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Original Article

Datura alba seed proteins effect on snake venom enzymes with antioxidant and antibacterial activities

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الملخص

أهداف البحث: هدفت هذه الدراسة إلى تقييم الإمكانات المثبطة للبروتينات المستخرجة من بذور الداتورة البيضاء على الإنزيمات السامة لسم الثعبان إلى جانب إمكاناتها المضادة للأكسدة والبكتيريا.

طريقة البحث: تم استخراج البروتينات الخام في مخازن بيولوجية مشتركة (20 مليمول أسيتات، 20 مليمول فوسفات و20 مليمول تريس) بنسبة 1:5 تليها ترسيب كبريتات الأمونيوم بنسبة 80٪، والغسيل الكلوي والتجفيف بالتجميد. ثم تم تحليل المستخلصات المجففة بالتجميد على هلام بنسبة 12٪. ثم تم تقييم مستخلص التريس الذي أظهر أقصى عدد من الأشرطة البروتينية على الهلام بشكل أكبر للأنشطة الحيوية المثبطة. تم إجراء طريقة انتشار بئر الأجار لنشاط تثبيط الفوسفوليباز أ2 والبروتياز وألفا-أميليز باستخدام 2٪ صفار البيض و5٪ حليب منزوع الدسم و1٪ نشا كركيزة على التوالي. تم أخذ سم الثعبان ولعاب الإنسان كمصدر لتثبيط إنزيم الفوسفوليباز أ2 والبروتياز والأميليز بواسطة المستخلص. تم إجراء النشاط المضاد للأكسدة باستخدام اختبار إزالة الجذور الحرة مع أخذ حمض الأسكوربيك كمعيار. تم تقييم النشاط المضاد للبكتيريا بواسطة طريقة انتشار الآجار باستخدام اي كولاي و ستاف اوريوس كمصدر للبكتيريا.

النتائج: أظهر مستخلص تريس من بروتينات البذور تثبيطا بنسبة 19٪ لفوسفوليباز أ2 في سم الثعبان عند تركيز 125 ميكروجرام / ميكرولتر بينما لم يتم ملاحظة تثبيط بروتياز السم والنشاط المضاد للبكتيريا عند أعلى التركيزات التي تم تحليلها. لوحظ نشاط مضاد للأكسدة كبير (44.9٪) عند تركيز 600 ميكروجرام / ميكرولتر بينما لوحظ نشاط تعزيز ألفا أميليز بطريقة تعتمد على التركيز.

الاستنتاجات: أظهرت الدراسة تحييد إنزيم فوسفوليباز أ2 في سم الثعبان وهو إنزيم سام رئيسي موجود في سموم الثعابين إلى جانب خصائص مضادة للأكسدة. تسلط هذه الدراسة الضوء على إمكانات بروتينات بذور الداتورة كمضاد للآفات مع تطبيقات علاجية أخرى مهمة.

الكلمات المفتاحية: الداتورة البيضاء؛ التثبيط؛ فوسفوليباز أ2؛ مستخلص البروتين؛ البذور؛ سم الثعبان

Abstract

Objective: This study assessed the inhibitory potential of proteins extracted from *Datura alba* seeds on snake venom toxic enzymes along with their potential antioxidant and antibacterial activities.

Methods: Crude proteins were extracted using common biological buffers (20 mM acetate, 20 mM phosphate and 20 mM Tris) at a ratio of 1:5 followed by 80 % ammonium sulfate precipitation, dialysis, and lyophilization. Then the lyophilized extracts were resolved on 15 % sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) gels. The Tris extract showing the maximum number of protein bands on the SDS gel was further assessed for inhibitory bioactivities. Specifically, the agar well diffusion method was performed to assess the inhibitory activities of phospholipase A2 (PLA2), protease, and α-amylase using 2 % egg yolk, 5 % skim milk and 1 % starch as substrates, respectively. *Naja naja, Echis carinatus* venom, and human saliva were used as sources of PLA2, protease, and amylase, respectively, to test the inhibitory activity of the extract on these enzymes. Antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay using ascorbic acid as a standard. Antibacterial activity was assessed by the agar well diffusion

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method using *Escherichia coli* and *Staphylococcus aureus* as bacterial sources.

Results: The Tris extract of seed proteins exhibited 19 % inhibition of snake venom PLA2 at a concentration of 125 μg/μL concentration, whereas no venom protease inhibition or antibacterial activity was observed at the highest concentrations analyzed. Significant antioxidant activity (44.9 %) was observed at 600 μg/μL, while α-amylase-enhancing activity in a concentration-dependent manner was noted.

Conclusion: The results of this study demonstrated snake venom PLA2 neutralization, which is a major toxic enzyme present in snake venom, along with significant antioxidant properties. This study highlights the potential of *Datura* seed proteins as an antiophidic along with other therapeutically important applications.

Keywords: *Datura alba*; Inhibition; Phospholipase A2; Protein extract; Seeds; Snake venom

Introduction

Plant-derived bioactive compounds include both phytochemicals (flavonoids, alkaloids, terpenoids *etc.*) and proteins/peptides. They possess broad-spectrum therapeutic potential to treat various illnesses.[1] The low abundance, poor absorption and bioavailability, and high toxicity of phytochemicals have driven researchers to explore proteins as an alternate source of therapeutic intervention.[2,3] A large number of proteins discovered from medicinal plants possess antimicrobial, antioxidant, anticancer, anti-HIV, neuromodulatory, and ribosomal-inactivating activities, among others.[3] In addition, protease inhibitors from medicinal plants such as Bowman-Birk protease inhibitors and Mistletoe lectin-1 are being investigated in clinical trials for the potential treatment of malignant melanoma, prostate cancer, and oral cancer.[4,5]

Bioactive compounds found in Solanaceae plant species are known for their antimicrobial, neurological, anticancer, antihypertensive, and insecticidal properties.[6,7] *Datura* is a flowering perennial herb, belonging to the family Solanaceae. It is a genus of 10 species that is widely distributed in Europe, America, Australia, Mexico, Pakistan, and many other tropical and subtropical parts of the world. The genus *Datura* includes several species such as *D. stramonium, D. innoxia, D. alba, D. fastuosa, D. suaveolens, D. wrightii, D. ferox, D. quercifolia, D. certocaula*, and *D. leichhardti*.[8] Important phytotherapeutic lead molecules have been identified in *Datura* species that have antidiabetic, antimicrobial, anticancer, anti-inflammatory, antioxidant, herbicidal, and insecticidal potential.[9] However, the bioactivities of protein content, especially from seeds of *D. alba*, have not been reported.

Seeds are the primary source of plant reproduction and are rich in fats and proteins, of which the latter ranges from 10 % to 40 % in cereals to legumes, respectively.[10] Seed proteins play a crucial role in the development of the plant embryo and its growth during germination. Likewise, these proteins also function in certain physiological processes such as the stress response. The proteins present in seeds are mainly enzymes, and storage and structural proteins that are produced during seed development and stored in the seed tissues for later use.[11]

Datura seeds are mainly oleiferous but are also rich in alkaloids, terpenes, glycosides as well as proteins present to a significant extent with broad-spectrum therapeutic applications.[12,13] The alkaloids present in the seeds are responsible for the toxic or harmful effects that are comparatively higher than other parts of the plant.[9] They cause paralysis of the parasympathetic nervous system by disturbing the innervating nerves to different organs of the body.[14] This property could be useful therapeutically as an analgesic, calming or even anthelmintic treatment as have been reported from *Datura* seeds for toothaches, stomach or intestine distress, and as a treatment for parasitic infection.[15] Also, an *in vitro* study demonstrated that a lectin-like protein purified from the seeds of *Datura* species exhibited anticancer effects on human colon cancer cells.[16]

Substantial research has been carried out to explore the potential benefits of phytochemicals from *Datura* plants; however, the therapeutic potential of its protein content has not been explored. In the current study, the bioactivities of seed protein extract from *D. alba* were evaluated, in particular, their potential to inhibit main snake venom toxic enzymes, an aspect that has not been previously studied.

Materials and Methods

Plant material

D. alba pods were collected from the surrounding area of Dow University of Health Sciences (Karachi, Pakistan). Identification of the plant was carried out at the Institute of Sustainable Halophyte Utilization, University of Karachi. The pods were washed thoroughly to remove dirt, and the seeds were manually retrieved, dried in the shade, and pulverized to fine powder. Then the seeds were defatted by maceration in n-hexane at a ratio of 1:5 (w/v) for 24 h with continuous stirring. The defatted seed flour was separated from n-hexane by gravity filtration using Whatman No.1 filter paper and air dried for 2 days at room temperature.

Protein extraction

The defatted seed flour was macerated in 20 mM sodium acetate (pH 5.0), 20 mM sodium phosphate (pH 7.0), and 20 mM Tris-HCl (pH 8.0) at a ratio of 1:5 (w/v) overnight at 4 °C with constant stirring.[17] The resulting homogenates were filtered by gravity filtration followed by cold centrifugation at 8000 rpm for 20 min. Then the collected supernatant of each extract was used for ammonium sulfate protein precipitation at 80 %. The pellets were obtained by centrifugation and resolubilized and dialyzed against deionized water using 10 kDa dialysis membrane

overnight at 4 °C. Each crude extract was collected, lyophilized, and stored at −20 °C until further analysis.

Gel electrophoresis

The crude protein extract of all three buffers was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Laemmli 1970.[18] The protein extracts were analyzed in a discontinuous system using 12 or 15 % resolving and 5 % stacking gels. About 20 µg of each protein extract was mixed with reducing/non-reducing sample buffer at a ratio of 1:1 (v/v). All samples were heated at 95 °C for 5 min, centrifuged, and loaded on the gel along with a standard molecular weight marker (BZ0011G; Bio-Basic, Inc., Markham, Ontario). The gels were run at 200 V until the tracking dye reached the bottom of the gel (Mini-PROTEAN Tetra Cell; Bio-Rad, Hercules, CA, USA). The gels were stained overnight with 0.2 % Coomassie Blue G-250 and destained until the blue protein bands were visible on a clear background.

Phospholipase A2 inhibition assay

Phospholipase A2 (PLA2) inhibitory activity of *D. alba* seed extract was analyzed by the agar egg yolk (2 %) plate method using 20 mM phosphate buffer (pH 7.0) containing 1 mM $CaCl_2$ and 0.01 % sodium azide.[19] The *Naja naja* venom (0.25 µg/µL) was taken as a standard, mixed and incubated with different concentrations of *D. alba* seed protein (7.8125, 15.625, 31.25, 62.5, 95, 125 µg/µL) for 30 min at 37 °C to a final volume of 20 µL. The test mixtures were then loaded into the wells and incubated overnight at 37 °C. The next day, the zone of inhibition by the protein extract was measured using a vernier caliper and was compared with the control. The zone of standard served as a positive control and the zone of water served as a negative control. The percentage inhibition of PLA2, α-amylase, and protease enzymes were calculated using the following formula:

$$\%\text{inhibition} = \text{Diameter (Control)} - \text{Diameter (Sample)} \times \frac{100}{\text{Diameter (Control)}}$$

Protease inhibition assay

The protease inhibition assay was performed by using the (5 %) skim milk agar plate method.[20] *Echis carinatus* venom (0.625 µg/µL) was mixed and incubated with different concentrations of *D. alba* seed protein (7.8125, 15.625, 31.25, 62.5, 95, 125 µg/µL) for 30 min at 37 °C to a final volume of 20 µL, loaded onto plates, and incubated. The next day, the zone of inhibition by the given samples was measured using the Vernier caliper and compared with the standard; percentage inhibition was calculated as noted for PLA2 activity.

α-Amylase inhibition assay

Amylase inhibitory activity of *D. alba* seed protein extracts was assessed by the (1 %) starch agar plate method under the same buffer conditions used to assess PLA2 activity.[21] After incubation of the respective concentration of extract with human saliva (0.003 µg/µL) for 30 min at 37 °C to a final volume of 20 µL, the plates were loaded and incubated overnight. The next day, they were stained with iodine solution until the clear zone of hydrolysis appeared, measured using the Vernier caliper, and compared with the standard (human saliva); percentage inhibition was calculated as noted for PLA2 activity.

DPPH antioxidant scavenging assay

Antioxidant activity of *D. alba* seed protein extract was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (RSA).[22] DPPH (0.1 mM solution) was prepared in 97 % absolute ethanol and stored in a dark bottle. Also, different dilutions of seed protein extract (100, 200, 400, and 600 µg/mL) were prepared in deionized water. Then 100 µL protein dilution was mixed with 1.9 mL DPPH solution. Ascorbic acid (25, 50,100, and 200 µg/mL) was used as a standard, and DPPH alone and DPPH with 100 µL water served as controls. All of the reaction mixtures were incubated for 30 min in the dark at room temperature. Then 200 µL from each tube was added to a 96-well plate and the absorbance was recorded at 517 nm in a microplate reader (BioTek Epoch 2, Agilent Technologies, Inc., Santa Clara, CA, USA). Percentage RSA was calculated using the following equation:

$$\%\text{RSA} = \text{Absorbance (Control)} - \text{Absorbance (Sample)} \times \frac{100}{\text{Absorbance (Control)}}$$

All experiments were performed in duplicate and on two independent days.

Agar well diffusion assay

Local Gram-negative (*Escherichia coli* [ZMS60]) and Gram-positive (*Staphylococcus aureus* [6538]) strains were selected for the antibacterial activity assay. An overnight bacterial culture was taken, and the OD600 was adjusted between 0.08 and 0.1 according to the McFarland principle.[23] Media and cells were poured and spread evenly over the surface of a nutrient agar plate by the overlay plate method and allowed to solidify for 30 min. Different concentrations of crude protein extract (31.25, 62.5, 125, 250 µg/µL) were prepared in sterile distilled water and loaded into the wells. Broad-spectrum antibiotics (ciprofloxacin and ampicillin; 10 µg/µL) were prepared and 20 µL was loaded into the wells as positive controls; sterile distilled water served as a negative control. Then the plate was incubated for 24 h at 37 °C. After incubation, zone diameter was measured using the Vernier caliper and percentage inhibition was calculated.

Results

Qualitative analysis of extracts by SDS-PAGE

D. alba seed proteins were extracted with common biological buffers (acetate, phosphate and tris) as described in

the Methods. The extracted protein profile was analyzed using 15 % SDS-PAGE gels. The best protein band pattern and resolution were observed in 20 mM Tris-HCl buffer (pH 8.0), as shown in Figure 1A. Therefore, inhibitory assays were performed using the Tris protein extract. The Tris extract was also analyzed under reducing and non-reducing sample buffer conditions on 12 % SDS gels to observe any change in protein profile. Both reducing and non-reducing conditions showed similar protein bands pattern, indicating that most proteins were monomeric in nature (Figure 1B).

PLA2 activity inhibition

PLA2 inhibitory activities of different concentrations of *D. alba* seed protein extract were tested for *Naja* venom inhibition activity on an egg yolk agar plate. The diameter of the activity was measured in millimeters (mm). The extract with concentrations of 7.8, 15.6, 31.2, 62.5, 95, and 125 µg/µL was incubated with 0.25 µg/µL *Naja* venom and loaded onto the agar plate. The zone of activity of extract plus venom was compared with the zone of activity by venom alone. It was observed that the crude protein extract of *D. alba* seeds decreased the enzymatic activity of *Naja* venom PLA2 enzymes in a concentration-dependent manner. The standard 0.25 µg/µL *Naja* venom produced an activity zone of ~18.5 mm that was reduced to ~16.5 mm by the seed proteins at the highest concentration (125 µg/µL), with percent inhibition of 11.1 % (Figure 2A).

Protease inhibition assay

Skim milk agar plates were prepared for analysis of inhibition of proteases predominantly found in *E. carinatus* venom by *D. alba* seed protein extract. The diameter of the activity was measured in millimeters (mm). The extract with concentrations of 7.8, 15.6, 31.2, 62.5, 95, and 125 µg/µL was incubated with 0.625 µg/µL *Echis* venom and loaded onto the agar plate. The zone of activity of extract plus venom was compared with the zone of activity by venom alone. The protein extract of *D. alba* exhibited no effect on protease activity, as the activity zones remained almost the same diameter as the standard venom (Figure 2B). This observation indicates that the crude protein extract of *D. alba* seeds has molecules that have no or neutral interactions with snake venom proteases, resulting in a lack of inhibition or activation of their protease enzymes.

α-amylase inhibition assay

Starch agar plates were prepared for analysis of inhibition of salivary amylase by *D. alba* seed protein extract. The diameter of the activity was measured in mm. The extract with concentrations of 7.8, 15.6, 31.2, 62.5, 95, and 125 µg/µL was incubated with 0.003 µg/µL saliva and loaded onto the agar plate. The zone of activity of extract plus saliva was compared with the zone of activity by saliva alone. Seed protein extract exhibited salivary α-amylase activity-enhancing properties rather than inhibition in a concentration-dependent manner on the starch agar plate. The standard 0.003 µg/µL salivary amylase produced an activity zone of ~22.0 mm that was increased to ~26.0 mm by the seed proteins at the highest concentration (125 µg/µL) utilized. This observation indicates that the crude protein extract of *D. alba* seeds has molecules that augment the enzymatic functionality of salivary α-amylase enzyme (Figure 2C).

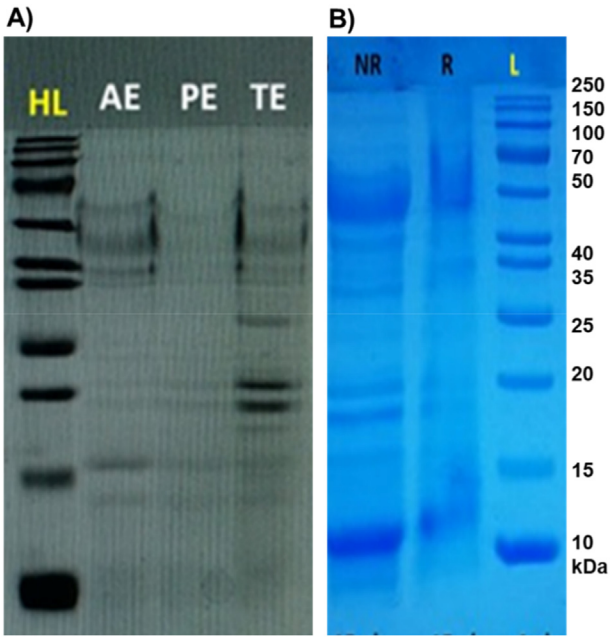

Figure 1: SDS-PAGE profile of *D. alba* seed extracts. (A) A 15 % SDS-PAGE gel of extracts in different biological buffers. (B) 12 % SDS-PAGE gel of Tris buffer protein extract under (NR) non-reducing and (R) reducing sample diluting buffer conditions. (HL/L); represents molecular weight ladder (AE); acetate buffer extract (PE); phosphate buffer extract (TE); tris buffer extract.

DPPH antioxidant assay

Different concentrations of *D. alba* seed protein extract (100, 200, 400 and 600 µg/mL) were examined for antioxidant activity with the DPPH free RSA. All concentrations of standard ascorbic acid (25, 50, 100, 200 µg/mL) showed 81 %–84 % RSA. Therefore, ascorbic acid at a concentration of 25 µg/mL, which exhibited 81.4 % RSA, taken as the control. The protein extract also exhibited antioxidant activity in a concentration-dependent manner. The highest RSA was found to be 45 % by seed protein extract at a concentration of 600 µg/mL, followed by 34 %, 28 %, and 19 % RSA by extract concentrations of 400, 200, and 100 µg/mL respectively (Figure 2D).

Antibacterial assay

There was no antibacterial activity observed with seed protein extract against local strains of *E. coli* and *S. aureus*, even at the highest concentration of 250 µg/µL (Figure 3). The standard antibiotics ampicillin and ciprofloxacin (10 µg/µL) showed a clear zone of bacterial growth inhibition on the agar plates. This observation indicates that the crude protein extract of *D. alba* seeds does not

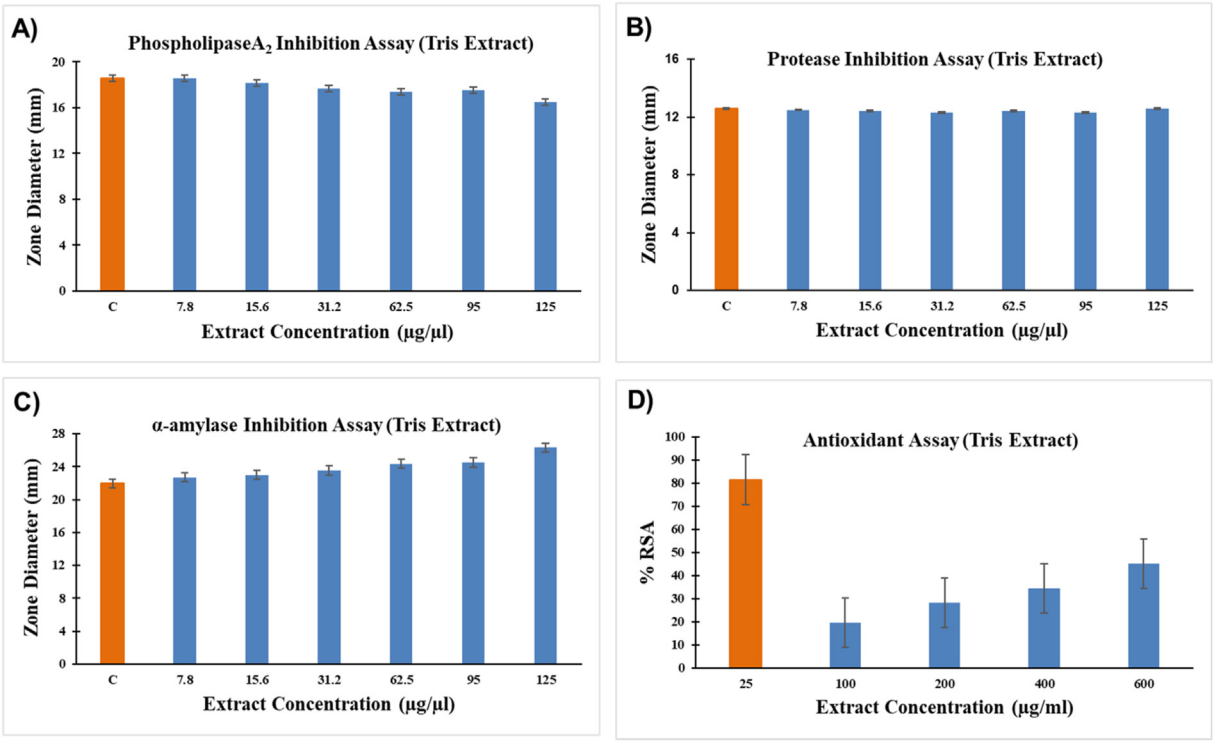

Figure 2: Inhibitory activities of *Datura* seed protein extracts. (A) Phospholipase A2 inhibition assay. (B) Protease inhibition assay. (C) α-amylase inhibition assay. (D) Antioxidant assay. (C) In each graph represents the respective control for each assay.

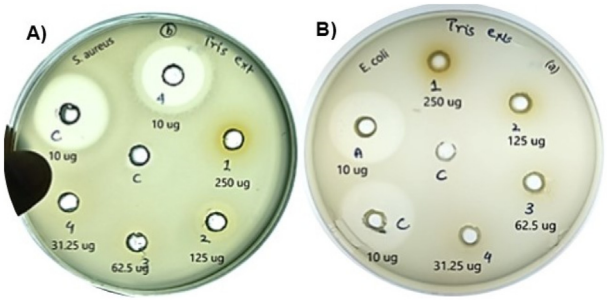

Figure 3: Antibacterial activity of *Datura alba* seed protein extract assessed by agar well diffusion assay. (A) Gram-positive *Staphylococcus aureus* (6538). (B) Gram-negative *Escherichia coli* (ZMS60). (C) represents ciprofloxacin (A) ampicillin (C in center); represents water as a negative control (1−4); different concentrations of protein extract.

contain an antibacterial protein or peptide against the selected two bacterial species.

Discussion

Medicinal plants serve as a rich source of promising protein therapeutics, which can be engineered and fine-tuned to obtain desirable characteristics with safety, efficacy, improved biocompatibility, and absorption over small molecule drugs.[24] The *Datura* plant is known for its broad pharmacological significance, particularly associated with the phytochemical constituents present in various parts of the plant. However, the biological significance of its proteins is not well established. Indo-Pak regions are affected by the climatic changes, which have caused increased rainfall and flooding. This in turn resulted in an increased number of envenomation cases and related deaths, especially in the population living in rural areas.[25] The study therefore explored the effects of *D. alba* seed proteins against two enzymes namely, PLA2 and proteases that constitute a major portion of the venom of the most common venomous snakes of Pakistan including *N. naja* and *E. carinatus*.[26] Also, the antioxidant and antibacterial potential has been assessed.

In our study, the *Datura* seed protein extract exhibited 11.1 % inhibition of *Naja* venom PLA2 activity. A 27 kDa acidic glycoprotein was isolated from the roots of *Withania somnifera* belonging to the family Solanaceae. It reduces *Naja* venom PLA2-induced edematogenicity and myotoxicity *in vivo* after removal of its glycosylated moiety. Also, it was able to neutralize the effects of PLA2 isoforms present in the venom.[27] However, the exact mechanism of its anti-PLA2 effect is not known. Another protein turmerin from *Curcuma longa* effectively inhibited PLA2-induced edema in a dose-dependent manner.[28] This suggests that plant proteins have potential to inhibit the activity of PLA2 enzymes found in the snake venom. Our results also indicated that the presence of proteins in the seed extract caused the inhibitory effect observed in the agar plate assay.

Similarly, proteinase inhibitors (PIs) are also ubiquitously found in plant seeds. Their inhibition strength and specificity depend on the defined inhibition site on the substrate protein.[29] Protein-based PIs such as potato

metallocarboxypeptidase inhibitors, Kunitz-type, and Potato type II inhibitors that target human proteases were discovered from plants in the Solanaceae family, namely, *Solanum tuberosum*, *Solanum lycopersicum*, and *Capsicum annuum* respectively.[30] A 21 kDa protease inhibitor was extracted from *Capsicum frutescens* leaves, which showed insecticidal activity by decreasing larval mass and interfering post-hatching development.[31] *D. alba* seed protein extract showed neither inhibitory nor enhancing activity towards proteases from *E. carinatus* venom. MP-4, a Kunitz-type protease inhibitor, was isolated from *Mucuna pruriens* seeds shown *in vivo E. carinatus* venom neutralization indirectly by an antibody-mediated mechanism rather than directly inhibiting venom proteases.[32] This suggests that *Datura* crude seed protein extracts do not possess direct inhibitory effects on venom proteinases and need further evaluation.

Alterations in the levels of α-amylases are significantly associated with diseases such as diabetes, periodontal, cardiometabolic conditions, obesity, metabolic syndrome, and renal failure. Therefore, salivary and serum amylases can serve as therapeutic targets and disease diagnostic tools.[33,34] The seed protein extract exhibited activity-enhancing effects on α-amylases (saliva) rather than inhibition. The highest concentration of seed protein extract (125 μg/μL) increased the α-amylase activity with percent enhancement of 19.65 %. The enhancement could be the result of amylases already found in plant seeds giving a synergistic effect in terms of enhanced activity. Amylases in plant seed are known to provide energy for the early development of plants during seed germination and maturation process.[35]

On the other hand, oxidative stress due to inadequate removal of reactive oxygen species is a leading cause of a number of disorders in humans. Antioxidant molecules are important in removing free radicals from the body and preventing diseases. Many antioxidant peptides from plant seeds such as hemp and Chinese leek seeds have been reported.[36] In the DPPH RSA, *Datura* seed protein extract showed strong activity in a concentration-dependent manner. The highest concentration of seed protein extract (125 μg/μL) exhibited 44.955 % RSA. It was previously reported that a protein fraction from *D. inoxia* leaves exhibited 45.89 % RSA at a 500 μg/mL concentration.[37] This shows that various parts of plants express proteins for oxidative stress management and normal physiological functions. The molecular weight, composition, nature of amino acids, and steric structure all influence the antioxidant activity of these proteins. Among these properties, protein sequence and amino acids such as cysteine, methionine, tyrosine, histidine, tryptophan, and phenylalanine have the most profound effects.[38]

In relation to antimicrobial proteins, it is known that all plant parts produce bioactive peptides that possess microbicidal properties. Some of these are involved in cellular signaling, playing a role in defense, homeostasis, senescence, and other activities.[39] It has been reported that the basic fractions of *D. stramonium* seed proteins exhibit antibacterial activity against *E. coli* and *K. pneumoniae*, whereas acidic fractions do no possess antibacterial activity.[40] Different protein fractions of *D. inoxia* leaves have been studied and fraction III was reported to exhibit antibacterial activity against *S. aureus*, *E. coli*, and *Vibrio cholera*.[37] In our study, the seed protein extract of *D. alba* exhibited no activity against Gram-negative *E. coli* (ZMS60) or Gram-positive *S. aureus* (6538). These results suggest the absence of antibacterial proteins against both bacterial strains assessed in this study. However, future studies are needed to confirm the results.

Conclusion

The current study indicated better *Datura* seed protein extraction in Tris buffer, which exhibited snake venom PLA2 inhibition, an enzyme mainly responsible for *Naja* venom toxicity. Also, significant antioxidant activity along with enhanced amylase activity were observed. However, the crude protein did not show venom protease inhibition or antibacterial activities. Further fractionation and purification should be carried out to identify potential antiophidic and other therapeutically important proteins from the *D. alba* seeds.

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Dow College of Biotechnology, Dow University of Health Sciences.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical approval

This study did not require clearance from an institutional review board. The authors declare no ethical concerns that require disclosure.

Authors contributions

WT and SMF performed all of the experiments. MM and SFM supervised the protein extraction, analysis, and venom enzyme inhibition assay. HW conceived and designed the study and supervised all of the *in vitro* biological assays. All of these authors made substantial contributions to the final manuscript and approved this submission.

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