ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2015; 1(9): 751-753
www.allresearchjournal.com
Received: 02-06-2015
Accepted: 06-07-2015

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Study on trypsin inhibitory activity by cassia tora seeds

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Abstract
The inhibitors may potentially be used for multiple therapeutic application in viral, bacterial, fungal disease and physiological disorder. In traditional Indian medicine system cassia tora is reportedly effective in treatment of skin and gastrointestinal disorder. The present work describe the isolation extraction purification characterization of trypsin inhibitor from cassia tora seeds. Purification of cassia torra seeds were done by ammonium sulphate precipitation and dialysis. Assay of trypsin inhibitors was performed by caseinolytic method and protein was estimated by Folin Lowry method.

Keyword: Cassia tora, ammonium sulphate, trypsin

1. Introduction
Cassia tora is a wild crop and grow in most part of the India as weed. According to ayurveda the leaves and the seeds are acrid, laxative, antiperiodic anthelmintic, liver tonic, cardio tonic and expectorant (Ahemad *et al.,* 1998) [1]. The leaves and seeds are useful in leprosy, ringworm, flatulence colic dyspepsia constipation cough bronchitis and cardiac disorder (Chain and Peria, 2001) [3] chemical components of cassia tora are anthraquinones chrysophanol emodin obtusifolin obtusin chryso-obtusin auronto-obtusin and their glycosides.
Seeds contains neptho-alfa-pyronetoralactone, chrysophanol physcon, emodin, rubrofusarin, chrysophonic acid -9-anthrone. emodin tricotan stigmasterol-β-sitosterol–β-D-glucoside, ferindlen, palmitic, steric, succinic and tartaric acid, uridine, quetcitrin, isoquetcitrin are isolated from seeds (Davis, 1994 and Desta, 1993) [5, 7] antibacterial, antiplatelet aggregation, hepatoprotective, campphosphodiesterases inhibitory activity antifungal, antieast, anti-inflammatory and antieterogenic, hypolipidemic, antimutagenic, and antioxidant activities have been evaluated (Devi *et al.,* 1994 [8]; Duke and Beckstrome 2002 [13] and Karaman *et al.,* 2003).
Literature survey revealed that the plant extract has yet not been screened for its traditional claim of protease inhibitory activity. Therefore the objective of this work was to explore the protease inhibitory activity of cassia tora seeds.
Trypsin play an important role in virulence of many human, plant and insect pathogens. The trypsin inhibitors of plant origin have been reported widely from many plant species. The inhibitors may potentially be used for multiple therapeutic applications in viral, bacterial, fungal diseases and physiological disorders. In traditional Indian medicine system, *Cassia tora* (Senna tora) is reportedly effective in treatment of skin and gastrointestinal disorders.
Trypsin constitute one of the largest functional group of proteins involved in many normal and pathological processes. Trypsin inhibition of pathogenic organisms may aid in control of several diseases (Supuran *et al.,* 2001).
The plant trypsin inhibitors are generally small proteins, which regulate significant physiological processes, and are also induced upon attack by insects or pathogens (Ryan C. A, 1990) [11].
Majority of trypsin inhibitors studied in plant kingdom originate from three main families namely leguminosae, solanaceae and gramineae (Richardson M. J., 1991) [10].

2. Material and Methods

The *Cassia tora* seeds were collected from fields of AKOLA region (MAHARASHTRA) during December 2014 to February 2015. The plant seeds were identified as *Cassia tora*.

2.2. Extraction of cassia tora seeds

Seeds were washed with tap water, shade dried powder in a kitchen blender and were stored in a air tight plastic bags. Then 30 gm of *Cassia tora* seed powder was homogenized with 300 ml of 0.1 M phosphate buffer (pH 7.6), stirred for 20 minutes and centrifuged at 12,000 g for 20 min at 4 °C. To the supernatant (185 ml), 72.2 g of ammonium sulphate was added.

After 18 h standing at 4 °C, the precipitate was collected by centrifugation at 12,000 g for 20 min, dissolved in 0.02 M phosphate buffer (pH 7.6), dialyzed against 5 litres of the same buffer for 18 h. This fraction (60 ml) was used for further studies.

2.3. Dialysis method

The sample were then dialysed by using dialysis membrane having pore 3.2u.

2.4. Enzyme assay

The trypsin activity was assayed by the casein digestion method. One ml of enzyme was incubated with 3.0 ml of 1% (w/v) casein (prepared in 100 mM Tris-HCl buffer; pH 8.0) at 37 ± 1 °C, and after 10 min, the reaction was stopped by addition of 3.0 ml of 10% (w/v) trichloroacetic acid (TCA). The mixture was centrifuged at 16,000 g for 10 min, and absorbance of supernatant was taken at 660 nm. Soyabean inhibitor was used as standard.

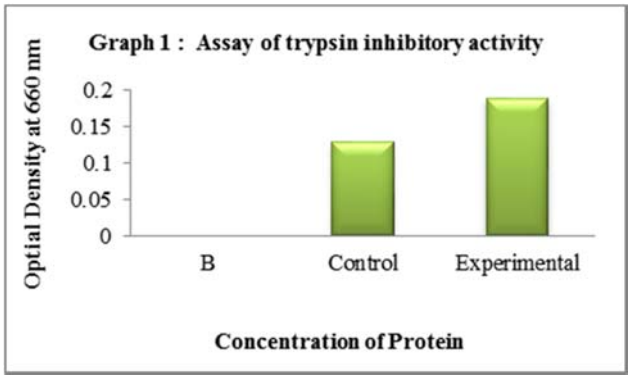
Protein estimation

This method is 10 time more sensitive than biuret method. The reagent is folin ciocalteu (phenol) is quite complex and contain phosphomolybdic acid and tungstate. The aromatic amino acid tyrosin and tryptophan present in protein react with dark blue colour complex. The colour formation is due to the presence of phosphomolybdic acid with tryptophan and tyrosine present in the protein reaction of alkaline copper with protein.

3. Results and Discussion

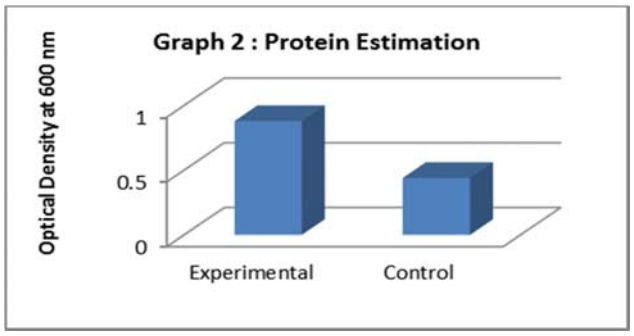
3.1. Enzyme assay

The concentration of protein in cassia tora extract found to be more in experimental than control i.e. 0.13 and 0.18 respectively. Because control contains inhibitor while experimental free from inhibitors.



3.2. Protein estimation

The concentration of protein in cassia tora extract found to be more in experimental than control due to presence of inhibitor.



4. Conclusion

The results revealed an unreported trypsin inhibitory activity in *Cassia tora* seeds. Traditionally, the plant is recommended in skin and gastrointestinal disorders. Inhibition of trypsin, bacterial and fungal proteases is indicative of its possible mechanism for varied therapeutic applications. The denaturing and activity gel electrophoresis revealed common protein(s) present in seed responsible for anti-tryptic activity. The *Cassia tora* seed extract moderately inhibited the spore germination of *Aspergillus flavus*. This can also be effectively used to bolster the plant defense response by employing biotechnological techniques in crop protection.

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