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PROTEASE ACTIVITY OF FLORAL EXTRACTS OF *CENTRATHERUM PUNCTATUM* CASS. A WOUND HEALING HERB

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ABSTRACT

Flowers of ***Centratherum punctatum* Cass**, are used in traditional medicine for quick wound healing. Protease, a potential aspirant in wound healing is not so for studied in *C.* ***punctatum* Cass** flowers. So an attempt was made to resolve the protease activity of floral extracts of ***C. punctatum* Cass**. Buffers of different pH range were used for extraction of the flowers to identify the best buffer for extraction of protease. Total protein content and protease activity were determined in the floral extracts. Floral extract showed higher protease activity when the extraction was carried out at pH 4.0. The results of the present study indicate that protease activity of the flower may be responsible for wound healing property of the flowers of ***C.punctatum* Cass**.

KEYWORD: *Centratherum punctatum* Cass, Wound Healing, Floral Extract, Protease.

INTRODUCTION

C.punctatum belongs to the family Asteraceae. Although cultivated in subtropical and warm temperate regions for its ornamental qualities, *C.punctatum* is valued as a medicinal plant as it is effective against number of ailments. The acetone extract of *Centratherum punctatum* Cass. exhibited the antimicrobial activity against a number of bacterial and fungal species.[1]

Besides they also depicted antioxidant and antiproliferative capabilities. Its purple flower essence is being used in the preparation of many herbal medicines. The plant extract was proved to possess some antimicrobial effects.[2] It is used in hair oil preparations and as skin whitening and antiageing agents. The cytotoxic and antioxidant potentials of ethanol extract of aerial parts of *Centratherum punctatum* Cass. (Family-Compositae were reported by.[3] Nutraceutical potentials of this drug source *Centratherum punctatum* Cass. was also evaluated by.[4] Traditionally it is used to increase libido, used as pain killer, used as an antidote for snake bite and tiger bite.[5] Protease, one of the key enzyme in wound healing is not so for been reported in flowers of *C. punctatum*. Proteases are effective in removing damaged and infected tissues from wounds and thus play an important role in the wound healing process. These enzymes may be used to debride both adherent slough and eschar. Enzymatic debridement is a primary technique in certain cases when surgical debridement is not feasible.[6] Proteases from various sources such as plant, microbes, maggots and animals were found to be useful in wound debridement.[7] Proteases occur naturally in all living organisms. Growth and development in all organisms occur as a result of an overall balance between protein synthesis and proteolysis. Commercially, proteolytic enzymes from the plant sources have received special attention because of their broad substrate specificity as well as activity in wide range of pH and temperature. As there is no report on the study of protease in *Centratherum*, an attempt was made to study proteases in floral extracts of *C.punctatum* which may play a role in wound healing property of the plant.

2. MATERIALS AND METHODS

Flowers of *C.punctatum* were collected and stored at -20° C immediately after weighing. The whole flowers were used as sample based on the experiment. The total protein content and protease activity were detected in the whole flowers with buffers of different pH range. Citrate phosphate buffer (50 mM) was used for the pH range- 4, 5 and 6 and sodium phosphate buffer (50 mM) was used for the pH range 7 and 8. The best extraction buffer was selected to determine the total protein content and protease activity in the flowers.

2.1 Extraction of Proteins from Flowers

Frozen samples were extracted with buffers of different pH range containing 0.01% (w/v) ascorbic acid. The buffers were used in the ratio 1:5 for extraction. The extracts were filtered through cheese cloth and centrifuged at 20, 000 × g for 15 min at 4°C. The clear supernatant was used as the protein and enzyme source.

2.2 Determination of Total Protein Content

The protein content of the floral extracts was determined by the dye binding method[8] with Bovine serum albumin fraction V (Sigma chemical Co., USA) as a standard. To 0.1 ml of enzyme solution, 0.9 ml of distilled water was added and to which 2 ml of Coomassie Brilliant Blue G reagent was added, mixed well and immediately read at 595 nm in a spectrophotometer. Protein content of the unknown sample was calculated from the standard graph. Respective buffer along with the reagent was used as blank.

2.3 Protease Assay

Protease assay was carried out following the procedure of Mc Donald and Chen.[9] The reaction mixture contained a known amount of protein in 0.35ml of buffer (50 mM, pH 5.0) and 0.35 ml of 0.5% (w/v) casein. The mixture was incubated at 37°C for one hr and the reaction was stopped by adding 0.7 ml of 10% (w/v) ice cold TCA. The undigested substrate was removed by centrifugation in a microfuge for 5 min at 10, 000×g. An aliquot (1 ml) of the supernatant was taken and to which 2.5 ml of the reagent (2.9% Na_2CO_3 and 0.3 N NaOH) and 0.75 ml of Folin Ciocalteu's phenol reagent (1:3 diluted with distilled water) were added. The samples were incubated at 37°C for 20 min and the liberation of tyrosine was read at 650 nm using a spectrophotometer. One unit of protease activity was defined as the amount of enzyme liberating one micromole of tyrosine equivalent under the assay conditions.

3. RESULTS AND DISCUSSION

3.1 Protein Content of Floral Extracts

Total protein content of the flowers of *C.punctatum* showed variation in response to different pH of the buffer that was used for extraction. Increase in protein content was observed with increase in pH of the buffer.

Maximum protein content was observed with pH 7.0. Further increase in pH showed decline in total protein extraction **(Fig 1).**

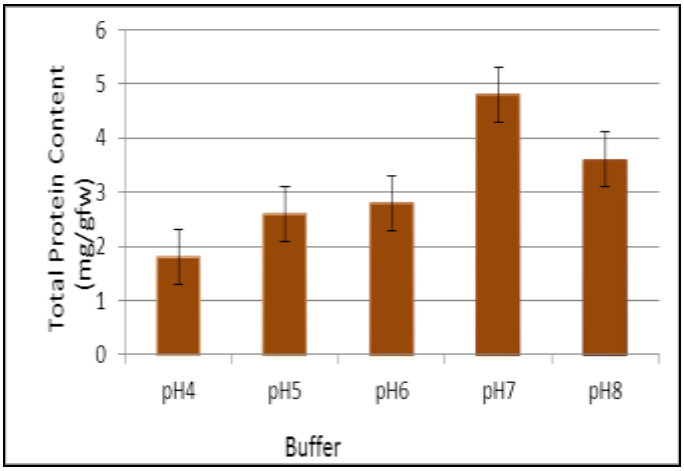

Fig 1: Protein activity of flowers of *C.punctatum*.

3.2 Protease Activity of Floral Extracts

Maximum protease activity/gfw was obtained when the buffer for extraction was citrate phosphate buffer (50 mM), pH 4.0 which was followed by extraction with citrate phosphate buffer (50 mM), pH 6.0 **(Fig 2).**

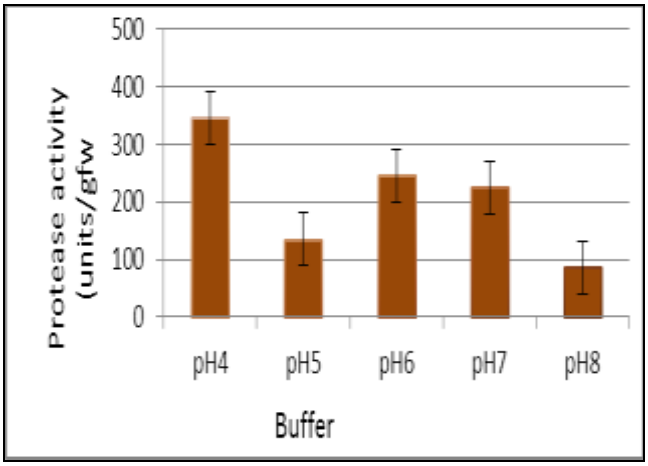

Fig 2: Protease activity of flowers of *C.punctatum*

Present study indicates that the presence of protease in the flowers of *C.punctatum* may also be responsible for the wound healing property of the plant. Studies on proteinase fraction from *Carica candamarcensis*[10] shows that proteinase can act as cell growth stimulators, as angiogenic promoters and possibly as debriding agents, thus fulfilling essential conditions for wound healing.

4. CONCLUSION

The present study helps to identify the level of protease activity in flowers of *C.punctatum*. As protease activity was more in the floral extracts this may be responsible for the wound healing property of the floral extract. However the isolation and purification of proteases

from this plant and *in vitro* and *in vivo* testing of the enzyme on wounds will help us understand the wound healing potential of the herb.

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