



Research article

In vitro screening of peptidase inhibitory activity in some plants of North India



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ARTICLE INFO

Keywords:

Peptidase inhibitor
 Screening
 Trypsin inhibitory activity
 Chymotrypsin inhibitory activity
 North India
 Food science
 Agricultural science
 Environmental science

ABSTRACT

In the present study, trypsin and chymotrypsin inhibitory activity of some plants of different families was evaluated. A total of 55 plants were screened, out of which six showed the maximum trypsin inhibitory activity namely *Acacia concinna*, *Caesalpinia bonducella*, *Lathyrus sativus*, *Mucuna pruriens*, *Psoralea corylifolia* and *Sapindus mukorossi*. Results suggested that the plants showing trypsin inhibitory activity (TIA) also have chymotrypsin inhibitory activity (CIA). Both trypsin and chymotrypsin inhibitory activities were high in seeds compared to leaves followed by flowers. It was also observed that TIA was maximally present in Sapindaceae family whereas CIA was maximum in fabaceae family followed by others.

1. Introduction

The Peptidase inhibitors (PIs) are regulatory proteins that occur in seeds, leaves, flowers and tubers (Mayasa et al., 2016). On the basis of active amino acid present in their reactive site, these are distributed in different families like serine, cysteine, aspartic and metallocarboxy peptidase inhibitors (Pesoti et al., 2015). Generally plant serine PIs are grouped into Kunitz, Kazal, *Streptomyces* subtilisin inhibitor, Soybean trypsin and proteinase inhibitor, Potato I inhibitor, Potato II inhibitor, *Ascaris* trypsin inhibitor and other (Laskowski and Kato, 1980). PIs are specific in nature as they inhibit only peptidases leaving other proteins unaffected. Studies have shown that plant peptidase inhibitors are more beneficial over chemical PIs as they are safer and more specific in their action (Shamsi et al., 2016).

PIs serve various functions viz. signal initiation mediator, cellular event's transmission and termination processes like apoptosis, inflammatory response, blood coagulation, hormone response pathway etc (Gomes et al., 2011). They regulate the development of insects, agricultural pests, plant and animal health (Shamsi et al., 2016). PIs accumulate in host plants in response to invading pathogens and thus have a defensive role to play in plants (Salzman et al., 2005). They affect the growth and development of insects which feed on various crops. Various peptidase inhibitors have been purified

from plants. In the present study the trypsin and chymotrypsin inhibitory activity of plants belonging to different families of North India was screened. The knowledge gained from this study can be explored further for identifying the plants with anti-insect potential, isolation and characterization of peptidase inhibitors peptidase .

2. Material and methods

2.1. Collection of plant material

Leaves, seeds and flowers of the plants were taken according to their availability from the different states of North India. The plant material was identified from the Department of Botanical and Environmental Sciences, G.N.D.U., Amritsar and Herbal Health Research Consortium Pvt. Ltd., Amritsar, Punjab, India.

2.2. Preparation of extract

Collected plant material (seeds, leaves, flowers) was washed with distilled water and then treated with mercuric chloride to remove any bacterial or fungal contamination. The treated plant parts were then washed with distilled water, air dried and crushed in liquid nitrogen until finely grounded. The fine powder was dissolved in distilled water (1:5 w/

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v) and stirred for 4 h on magnetic stirrer at 4 °C. The suspension was then centrifuged at 8000 rpm for 30 min at 4 °C and supernatant was collected. The supernatant was termed as Crude Extract (CE) and stored at -20 °C till further use.

2.3. Trypsin and chymotrypsin inhibitory assay

Trypsin inhibitory activity was measured by following the protocol of Vasudev and Sohal (2016). Trypsin and chymotrypsin inhibitory activity was measured using BAPNA (*N*-benzoyl DL-arginine *p*-nitroanilide) (prepared in 0.1mM DMSO) and casein (1%) respectively, as substrate. Twenty microlitres of enzyme (trypsin/chymotrypsin) was pre-incubated with 50µl of buffer (0.05M Tris HCl, pH - 8.2) and 30µl of inhibitor at 37 °C for 10 min. Then 100µl of substrate was added and the absorbance change was continuously monitored for 10 min at 410 nm at an interval of 1 min. The calibration curve was constructed using different concentrations of *p*-nitrophenol for expressing trypsin and chymotrypsin activity as µmol/min/mg protein. Formula used for calculation of inhibitory activity is given as:

$$\text{Inhibitory activity} = [1 - (\text{Absorbance of Sample} / \text{Absorbance of Control})] \times 100$$

2.4. Statistical analysis

All results were reported as means with the standard deviation. For each set of results, Analysis of variance (ANOVA) was applied using SPSS software followed by the Tukey's test.

3. Results

The presence or absence of trypsin and chymotrypsin inhibitory activity in the extract of 55 different plants is listed in Table 1.

3.1. Bignoneaceae family

In Bignoneaceae family five plants were evaluated for trypsin inhibitory activity (TIA) and chymotrypsin inhibitory activity (CIA), out of which leaves of *Kigelia pinnata* and seeds of *Tecoma argentina* and *Tecoma stans* gave ~10% TIA and CIA. However, seeds of *Tecoma grandiflora* gave 7.92 ± 0.95% and 1.02 ± 1.69% and leaves of *T. stans* showed 6.24 ± 0.05% and 3.62 ± 0.55% of TIA and CIA, respectively (Table S1).

3.2. Combretaceae family

In Combretaceae family, *Terminalia arjuna* and *Terminalia chebula* showed neither trypsin nor chymotrypsin inhibitory activity.

3.3. Euphorbiaceae family

In Euphorbiaceae family, 32.16 ± 2.58% of TIA and 26.63 ± 1.04% of CIA was observed in the seeds of *Phyllanthus embilica*, whereas seeds of *Ricinus communis* showed 16.7 ± 0.58% of TIA and 10.26 ± 1.02% of CIA. None of the inhibitory activities were present in the leaves of *Jatropha curcas*.

3.4. Fabaceae family

Out of 31 plants of Fabaceae family, leaves of 19 and seeds of 18 plants were screened for their inhibitory activity. The maximum inhibitory activity was observed in leaves of the plants namely; *Bauhinia variegata* (with 42.44 ± 1.25% TIA & 40.26 ± 0.25% CIA), *Bauhinia alba* (42.44 ± 1.36% TIA & 38.25 ± 1.02% CIA), *Bauhinia acuminata* (40.62 ± 0.95% TIA & 28.98 ± 0.002% CIA), *Caesalpinia pulcherrima* (35.32 ± 0.69% TIA & 36.75 ± 1.02% CIA), *Butea monosperma* (32.02 ± 1.85% TIA & 29.63 ± 0.25% CIA) and *Cassia glauca* (28.44 ± 1.02% TIA & 25.69 ± 1.02% CIA).

The proteinaceous extract of leaves of some plants gave moderate peptidase inhibitory effect like *Acacia auriculiformis* (24.00 ± 0.25% TIA & 10.36 ± 1.32% CIA), *Cassia biflora* (19.16 ± 0.36% TIA & 10.24 ± 0.78% CIA), *Cassia fistula* (21.61 ± 0.14% TIA & 19.86 ± 0.63% CIA), *Cassia occidentalis* (having 21.26 ± 1.25% of TIA & 29.36 ± 0.52% of CIA) and *Delonix regia* (24.23 ± 0.25% TIA & 20.69 ± 1.02% CIA). However, very little inhibitory effect was noticed from leaves of *Acacia nilotica* (4.15 ± 1.52% CIA & 0.78 ± 0.25% TIA), *Bauhinia tomentosa* (6.16 ± 2.01% TIA & 1.26 ± 1.52% CIA), *Cassia siamea* (6.24 ± 0.69% of TIA & 10.20 ± 1.58% of CIA), *Pongamia glabra* (0.95 ± 3.02% CIA & 0.12 ± 0.95% TIA) and *Prosopis juliflora* (2.89 ± 1.05% CIA & 0.95 ± 0.69% TIA).

The maximum inhibitory activity observed in proteinaceous extract prepared from seeds of plants were *Acacia concinna* (47.29 ± 0.85% TIA & 40.36 ± 1.69% CIA), *Abrus precatorius* (39.90 ± 2.26% TIA & 20.45 ± 1.05% CIA), *Caesalpinia bonducella* (44.00 ± 1.25% TIA & 45.89 ± 0.95% CIA), *Enterolobium consortium* (39.65 ± 1.02% TIA & 30.65 ± 0.69% CIA), *Lathyrus sativus* (45.00 ± 1.11% TIA & 42.36 ± 1.52% CIA), *Mucuna pruriens* (48.01 ± 0.23% TIA & 39.89 ± 1.25% CIA), *Psoralea corylifolia* (46.78 ± 0.95% of TIA & 42.26 ± 0.32% of CIA) and *Tamarindus indica* (37.00 ± 0.26% TIA & 35.26 ± 0.14% CIA).

Other plants with comparatively lesser inhibitory effect were *Cassia absus* (9.72 ± 0.14% TIA & 5.26 ± 0.63% CIA), *Phaseolus vulgaris* (12.92 ± 1.03% TIA & 10.29 ± 1.14% CIA) and *Pongamia pinnata* (8.92 ± 0.05% of TIA & 12.36 ± 0.87% CIA).

3.5. Laminiaceae family

In Laminiaceae family, *Vitex negundo* seeds did not show any activity.

3.6. Malvaceae family

In Malvaceae family, flowers and leaves of *Bombax ceiba* showed neither trypsin nor chymotrypsin inhibitory activity whereas, *Pterospermum acerifolium* leaves (2.64 ± 1.02% TIA, 1.32 ± 0.45% CIA) showed both inhibitory activities.

3.7. Putranjivaceae family

The seeds of *Putranjiva roxburghii* from Putranjivaceae family showed both trypsin and chymotrypsin inhibitory activities (29.64 ± 0.25% TIA, 29.63 ± 0.31% CIA).

3.8. Phytolaccaceae and Rhamnaceae family

The seeds of *Rivina humilis* from Phytolaccaceae and *Ziziphus jujube* from Rhamnaceae family showed neither trypsin nor chymotrypsin inhibitory activity.

3.9. Rutaceae family

The seeds and leaves of *Aegle marmelos* and leaves of *Citrus medica*, *Murraya exotica*, *Murraya koengi* from Rutaceae family showed neither trypsin nor chymotrypsin inhibitory activity.

3.10. Sapoteaceae, Sapindaceae and Theaceae family

Leaves of *Mimusops elengi* (Sapoteaceae family) and *Acer oblongum* (Sapindaceae family) showed both TIA (5.06 ± 0.12%, 13.38 ± 1.03%) and CIA (0.45 ± 0.74%, 5.62 ± 1.52%) respectively, whereas, seeds of *Sapindus mukorossi* of Sapindaceae family showed 48.25 ± 0.98% TIA and 47.89 ± 0.96% CIA. On the other hand, seeds and leaves of *Camellia sinensis* of theaceae family showed neither activity.

Also, flowers of *Bombax ceiba* (Malvaceae family) did not show either activity whereas, both trypsin and chymotrypsin inhibitory activity was observed with flowers of plants from fabaceae family viz. *B. monosperma*

Table 1. Trypsin and Chymotrypsin inhibitory activities of extracts of some plants of different families.

S. No.	Plant Name	Family	Part used			Trypsin inhibitory activity	Chymotrypsin inhibitory activity
			Leaves	Seeds	Flowers		
1.	<i>Abrus precatorious</i>	Fabaceae		✓		+	+
2.	<i>Acacia auriculiformis</i>	Fabaceae	✓			+	+
3.	<i>Acacia modesta</i>	Fabaceae	✓			-	-
4.	<i>Acacia concinna</i>	Fabaceae		✓		+	+
5.	<i>Acacia sp.</i>	Fabaceae	✓			-	-
6.	<i>Acacia nilotica</i>	Fabaceae	✓			+	+
7.	<i>Acer oblongum</i>	Sapindaceae	✓			+	+
8.	<i>Aegle marmelos</i>	Rutaceae	✓			-	-
				✓		-	-
9.	<i>Bauhinia acuminata</i>	Fabaceae	✓			+	+
10.	<i>Bauhinia alba</i>	Fabaceae	✓			+	+
11.	<i>Bauhinia tomentosa</i>	Fabaceae	✓			+	+
12.	<i>Bauhinia variegata</i>	Fabaceae	✓			+	+
13.	<i>Bombax ceiba</i>	Malvaceae	✓			-	-
					✓	-	-
14.	<i>Butea monosperma</i>	Fabaceae	✓			+	+
					✓	+	+
15.	<i>Caesalpinia bonducella</i>	Fabaceae		✓		+	+
16.	<i>Caesalpinia pulcherrima</i>	Fabaceae	✓			+	+
17.	<i>Camellia sinensis</i>	Fabaceae	✓			-	-
				✓		-	-
18.	<i>Cassia absus</i>	Fabaceae		✓		+	+
19.	<i>Cassia biflora</i>	Fabaceae	✓			+	+
20.	<i>Cassia fistula</i>	Fabaceae	✓			+	+
21.	<i>Cassia glauca</i>	Fabaceae	✓			+	+
22.	<i>Cassia occidentalis</i>	Fabaceae	✓			+	+
23.	<i>Cassia siamea</i>	Fabaceae	✓			+	+
24.	<i>Citrus medica</i>	Rutaceae	✓			-	-
25.	<i>Dalbergia sisso</i>	Fabaceae		✓		-	-
26.	<i>Delonix regia</i>	Fabaceae	✓			+	+
					✓	-	-
27.	<i>Enterolobium consortium</i>	Fabaceae		✓		+	+
28.	<i>Jacoranda mimosi</i>	Bignoniaceae		✓		-	-
29.	<i>Jatropha curcas linn</i>	Euphorbiaceae	✓			-	-
30.	<i>Kigelia pinnata</i>	Bignoniaceae	✓			+	+
31.	<i>Lathyrus sativus</i>	Fabaceae		✓		+	+
32.	<i>Milletia ovalifolia</i>	Fabaceae	✓			-	-
33.	<i>Mimusops elengi</i>	Sapotaceae	✓			+	+
34.	<i>Mucuna Pruriens</i>	Fabaceae		✓		+	+
35.	<i>Murraya exotica</i>	Rutaceae	✓			-	-
36.	<i>Murraya koengii</i>	Rutaceae	✓			-	-
37.	<i>Phaseolus vulgaris</i>	Fabaceae		✓		+	+
38.	<i>Phyllanthus embilica</i>	Euphorbiaceae		✓		+	+
39.	<i>Pongamia glabra</i>	Fabaceae	✓			+	+
40.	<i>Pongamia pinnata</i>	Fabaceae		✓		+	+
41.	<i>Prosopis juliflora</i>	Fabaceae	✓			+	+
42.	<i>Psoralea corylifolia</i>	Fabaceae		✓		+	+
43.	<i>Pterospermum acerifolium</i>	Malvaceae	✓			+	+
44.	<i>Putranjiva roxburghii</i>	Putranjivaceae		✓		+	+
45.	<i>Ricinus communis</i>	Euphorbiaceae		✓		+	+
46.	<i>Rivina humilis</i>	Phytolaccaceae		✓		-	-
47.	<i>Sapindus mukorossi</i>	Sapindaceae		✓		+	+
48.	<i>Tamarindus indica</i>	Fabaceae		✓		+	+
49.	<i>Tecoma argentina</i>	Bignoniaceae	✓			+	+
50.	<i>Tecoma grandiflora</i>	Bignoniaceae		✓		+	+
51.	<i>Tecoma stans</i>	Bignoniaceae	✓			+	+
52.	<i>Terminalia arjuna</i>	Combretaceae		✓		-	-

(continued on next page)

Table 1 (continued)

S. No.	Plant Name	Family	Part used			Trypsin inhibitory activity	Chymotrypsin inhibitory activity
			Leaves	Seeds	Flowers		
53.	<i>Terminalia chebula</i>	Combretaceae		✓		-	-
54.	<i>Vitex negundo</i>	Lamiaceae		✓		-	-
55.	<i>Ziziphus jujuba</i>	Rhamnaceae		✓		-	-

"+" = Present "-" = Absent.

(18.26 ± 2.01% TIA & 15.29 ± 1.58% CIA) and *D. regia* (5.42 ± 1.25% TIA & 15.76 ± 0.25% CIA).

3.11. Comparison of peptidase inhibitory activity in leaves, flowers, seeds and in different families

Different parts of the plant, on comparison showed that seeds had the significant highest trypsin ($F_{6, 35} = 121.35$; $P < 0.01$) and chymotrypsin ($F_{6, 35} = 198.86$; $P < 0.01$) inhibitory activity followed by leaves and flowers (Figure 1). Among families, plants from Sapindaceae showed the maximum trypsin inhibitory activity followed by Putranjivaceae, Fabaceae, Euphorbiaceae, Rutaceae, Bignoniaceae, Sapotaceae and Malvaceae. Whereas, plants of Putranjivaceae showed the maximum chymotrypsin inhibitory activity followed by Sapindaceae, Fabaceae, Euphorbiaceae, Rutaceae, Bignoniaceae, Malvaceae and Sapotaceae. Plants belonging to Combretaceae, Lamiaceae, Phytolaccaceae, Rhamnaceae and Theaceae showed none of the activities (Figure 2). Also the plants which showed the TIA also possessed CIA.

4. Discussion

Over the past few decades, there has been increasing interest in identifying, purifying and characterizing novel PIs. They are leading candidates with various applications across medicinal biotechnology and agriculture. Plants are excellent sources of PIs which help them to combat various diseases, insects, pests and herbivores (Ryan, 1990). TIA and CIA of various PIs are required for the control of various insect pests which act by the inhibition of their midgut peptidases. The PIs are present in different organisms viz. microorganisms, plants and animals. These are present in seeds, leaves and flowers of plants which inhibit the digestive enzymes of the insects/pests (Fan and Wu, 2005). A number of plant seeds of leguminaceae family have been identified for their peptidase

inhibitor activity (Tamir et al., 1996). In this study 55 plants were studied which belonged to 13 different families viz. Bignoniaceae, Combretaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Malvaceae, Putranjivaceae, Phytolaccaceae, Rhamnaceae, Rutaceae, Sapotaceae, Sapindaceae and Theaceae.

Maximum trypsin and chymotrypsin inhibitory activity was reported from the seeds of *S. mukorossi* which belongs to sapindaceae family. Gandreddi et al. (2015) isolated and purified trypsin inhibitor from soap nut (*Sapindus trifoliatus* L. Var. *Emarginatus*) seeds and evaluated its role against larval gut peptidases of *Helicoverpa armigera* and *Spodoptera frugiperda*.

Maximum number of plants was screened from leguminaceae family, in which maximum peptidase inhibitory activity was observed from the seeds of *M. pruriens* followed by *A. concinna*, *P. corylifolia*, *L. sativus* and *C. bonducella*. There are previous reports on purification of trypsin inhibitors from the seeds of *M. pruriens*, *P. corylifolia*, *L. sativus* and *Trigonella foenum graecum* respectively, which is in support of our results (Borde et al., 2012; Yang et al., 2006; Ramakrishna et al., 2010 and Oddepally et al., 2013). Zhou et al. (2020) studied the X-ray structure of trypsin inhibitor purified from *Cassia obtusifolia* and gave inhibitory activity comparable with that of soybean trypsin inhibitor against midgut trypsin from *Pieris rapae*. Ferreira et al. (2019) purified two recombinant PIs namely, cruzipain inhibitor (rBbCI) and kallikrein inhibitor (rBbKI) from *Bauhinia bauhinoides* and analyzed its insecticidal activity against soldiers and workers of *Nasutitermes*. Ahmad et al. (2020) purified a trypsin inhibitor from *Arachis hypogaea* and evaluated its anticarcinogenic effect.

There are reports on the isolation and purification of trypsin inhibitors from several other families. Patriota et al. (2016) purified trypsin inhibitor from bignoniaceae family (*Tecoma stans*) which had an inhibitory effect on growth and promotes ATP depletion and lipid peroxidation in *Candida albicans* and *Candida krusei*. Shahid et al. (2008) purified a novel protein from *Croton tiglium* belonging to Euphorbiaceae family with antifungal and antibacterial activities. Similarly, Lone et al. (2017) and

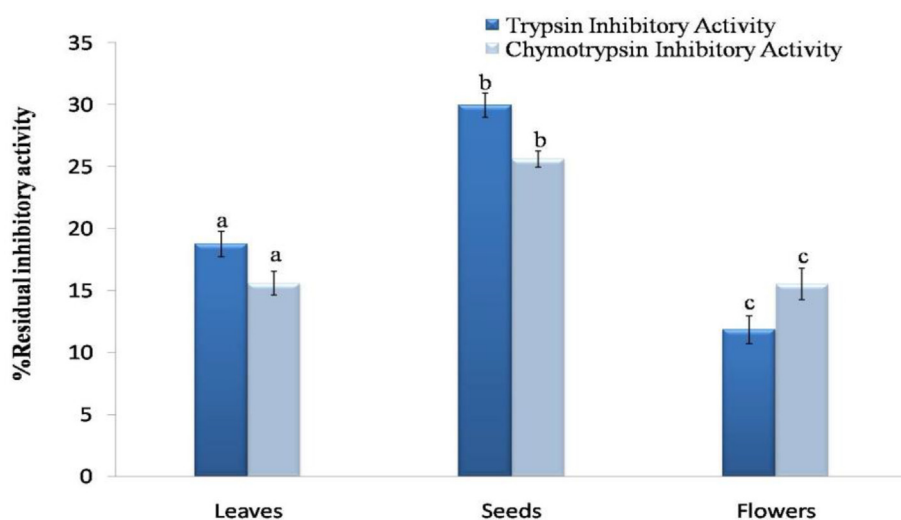


Figure 1. Mean % Trypsin and Chymotrypsin inhibitory activity of leaves, seeds and flowers of different plants. Treatments with same letter indicate no significant difference $p < 0.01$. TIA ($F = 121.35^{**}$, $HSD = 2.56$); CIA ($F = 198.86^{**}$, $HSD = 3.22$) as depicted by one way ANOVA and Tukey's test. * and ** indicates significance at $p < 0.05$ and $p < 0.01$, respectively. HSD = Honestly Significant Difference

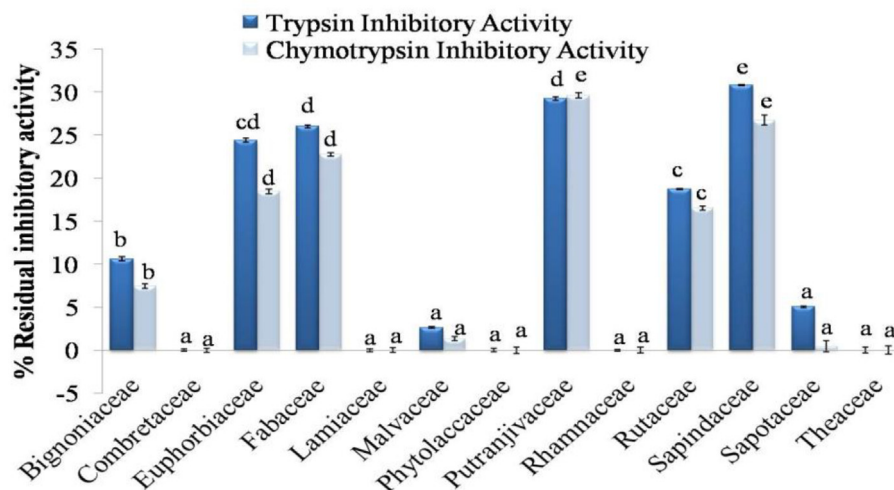


Figure 2. Comparison of % Trypsin and Chymotrypsin inhibitory activity of different plant families. Treatments with same letter indicate no significant difference $p < 0.01$. TIA ($F = 152.65^{**}$, $HSD = 6.25$); CIA ($F = 125.36^{**}$, $HSD = 6.25$) as depicted by one way ANOVA and Tukey's test. * and ** indicates significance at $p < 0.05$ and $p < 0.01$, respectively. HSD = Honestly Significant Difference

Soomro et al. (2007) also isolated and characterized peptidase inhibitor from the seeds of a plant *Ricinus communis* of Euphorbiaceae family.

Chaudhary et al. (2008) had purified and characterized trypsin inhibitor from the seeds of *Putranjiva roxburghii* of Putranjivaceae family by acid precipitation, cation-exchange and anion-exchange chromatography. Trypsin inhibitor was previously isolated by Shee and Sharma (2007) from the seeds of *Murraya koenigii* which belongs to rutaceae family. Rathinavelusamy et al. (2014) previously reported a protein with α -amylase inhibition potential and antidiabetic activity from the bark of *Pterospermum acerifolium* belonging to malvaceae family.

Bijina et al. (2011) observed that the crude extract of leaves of *Moringa oleifera* showed maximum percent of inhibition (77%) followed by the seed extract (63%). Norioka et al. (1988) reported that the seeds of the leguminous species contained mainly the Kunitz family inhibitors and those of the more advanced ones had the Bowman-Birk family inhibitors. It was also reported by the same group that the Kunitz family inhibitors in leguminous seed have gradually been replaced by the Bowman-Birk family inhibitors in the process of evolution. Ryan (1990) had reported that PIs have high inhibitory activity against phytophagous insects as they possess alkaline guts where serine peptidases are dominantly required for digestion of food and therefore this can be effectively used as defense tool because of anti nutritional interaction.

5. Conclusion

The aim of this study was to screen Indian plants for the presence of trypsin and chymotrypsin inhibitory activity where, *A. concinna*, *C. bonducella*, *L. sativus*, *M. pruriens*, *P. corylifolia* and *S. mukorossi* exhibited maximum PI activity. The present study has also revealed that seeds had maximum trypsin and chymotrypsin inhibitory activity as compared to leaves and flowers. Work is further being undertaken to explore the plants exhibiting maximum TIA and CIA for purification and evaluation of insecticidal activity.

Declarations

Author contribution statement

Samiksha: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the manuscript.

Satwinder Kaur Sohal: Conceived and designed the experiments; Analyzed and interpreted the data.

Funding statement

This work was supported by UGC New Delhi under the scheme "University with Potential for Excellence (UPE)".

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2020.e05203>.

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