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Antiproteinase and Antibacterial Activity of *Pueraria phaseoloides* Parts Collected from the Ovitrap with Mosquito Larvae

P. Blessy [a++*] and E. Grace Marin [a#]

[a] *Department of Zoology, Scott Christian College (A), Nagercoil, Affiliated with Manonmaniam Sundaranar University, Tirunelveli, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The globe is progressively turned to find herbal products which are known to be effectual against a wide range of mosquito borne diseases. The present study was aimed to assess the antiproteinase and antibacterial activity of *Pueraria phaseoloides* plant parts collected from the Ovitrap with mosquito larvae. The antiproteinases are proteins that inhibit protease and modulate immune responses of a living organism. The antiproteinase activity of 6.29 to 92.55 was observed. The *Staphylococcus aureus* showed minimum inhibition zone of5.1±0.00mm to 37±0.10mm in ethanol extract tested against *Bacillus subtilis.* The obtained data revealed the anti-inflammatory and antibacterial properties of the sample rich in pharmacological worth.

[++]*Research Scholar;*
[#]*Assistant Professor;*
**Corresponding author: Email: rmsuja.83@gmail.com;*

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Keywords: Mosquito; larvae; Ovitrap; Neustanthus phaseoloides; antiproteinase and inhibition.

1. INTRODUCTION

"Mosquitoes are the most important vector of human disease in the tropic and are notoriously responsible for causing human health aliments" (Qureshi *et al*., 2017). "The repellents manufactured from herbals are not known to cause any notable deprecating effects and they are available in shops with good affordable prices too. The plant remedies are effective but they have to be selected properly and taken under proper medical supervision. The scientific assessment of preliminary studies are essentialto assess the effect of the sample either it is creditable as a bio-repellent or medicine. Tropical kudzu is a monotypic genus of flowering plants bear vigorous twining and climbing nature is a perennial legume deeply rooted to 2 m depth bear hairy, slender stems may be 6-20 m long. The stem portions grown into root from the nodes were developing into many branches" (FAO, 2015; Cook *et al*., 2005; Halim, 1997). "In the Tropical area the forage and cover crops of deep rooting systemare used for nitrogen accumulation and improvement of the soil structure" (Guanglong *et al*., 2000).

The inflammation is a bodily response to injury and infection. The effect of antiproteinase inhibitors was studied on the proteolytic activity of crude extracts of mosquito *Aedesaegypti* (L.). Yeates and Prasitenkd, (1980) stated that the antiproteinase activity are partially inhibited by lima bean trypsin inhibitor or by EDTA. The earlier experiments showed that the mixed inhibitor effects are used to indicate the trypsin like enzyme. In LBTI resistant serine proteinase can be inhibited only by ovomucoid inhibitor is a newly described proteinase inactive against several model substrates developed for the mammalian enzymes and they are partially separable by ion-exchange chromatography techniques. "The crude plant extracts and phytochemicals of known antimicrobial properties of great significance in the therapeutic treatments. The screening of plant products for antimicrobial activity have shown that the higher plants represent a potential source of novel antibiotic prototypes There has been an increasing prevalence of multiple resistances in human pathogenic microorganisms was largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced the scientists to search for new antimicrobial substances from various natural resources" (Wu *et al*., 1999).

2. MATERIALS AND METHODS

The antiproteinase test was carried out according to the modified method of Oyedepo and Femurewa *et al*., (1999) and Sakat *et al*., (2010). The reaction mixture (2 ml) containing 1 ml of mM Tris HCl buffer (pH 7.4), 0.06 mg trypsin and 1 ml test sample and standard Aspirin of different concentrations (10-100µg/ml) was added. The mixture was kept warm at 37^0C for 5 minutes. To this 1ml of 0.8% (w/v) casein was added. The mixtures were kept warm for 20 minute then add 2ml of 70% chloric acid to arrest the reaction. The reaction was followed by the cloudy suspension was centrifuged. Then the absorbance of the supernatant was read at 210 nm against the buffer as blank. The experiment was repeated thrice and the percentage inhibition of proteinase inhibitory activity was computed as follows.

> Percentage inhibition = (Abs control –Abs sample) x 1000/Abs control

Antibacterial activity was carried out by agar disc diffusion method. To 0.1ml of 24 hrs old bacterial culture was spread in Muller -Hinton agar plate by spread plate technique. The sterile disc containing respective solvent extract was placed on the surface of the medium and the antibiotic disc was used as positive control. The plates were incubated at 37◦ C for 24hrs. The plates were observed after 24 hrs for zone of inhibition. The bacterial strains collected from the scientific laboratory namely *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Klebsiella pneumonia* are scientifically assessed and the zone of inhibition was measured and tabulated as per the standard protocol (Bauer *et al*., 1966)

3. RESULTS AND DISCUSSION

The earlier studies on antiproteinase activity were reported by Saikat Sen *et al*., (2015) on *in vitro* anti-inflammatory activity of methanol and butanol extract of *A. caudatus* L. They stated that the plant materials are capable to stabilize lysozomal membranes an important step to limit inflammatory mediators responsible for inflammation through membrane stabilizing effect. In the present study anti-proteinase activity of *Neustanthus phaseolodies* leaf showed

a minimum inhibition of 7.02 at 10 µg/ml to 80.72 at 100µg/ml; stem portion of 6.46 at 10µg/ml to 82.50 at100µg/ml;flower parts of 6.22 at 10µg/ml to 84.64 at 100µg/ml;seed of 17.01 at 10µg/ml to 88.31 at 100µg/ml;pod of 12.76 at 10 µg/ml to 92.55 at 100µg/ml and root of 6.29 at 10µg/ml to 9.37 at100 µg/ml was noticed which highlight the antiproteinase effect of the tested samples. The earlier studies on flavonoid derivatives have shown a wide range of antibacterial, antiviral, anti-inflammatory, anticancer and anti-allergic activities (Carlo *et al.,* 1999) and the antimicrobial activity of essential oil tested against two bacteria *Enterococcus faecalis* and *Staphylococcus aureus* using microdilution broth susceptibility assay(Dai *et al*., 2015). In the present study antibacterial activity of *Pueraria phaseoloides* pod tested against *E.coli* showed a minimum inhibition zone of 11±0.36mm in acetone extract to 27±0.72mm in aqueous extract; chloroform extract of 14±0.84mm to 34±0.24mm in methanol extract tested against *Klebsiella pneumonia;* ethanol extract of5.1±0.00mm to 18.3±0.41mm in methanol extract tested against *Staphylococcus aureus* and aqueous extract of 27.4±0.72mm to 37±0.10mm in ethanol extract tested against *Bacillus subtilis.*

4. CONCLUSION

The present study demonstrate that the antiproteinase activity of pod and antibacterial activity of aqueous extract of pod revealed that further research should be done to elucidate to discover an effective herbal pharmacological agent or herbal bio-repellent.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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