAR-2- trypsin like serine proteases [3]. Proteases especially those involved in the activation of PARS are attractive targets for therapeutic drug development.

Mikania micrantha commonly known as 'mile a minute' is a perennial creeper belonging to the family Asteraceae. It is native to Central and South America but the plant is now widely distributed in China, India, Southeast Asia etc [4]. The plant is used in traditional medicine in many areas of the world but not much study has been conducted on its medicinal properties. In India people are not aware of the medicinal properties of this plant and it is considered as a weed. In this study we have evaluated trypsin inhibiting activity of methanolic extract of aerial parts of *Mikania micrantha*.

MATERIALS AND METHODS

Plant material

Aerial parts of *Mikania micrantha* were collected from its natural habitat during the flowering season from the district of Kottayam, Kerala state, India. Plant material was washed thoroughly in running water, shade dried at room temperature and powdered in a kitchen blender.

Solvent extraction

Powdered plant material was extracted with methanol at room temperature in an orbital shaker for seven days. The extract was filtered using Whatman number 1 filter paper, evaporated to dryness using rotary evaporator and kept in sterile bottles at 4°C in a refrigerator until use.

Trypsin inhibiting activity

The assay was performed according to modified method of Alam *et al.*, 2011[5]. Bovine serum albumin (BSA) was used as the substrate for trypsin. If the test sample is having trypsin inhibiting activity, hydrolysis of albumin will not take place in its presence. The activity was tested for 200-1000µg dry weight extract /ml of medium. The reaction mixture contained 100 µg of trypsin and different concentrations of extract made up to 1ml with 50 mM Tris – HCl buffer (pH 7.8) containing 1mM calcium chloride. The mixture was incubated at room temperature for 10 minutes. Then added one ml BSA (4%) to all tubes and digestion was carried out at

37°C in a water bath for 20 minutes. Three ml of 5 % TCA (Trichloro acetic acid) was added to stop the reaction, centrifuged at 2500 rpm and the absorbance of the supernatant was read at 280 nm. Appropriate controls and blanks were also run.

Trypsin cleaves peptides on the C-terminal side of lysine and arginine amino acid residues. On adding TCA, unhydrolysed BSA gets precipitated and the peptide fragments formed by cleavage remain in the supernatant whose absorbance is read at 280 nm. The percentage of inhibition was calculated as,

$$\frac{(\text{absorbance of control} - \text{absorbance of test}) \times 100}{\text{absorbance of control}}$$

Control and tests were done in triplicate.

Statistical analysis

Results are expressed as mean \pm SD, where n=3 (Calculated using Microsoft Office Excel 2007).

RESULT AND DISCUSSION

Percentage inhibition of trypsin activity by the methanolic extract of aerial parts of *Mikania micrantha* were 27.33 \pm 0.79, 39.14 \pm 1.02, 48.48 \pm 2.62, 57.40 \pm 3.50 and 70.12 \pm 2.19 respectively for 200,400, 600, 800 and 1000 mg of extract (Figure 1). In addition to the traditional role of protein degradation during digestion proteinaes are now known as signalling molecules in a number of physiological and pathophysiological processes. Among the different classes of proteinases serine protease mainly mediate cell signalling through PAR family of receptors. Among the four PARs identified PAR-1, PAR-3 and PAR-4 are activated by thrombin and PAR-2- trypsin like serine proteases [3]. PAR-2 is a prominent member of the group and is widely distributed throughout human body such as skin, sensory neurons, inflammatory cells such as macrophages, neutrophils, monocytes, mast cells etc. Emerging evidence indicates that PAR-2 plays important role in various physiological and pathophysiological processes in different organ systems including cardiovascular system, the pulmonary system, gastrointestinal tract, skin etc [6]. Acute activation of PAR-2 helps in normal physiological processes but hyperactivation is deleterious and leads to inflammation. Studies have shown that PAR-2 plays important role in inflammation and pain sensation [3]. Additionally PAR-2 is an important sensor for exogenous proteases from various allergens. Since PAR-2 is activated by proteinase, proteinase inhibitors are emerging drugs to treat inflammatory and allergic diseases. Methanolic extract of *Mikania micrantha* exhibited excellent trypsin inhibiting activity. Hence the plant is an authentic source for the development of new drugs against inflammatory and allergic diseases in traditional as well as modern medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

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REFERENCES

- [1] I. Richard. The genetic and molecular bases of monogenic disorders affecting proteolytic systems. J Med Genet., 2005, 42: 529-539.
- [2] R. Ramachandran, M. D. Hollenberg. Proteinases and signalling: pathophysiological and therapeutic implications via PARs and more. Br J Pharmacol., 2008, 153: S263-S282.
- [3] S. R. Macfarlane, M. J. Seatter, T Kanke, G. D Hunter, R Plevin. Proteinase- Activated Receptors. Pharmacol Rev. 2001, 53: 245-282.
- [4] M. C. Perez-Amador, V. M. Ocotero, R. I. Balcazar, F. G. Jimenez. Phytochemical and pharmacological studies on Mikania micrantha H.B.K. PHYTON-International Journal of Experimental Botany., 2010, 79: 77-80.
- [5] MB Alam, MS Hossain, ME Haque. Antioxidant and anti-inflammatory activities of the leaf extract of Brassica nigra. Int j pharm sci res., 2011, 2(2): 303-310.
- [6] A. Coelho, V. Ossovskaya, N. W. Bunnett. Proteinase_Activated Receptor-2: Physiological and Pathophysiological roles. Curr.Med.Chem. – Cardiovascular & Hematological Agents., 2003, 1: 61-72.

Figure Legends

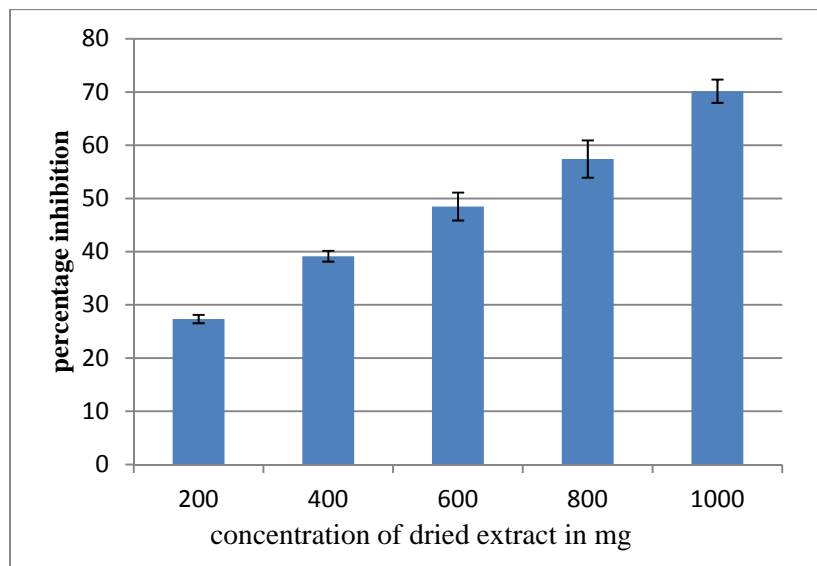


Figure 1: Trypsin inhibiting activity of methanolic extract of aerial parts of *Mikania micrantha*. Values are the mean±SD (n=3)

Excel Table

A						
200	27.33					
400	39.14	0.79	1.02	2.62	3.5	2.19
600	48.48					
800	57.4					
1000	70.15					