Research Article

Anti-5′-Nucleotidases (5′-ND) and Acetylcholinesterase (AChE) Activities of Medicinal Plants to Combat Echis carinatus Venom-Induced Toxicities

Nazia Aslam,1 Syeda Fatima,1 Sofia Khalid,1 Shahzad Hussain,2 Mughal Qayum,3 Khurram Afzal,4 and Muhammad Hassham Hassan Bin Asad5,6

1Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan
2Drugs Control & Traditional Medicines Division, National Institute of Health, Islamabad, Pakistan
3Department of Pharmacy, Kohat University of Science and Technology, Kohat 26000, Pakistan
4Institute of Food Sciences and Nutrition, Bahauddin Zakariya University, Multan, Pakistan
5Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus 22060, KPK, Pakistan
6Institute of Fundamental Medicine and Biology, Department of Genetics, Kazan Federal University, Kazan 420008, Russia

Correspondence should be addressed to Muhammad Hassham Hassan Bin Asad; hasshamasad@yahoo.com

Received 3 November 2020; Revised 11 January 2021; Accepted 23 January 2021; Published 4 February 2021

Academic Editor: Ihsan ul Haq

Copyright © 2021 Nazia Aslam et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Echis carinatus is one of the highly venomous snakes of Pakistan that is responsible for numerous cases of envenomation and deaths. In Pakistan, medicinal plants are commonly used traditionally for snakebite treatment because of their low cost and easy availability in comparison with antivenom. The current research is aimed at evaluating the inhibitory activity of Pakistani medicinal plants against acetylcholinesterase and 5′-nucleotidases present in Echis carinatus venom. Acetylcholinesterase and 5′-nucleotidase enzymatic assays were performed at different venom concentrations to check the activity of these enzymes. Methanolic extracts from different parts of plants were used for in vitro determination of their inhibitory activity against 5′-nucleotidases in snake venom. Active methanolic extracts were subsequently fractioned using different solvents, and these fractions were also assessed for their anti-5′-nucleotidase activity. Results of this study exhibited that Eugenia jambolana Willd. ex O. Berg, Rubia cordifolia L., Trichodesma indicum (L.) R. Br., Calotropis procera (Wild.) R. Br., Curcuma longa L., and Fagonia arabica L. were able to significantly (p > 0.5) neutralize the 5′-nucleotidase activity by 88%, 86%, 86%, 85%, 83.7%, and 83%, respectively, compared with a standard antidote (snake venom antiserum). Thus, this study indicates that these plants possess the potential to neutralize one of the toxic enzymatic components of Echis carinatus venom and hence can help to augment the future efforts of developing alternative therapy for the management of snakebites.

1. Introduction

Snake envenomation is a global medical problem that has always had serious implications for the health and welfare of human beings [1]. It is specifically more prevalent in the poor and rural regions of South and Southeast Asia, Latin America, sub-Saharan Africa, and Papua New Guinea [2, 3]. South Asia is predominantly the most affected region which experiences approximately 121,000 cases of envenoming and 14,000 deaths each year. This densely populated region is a hotspot of venomous snake species. Sociodemographic as well as occupational profile of people has major contribution in increasing the risk of human and snake interactions. In addition, inadequate first aid along with delayed and suboptimal treatment of snakebites has further aggravated the situation in this region [3–5].

Echis carinatus is one of the four main medically imperative snakes (referred to as "Big Four") in South Asia [6], which is responsible for numerous cases of morbidity and mortality in the Indian subcontinent including Pakistan (Astola Island in Makran) [7]. Envenomation caused by Echis carinatus is mainly characterized by severe local tissue
2. Materials and Methods

2.1. Snake Venom and Chemicals. Lyophilized venom of *Echis carinatus* was kindly given by the National Institutes of Health (NIH), Islamabad, Pakistan. Venom was stored at 2 to 8°C in a light-resistant bottle. All other chemicals were purchased from Merck unless otherwise described.

2.2. Medicinal Plants. Plants having ethnobotanical evidence for antivenom activity were selected for this study. These plants were collected from various regions of Pakistan, while few were acquired from a local market in Rawalpindi. Plants were identified by an expert plant taxonomist, and voucher specimens were submitted to the herbarium of the Department of Botany, Bahauddin Zakariya University, Multan, Pakistan. Details of selected medicinal plants have been presented in Table 1.

2.3. Plant Extraction Process. After thoroughly washing, shade drying, and chopping, different parts of the plants were soaked in methanol for a period of about four weeks at ambient temperature. After that, the filtration process was carried out firstly with ordinary filter paper and then using Whatman filter paper 41. Subsequently, plant extracts were dried and stored in amber glass vials at 8°C in a refrigerator [33].

2.4. Enzymatic Assay for Acetylcholinesterase. Acetylcholinesterase activity in *Echis carinatus* venom was assessed using acetylthiocholine iodide as a substrate. Briefly, the reaction mixture containing venom (1–8 mg), phosphate buffer (pH 8.0), 10 mmol DTNB (5,5′-dithiobis(2-nitrobenzoic acid)), and acetylthiocholine iodide was incubated at 37°C for a period of 10 min. Hydrolysis of acetylthiocholine iodide by acetylcholinesterase enzyme of snake venom is depicted by the appearance of yellow color, which is produced due to the reaction of thiocholine with DTNB. The amount of yellow color produced was measured at 412 nm using a spectrophotometer (UV-1280 by Shimadzu) [36–38].

2.5. Enzymatic Assay for 5′-Nucleotidases. To perform the 5′-nucleotidase assay, adenosine 5′-monophosphate (5′-AMP) was used as substrate. Concisely, reaction mixture containing 5′-AMP (0.02 M, 0.5 mL), glycine buffer (0.2 M, 0.5 mL), magnesium sulfate (0.1 M, 0.1 mL), and venom (10-40 μg) was incubated for 10 minutes at 37°C. After that, the reaction was stopped by adding 1.5 mL of 10% trichloroacetic acid (TCA). The concentration of inorganic phosphate released in the reaction mixture was analyzed using the ascorbic acid reagent by adopting the protocol as described by Tan et al. [39]. The reaction mixture was allowed to stand at room temperature for 30 min, and absorbance was then measured at 820 nm. A standard curve was also constructed using known concentrations of Pakistani medicinal plants against 5′-nucleotidases and acetylcholinesterase enzymes present in *Echis carinatus* venom.
of inorganic phosphate [39]. For evaluation of anti-5′-nucleotidase activity, venom was preincubated with plant extracts at 37°C for 15 min [40].

2.6. Fractionation of Active Plant Extracts. Fractionation of active methanolic plant extracts (they have shown antienzymatic activity in their crude form of extract) was carried out using four different solvents, i.e., n-hexane, chloroform, dichloromethane, and ethyl acetate, based on their ascending polarity, respectively (relevant constituents dissolved in their relevant polarity of solvents) [41, 42]. These fractions, after filtration and drying, were again tested for their inhibitory activity against 5′-nucleotidase enzymes of Echis carinatus venom.

2.7. Phytochemical Analysis. Active methanolic plant extracts as well as their active fractions were analyzed qualitatively for the presence of different phytochemical constituents using standard procedures [43].

2.8. Statistical Analysis. All results were expressed as the mean. The Student t-test (SPSS) was used to compare the significance of the experimental results with the standard

| Sr. no. | Medicinal plants (voucher number) | Family | Part used | References |
|---------|----------------------------------|--------|-----------|------------|
| 1       | Adiantum capillus-veneris L. (R.R.Stewart F.W.Pak.4(2)) | Pteridaceae | Whole plant | [28] |
| 2       | Albizia lebbeck (L.) Benth. (R.R.Stewart F.W.Pak.381(9)) | Fabaceae | Seeds | [28] |
| 3       | Althaea officinalis L. (R.R.Stewart F.W.Pak.477(6)) | Malvaceae | Roots | [29] |
| 4       | Calotropis procera (Wild.) R.Br. (R.R.Stewart F.W.Pak.566(6)) | Apocynaceae | Flower | [28] |
| 5       | Citrullus colocynthis (L.) Schrad. (R.R.Stewart F.W.Pak.702(10)) | Cucurbitaceae | Fruit | [28] |
| 6       | Curcuma longa L. (R.R.Stewart F.W.Pak.66(3)) | Zingiberaceae | Rhizome | [30] |
| 7       | Eclipta prostrata (L.) L.Mint (R.R.Stewart F.W.Pak.743(5)) | Asteraceae | Whole plant | [28] |
| 8       | Eugenia jambolana Willd. ex O. Berg (R.R.Stewart F.W.Pak.504(2)) | Myrtaceae | Fruit | [28] |
| 9       | Fagonia arabica L. (R.R. Stewart F.W. Pak.433(2)) | Zygophyllaceae | Leaves and twigs | [31] |
| 10      | Lepidium sativum L. (R.R. Stewart F.W.Pak.319(4)) | Brassicaceae | Whole plant | [32] |
| 11      | Matthiola incana (L.) R.Br. (R.R. Stewart F.W.Pak.322(2)) | Brassicaceae | Seeds | [33] |
| 12      | Momordica charantia L. (R.R. Stewart F.W. Pak.706(1)) | Apocynaceae | Flowers | [28] |
| 13      | Psoralea corylifolia L. (R.R. Stewart F.W. Pak.418(1)) | Zygophyllaceae | Seeds | [33] |
| 14      | Rubia cordifolia L. (R.R. Stewart F.W. Pak.689(4)) | Rubiaceae | Roots | [28] |
| 15      | Sapindus mukorossi Gaertn. (R.R. Stewart F.W. Pak.463(3)) | Sapindaceae | Fruits | [28] |
| 16      | Swertia chirayita (Roxb.ex Flem.) Karst. (R.R.Stewart F.W.Pak.561(4)) | Gentianaceae | Stems | [34] |
| 17      | Terminalia arjuna Wight & Arn (R.R. Stewart F.W.Pak.502(4)) | Combretaceae | Bark | [28] |
| 18      | Trichodesma indicum (L.) R.Br. (R.R. Stewart F.W.Pak.604(3)) | Boraginaceae | Leaves | [35] |

| Concentration of venom used | Absorbance (mean ± S.D.) | Enzyme activity (units/mg) |
|-----------------------------|--------------------------|----------------------------|
| 0.04 mg                     | 0.045 ± 0.0092           | 0.0808                     |
| 0.03 mg                     | 0.044 ± 0.0102           | 0.0790                     |
| 0.02 mg                     | 0.039 ± 0.0141           | 0.0701                     |
| 0.01 mg                     | 0.030 ± 0.0078           | 0.0539                     |

Table 1: List of medicinal plants used to evaluate inhibitory activity against 5′-nucleotidases of Echis carinatus venom.

![Standard curve for inorganic phosphate to generate a standard curve.](image)

**Figure 1:** Standard curve for inorganic phosphate to generate a standard curve.

| Concentration of venom used | Absorbance (mean ± S.D.) | Enzyme activity (units/mg) |
|-----------------------------|--------------------------|----------------------------|
| 0.04 mg                     | 2.712 ± 0.2202           | 386                        |
| 0.03 mg                     | 2.149 ± 0.1307           | 303                        |
| 0.02 mg                     | 1.601 ± 0.1594           | 223                        |
| 0.01 mg                     | 1.063 ± 0.1296           | 145                        |

Table 3: Enzymatic activity of 5′-nucleotidases at different concentrations of Echis carinatus venom.
antidote (snake venom antiserum). The level of significance was set at $p > 0.5$.

3. Results

Acetylcholinesterase enzyme induces hydrolysis of acetylcholine which results in the liberation of choline and acetic acid. The acetylcholinesterase assay was performed using acetylthiocholine iodide as a substrate. Different concentrations of *Echis carinatus* venom were used to check the activity of the acetylcholinesterase enzyme. Results indicate that there was no significant increase in acetylcholinesterase activity with the increase in venom concentration. Even at 8 mg venom dose, very low acetylcholinesterase activity was observed in snake venom (Table 2). Hence, it can be said that *Echis carinatus* venom contains a very low amount of acetylcholinesterase enzyme. So, this assay was rejected and not proceeded further.

5′-Nucleotidases induced hydrolytic cleavage of adenosine monophosphate, which results in the liberation of inorganic phosphate. A standard curve was constructed with a known concentration of inorganic phosphate (Figure 1). 5′-Nucleotidase activity was assessed at different concentrations of *Echis carinatus* venom. Enzymatic activities at venom concentrations of 10 μg, 20 μg, 30 μg, and 40 μg were found to be 145, 223, 303, and 386 units/mg, respectively (Table 3). A fixed venom concentration (10 μg) was then used to evaluate the inhibitory potential of Pakistani medicinal plants against 5′-nucleotidase enzymes of *Echis carinatus* venom. In this study, snake venom antiserum was used as the reference standard.

Results showed that among eighteen selected medicinal plants, six plants were able to significantly neutralize the 5′-nucleotidase activity of *Echis carinatus* venom. Maximum inhibition was shown by *Eugenia jambolana* Willd. ex O. Berg (88%, $p > 0.5$), followed by *Rubia cordifolia* L. (86%, $p > 0.5$), *Trichodesma indicum* (L.) R.Br. (85%, $p > 0.5$), *Calotropis procera* (Wild.) R.Br. (85%, $p > 0.5$), *Curcuma longa* L. (83.7%, $p > 0.5$), and *Fagonia arabica* L. (83%, $p > 0.5$). Other plants show moderate to low anti-5′-nucleotidase activities. Inhibitory activities of all medicinal plants against 5′-nucleotidase enzymes have been given in Table 4. Fractions of active plant extracts were also analyzed for their neutralizing potential against 5′-nucleotidase enzymes of *Echis carinatus* venom. Inhibitory activity of different fractions of active methanolic plant extracts has been shown in Table 5.

Fractionation results showed that all four fractions of *Eugenia jambolana* Willd. ex O. Berg showed inhibitory activities comparable to the crude extract which were as
follows: n-hexane 86.8%, chloroform 88%, dichloromethane 80.5%, and ethyl acetate 90%. In the case of Calotropis procera (Wild.) R.Br., two fractions were effective; chloroform fraction inhibited 5' nucleotidase activity by 84% and dichloromethane fraction by 76.5%. For Curcuma longa L., dichloromethane fraction (85%), and for Fagonia arabica L., ethyl acetate fraction (85.5%), showed inhibition close to the crude extract. Dichloromethane and ethyl acetate fractions of Rubia cordifolia L. exhibited 89% and 88% inhibition.
respectively. For *Trichodesma indicum* (L.) R. Br., only chloroform fraction showed percentage inhibition (88%) comparable to the crude extract. Phytochemical screening was also performed for active methanolic plant extract as well as their active fractions. Phytochemical analysis results have been presented in Tables 6–10.

### 4. Discussion

In Pakistan, the increased frequency of snakebites is usually attributed to the destruction of snakes’ habitats and subsequent migration of these venomous animals to human settlements [44]. *Echis carinatus* is one of the highly venomous snakes in South Asia including Pakistan that is responsible for more bites and deaths among the human population than any other snake species [45]. 5′-Nucleotidases are one of the enzymatic components of *Echis carinatus* venom [6, 11]. 5′-Nucleotidase enzymes act as a cofactor of hemorrhagic toxins and affect homeostasis through modulation of platelet function [13, 46, 47]. They have been described as the most potent platelet aggregation inhibitors [48]. Inhibition of platelet aggregation caused by 5′-nucleotidase enzymes subsequently leads to inhibition of blood coagulation [49]. Snake venom acetylcholinesterase is quite stable compared to acetylcholinesterase enzymes from other sources. It is believed that this enzyme principally affects the nervous system of prey/victim through disruption of cholinergic transmission [20].

Medicinal plants used for the treatment of snakebites are commonly found worldwide, particularly in the regions of Asia, Africa, and America [50–52]. In developing countries like Pakistan, the advanced allopathic medication framework is either exorbitant or lacking, so people in rural areas are mostly dependent on plants for primary healthcare [53]. Many indigenous communities use plants as an alternative remedy in an attempt to treat or reduce the toxic effects of snake venom like edema and hemorrhage [54]. Plant extracts together with their fractions and isolates have been reported to have neutralizing ability against snake venom as well as its neutralization by Pakistani medicinal plants.

In this study, *Echis carinatus* venom showed very low activity of acetylcholinesterase enzyme. Similar results were reported by a previous study where significant activity of acetylcholinesterase enzyme was found in the elapid venom while no acetylcholinesterase activity was detected in the viperid venom [57]. Another study conducted by Hashmi et al. [6] also revealed very low or no activity of acetylcholinesterase enzyme in the venom of *Echis carinatus* and *Daboia russelii*, whereas considerable activity was observed in the venom of *Bungarus caeruleus* and *Naja naja*. Observations of aforementioned studies reveal that snakes belonging to the family Viperidae contain a low or negligible amount of acetylcholinesterase enzyme in their venom. Accordingly, *Echis carinatus*, being the member of the family Viperidae, showed extremely low acetylcholinesterase activity.

5′-Nucleotidase activity was observed in a dose-dependent manner in Pakistani *Echis carinatus* venom. Inhibition study results revealed that, among eighteen medicinal plants, six plants showed anti-5′-nucleotidase activity comparable to standard antidote (p > 0.5). Some fractions of these active plant extracts also showed noteworthy inhibitory activity against 5′-nucleotidases present in snake venom. Previous studies have also reported such neutralizing ability of medicinal plants against nucleotidase activity of *Echis carinatus* venom. A study showed that different extracts of the *Tabernaemontana alternifolia* root (ethyl acetate, acetone, ethanol, methanol, and water) were able to completely inhibit the 5′-nucleotidase activity of *Echis*

### Table 8: Qualitative analysis of phytochemicals in the active fractions of *Calotropis procera* (Wild) R. Br. and *Curcuma longa* L. crude extracts.

| Phytochemicals          | *Calotropis procera* (Wild) R. Br. | *Curcuma longa* L. | Chloroform | Dichloromethane | Chloroform | Dichloromethane |
|-------------------------|-----------------------------------|-------------------|------------|----------------|------------|----------------|
| Alkaloids               | +                                 |                   | +          | −              | +          | −              |
| Carbohydrates           | −                                 |                   | −          | −              | −          | −              |
| Fatty acids             | −                                 |                   | −          | −              | −          | −              |
| Flavonoids             | +                                 |                   | −          | −              | −          | −              |
| Glycins                | −                                 |                   | −          | −              | −          | −              |
| Phenols/tannins         | +                                 |                   | −          | +              | −          | +              |
| Proteins               | −                                 |                   | −          | −              | −          | −              |
| Saponins               | +                                 |                   | −          | −              | +          | −              |
| Terpenoids/steroids     | −                                 |                   | −          | +              | +          | +              |

Note: (+) indicates the presence and (−) indicates the absence of phytochemicals.


**Table 9**: Qualitative analysis of phytochemicals in active fractions of *Eugenia jambolana* Willd. ex O. Berg crude extract.

| Phytochemicals       | *Eugenia jambolana* Willd. ex O. Berg | n-Hexane | Chloroform | Dichloromethane | Ethyl acetate |
|----------------------|--------------------------------------|----------|------------|-----------------|---------------|
| Alkaloids            | +                                    | +        | +          |                 |               |
| Carbohydrates        | −                                    | −        | −          |                 | −             |
| Fatty acids          | −                                    | −        | −          |                 | −             |
| Flavonoids           | +                                    | +        | +          |                 | +             |
| Glycosides           | +                                    | +        | +          |                 | +             |
| Phenols/tannins      | −                                    | +        | −          |                 | −             |
| Proteins             | −                                    | −        | −          |                 | −             |
| Saponins             | +                                    | +        | +          |                 | +             |
| Terpenoids/steroids  | −                                    | +        | −          |                 | +             |

Note: (+) indicates the presence and (−) indicates the absence of phytochemicals.

**Table 10**: Qualitative analysis of phytochemicals in active fractions of *Fagonia arabica* L., *Rubia cordifolia* L., and *Trichodesma indicum* (L.) R. Br. crude extracts.

| Phytochemicals       | *Fagonia arabica* L. | *Rubia cordifolia* L. | *Trichodesma indicum* (L.) R. Br. |
|----------------------|----------------------|-----------------------|----------------------------------|
|                      | Ethyl acetate        | Dichloromethane       | Chloroform                        |
| Alkaloids            | +                    | +                     | +                                 |
| Carbohydrates        | −                    | −                     | −                                 |
| Fatty acids          | −                    | −                     | −                                 |
| Flavonoids           | +                    | +                     | +                                 |
| Glycosides           | +                    | +                     | +                                 |
| Phenols/tannins      | +                    | +                     | +                                 |
| Proteins             | −                    | +                     | +                                 |
| Saponins             | +                    | +                     | +                                 |
| Terpenoids/steroids  | +                    | +                     | +                                 |

Note: (+) indicates the presence and (−) indicates the absence of phytochemicals.

**5. Conclusion**

This study revealed that *Calotropis procera* (Wild.) R. Br., *Curcuma longa* L., *Eugenia jambolana* Willd. ex O. Berg, *Fagonia arabica* L., *Rubia cordifolia* L., and *Trichodesma indicum* (L.) R.Br. possess the ability to neutralize the 5′-nucleotidase enzymes present in Pakistani *Echis carinatus* venom. So, based on this study, it can be concluded that these plants can serve as the potent source of bioactive compounds with antivenom property for managing the toxicities of snakebites, particularly the effects of 5′-nucleotidase enzymes which are the potent inhibitor of platelet aggregation in victims.

**Data Availability**

Data used to support this study finding have been included in the article and could be provided upon request from first author Nazia Aslam (nazia.3284@gmail.com).

**Conflicts of Interest**

The authors declare that there is no conflict of interest.
Acknowledgments

The authors highly acknowledge Dr. Muhammad Hassham Hassan Bin Asad (KFU, Russia; CUI, Pakistan) for their valuable support to complete this research work. Moreover, the authors are thankful to the NIH, Islamabad, Pakistan, for the provision of *Echis carinatus* venom.

References

[1] M. A. Bittenbinder, C. N. Zdenek, B. op den Brouw et al., "Coagulotoxic cobras: clinical implications of strong anticoagulant actions of African spitting *Naja* venoms that are not neutralised by antivenom but are by LY315920 (Varespladib)," *Toxins*, vol. 10, no. 12, p. 516, 2018.

[2] J. M. Gutiérrez, J. J. Calvete, A. G. Habib, R. A. Harrison, D. J. Williams, and D. A. Warrell, "Snakebite envenoming," *Nature Reviews Disease Primers*, vol. 3, no. 1, article 17063, 2017.

[3] R. Ralph, S. K. Sharma, M. A. Faiz et al., "The timing is right to end snakebite deaths in South Asia," *BMJ*, vol. 364, article k3317, 2019.

[4] E. Alitrol, S. K. Sharma, H. S. Bawaskar, U. Kuch, and F. Chappuis, "Snake bite in South Asia: a review," PLoS Neglected Tropical Diseases, vol. 4, no. 1, article e603, 2010.

[5] A. M. F. Oh, C. H. Tan, K. Y. Tan, N. H. Quraishi, and N. H. Tan, "Venom proteome of *Bungarus sinduanus* (Sind krait) from Pakistan and in vivo/ cross-neutralization of toxicity using an Indian polyvalent antivenom," *Journal of Proteomics*, vol. 193, pp. 243–254, 2019.

[6] S. U. Hashmi, A. Alvi, I. Munir et al., "Functional venomics of the big-4 snakes of Pakistan," *Toxicon*, vol. 179, pp. 60–71, 2020.

[7] A. Savanur, S. A. Ali, I. Munir, A. Abbasi, M. Alam, and H. A. Shai kh, "Pharmacological and biochemical studies on the venom of a clinically important viper snake (*Echis carinatus*) of Pakistan," *Toxicon*, vol. 80, pp. 47–57, 2014.

[8] Y. H. Mahadeswaraswamy, S. Nagaraju, K. S. Girish, and K. Kemaparaju, "Local tissue destruction and procoagulation properties of *Echis carinatus* venom: inhibition by *Vitis vinifera* seed methanol extract," *Phytotherapy Research*, vol. 22, no. 7, pp. 963–969, 2008.

[9] T. Escalante, A. Rucavado, J. W. Fox, and J. M. Gutiérrez, "Key events in microvascular damage induced by snake venom hemorrhagic metalloproteinases," *Journal of Proteomics*, vol. 74, no. 9, pp. 1781–1794, 2011.

[10] J. M. Alam, R. Qasim, and S. M. Alam, "Enzymatic activities of some snake venoms from families Elapidae and Viperidae*, Pakistan Journal of Pharmaceutical Sciences*, vol. 9, no. 1, pp. 37–41, 1996.

[11] N. R. Casewell, R. A. Harrison, W. Wüster, and C. W. Wagstaff, "Comparative venom gland transcriptome surveys of the saw-scaled vipers (Viperidae: *Echis*) reveal substantial intra-family gene diversity and novel venom transcripts," *BMC Genomics*, vol. 10, no. 1, p. 564, 2009.

[12] B. L. Dhananjaya, A. Nataraju, C. R. Gowda, B. K. Sharath, and C. J. M. D’ souza, "Vanillic acid as a novel specific inhibitor of snake venom 5′-nucleotidase: a pharmacological tool in evaluating the role of the enzyme in snake envenomation," *Biochemistry (Moscow)*, vol. 74, no. 12, pp. 1315–1319, 2009.

[13] S. D. Aird, "Ophidian envenomation strategies and the role of purines," *Toxicon*, vol. 40, no. 4, pp. 335–393, 2002.

[14] M. B. Hargreaves, S. M. Stogall, and M. G. Collis, "Evidence that the adenosine receptor mediating relaxation in dog lateral saphenous vein and guinea pig aorta is of the A2 subtype," *British Journal of Pharmacology*, vol. 102, p. 198P, 1999.

[15] C. Seligmann, C. Kupatt, B. F. Becker, S. Zahler, and S. Beblo, "Adenosine endogenously released during early reperfusion mitigates postischemic myocardial dysfunction by inhibiting platelet adhesion," *Journal of Cardiovascular Pharmacology*, vol. 32, no. 1, pp. 156–163, 1998.

[16] L. Sobrevia, D. L. Yudilevich, and G. E. Mann, "Activation of A2A-purinoceptors by adenosine stimulates L-arginine transport (system y+) and nitric oxide synthesis in human fetal endothelial cells," *The Journal of Physiology*, vol. 499, no. 1, pp. 135–140, 1997.

[17] V. Ralevic and G. Burnstock, "Receptors for purines and pyrimidines," *Pharmacological Reviews*, vol. 50, no. 3, pp. 413–492, 1998.

[18] R. S. Redman and E. M. Silinsky, "A selective adenosine antagonist (8-cyclopentyl-1,3-dipropylxanthine) eliminates both neuromuscular depression and the action of exogenous adenosine by an effect on A1 receptors," *Molecular Pharmacology*, vol. 44, no. 4, pp. 835–840, 1993.

[19] M. Vigny, S. Bon, J. Massoulie, and F. Leterrier, "Active-site catalytic efficiency of acetylcholinesterase molecular forms in Electrophorus, Torpedo, rat and chicken," *European Journal of Biochemistry*, vol. 85, no. 2, pp. 317–323, 1978.

[20] M. Ahmed, J. B. T. Rocha, V. M. Morsh, and M. R. C. Schetin ger, "Snake venom acetylcholinesterase," in *Handbook of Venoms and Toxins of Reptiles*, pp. 207–219, CRC press, 2009.

[21] C. Xie, J. Slagboom, L. O. Albulascu et al., "Antivenom neutralization of coagulopathic snake venom toxins assessed by bioactivity profiling using nanofractionation analytics," *Toxins*, vol. 12, no. 1, p. 53, 2020.

[22] R. M. Kini, S. S. Sidhu, and A. H. Laustsen, "Biosynthetic oligolcinal antivenom (BOA) for snakebite and next-generation treatments for snakebite victims," *Toxins*, vol. 10, no. 12, p. 534, 2018.

[23] J. Parker-Cote and W. J. Meggs, "First aid and pre-hospital management of venomous snakebites," *Tropical Medicine and Infectious Disease*, vol. 3, no. 2, p. 45, 2018.

[24] S. A. Gilani, Y. Fuji, Z. K. Shinwari, M. Adnan, A. Kikuchi, and K. N. Watanahe, "Phytotoxics studies of medicinal plant species of Pakistan," *Pakistan Journal of Botany*, vol. 42, no. 2, pp. 987–996, 2010.

[25] M. Ahmad, S. Sultan, S. Fazl-i-Hadi et al., "An ethnobotanical study of medicinal plants in high mountainous region of Chail valley (District Swat- Pakistan)," *Journal of Ethnobiology and Ethnomedicine*, vol. 10, no. 1, p. 36, 2014.

[26] M. Hamayun, "Ethnobotanical studies of some useful shrubs and trees of District Buner, NWFP, Pakistan," *Ethnobotanical Leaflets*, vol. 3, no. 1, p. 12, 2003.

[27] M. H. Borges, A. M. Soares, V. M. Rodrigues et al., "Neutralization of proteases from *Bothrops* snake venoms by the aqueous extract from *Cascaria sylvestris* (Flacourtiaceae)," *Toxicon*, vol. 39, no. 12, pp. 1863–1869, 2001.

[28] M. A. Butt, M. Ahmad, A. Fatima et al., "Ethnomedicinal uses of plants for the treatment of snake and scorpion bite in Northern Pakistan," *Journal of Ethnopharmacology*, vol. 168, pp. 164–181, 2015.

[29] S. Z. Husain, R. N. Malik, M. Javaid, and S. Bibi, "Ethnobotanical properties and uses of medicinal plants of Morgah..."
biodiversity park, Rawalpindi,” *Pakistan Journal Botany*, vol. 40, no. 5, pp. 1897–1911, 2008.

[30] R. P. Samy, M. M. Thwin, P. Gopalakrishnakone, and S. Ignacimuthu, “Ethnobotanical survey of folk plants for the treatment of snakebites in Southern part of Tamilnadu, India,” vol. 115, no. 2, pp. 302–312, 2008.

[31] M. T. Razi, M. H. B. Asad, T. Khan et al., “Antihaemorrhagic potentials of *Fagonia cretica* against *Naja naja* karachensis (black Pakistan cobra) venom,” *Natural Product Research*, vol. 25, no. 20, pp. 1902–1907, 2011.

[32] A. Jabeen, S. Rani, M. Ibrahim, and A. S. Mohammed, “A review on *Lepidium sativum*,” *Indo American Journal of Pharmaceutical Sciences*, vol. 4, no. 8, pp. 2223–2227, 2017.

[33] B. A. MH, M. Iqbal, M. R. Akram et al., “5′-nucleotidases of *Naja naja karachensis* snake venom: their determination, toxicities and remedial approach by natural inhibitors (medicinal plants),” *Acta Poloniae Pharmaceutica*, vol. 73, pp. 667–673, 2016.

[34] V. Kumar and J. Van Staden, “A review of *Swertia chirayita* (Gentianaceae) as a traditional medicinal plant,” *Frontiers in Pharmacology*, vol. 6, p. 308, 2016.

[35] A. Dey and J. N. De, “Traditional use of plants against snakebite in Indian subcontinent: a review of the recent literature,” *African Journal of Traditional, Complementary and Alternative Medicine*, vol. 9, no. 1, pp. 153–174, 2012.

[36] M. Ahmed, A. Razaq, A. Razaq, N. Mushtaq, and R. A. Khan, “In vitro kinetics and inhibition of krait snake’s venom acetylcholinesterase by *Calligonum polygonoides* extract in relation to the treatment of Alzheimer’s disease,” *Iranian Journal of Basic Medical Sciences*, vol. 21, no. 8, pp. 869–872, 2018.

[37] G. L. Ellman, K. D. Courtney, V. Andres Jr., and R. M. Featherstone, “A new and rapid colorimetric determination of acetylcholinesterase activity,” *Biochemical Pharmacology*, vol. 7, no. 2, pp. 88–95, 1961.

[38] B. Janardhan, V. M. Shrikanth, K. K. Mirajkar, and S. S. More, “In vitro screening and evaluation of antivenom phytochemicals from *Azima tetracantha* Lam. leaves against *Bungarus caeruleus* and *Vipera russelli*,” *Journal of Venomous Animals and Toxins including Tropical Diseases*, vol. 20, no. 1, p. 12, 2014.

[39] C. H. Tan, S. M. Sim, C. A. Gnanathasan et al., “Enzymatic and toxicological activities of *Hypna ple plena* (hump-nosed pit viper) venom and its fractionation by ion exchange high performance liquid chromatography,” *Journal of Venomous Animals and Toxins Including Tropical Diseases*, vol. 17, no. 4, pp. 473–485, 2011.

[40] S. Ushanandini, S. Nagaraju, K. H. Kumar et al., “The anti-snake venom properties of *Tamarindus indica* (leguminosae) seed extract,” *Phytotherapy Research*, vol. 20, no. 10, pp. 851–858, 2006.

[41] J. Hussain, L. Ali, A. Khan et al., “Isolation and bioactivities of the flavonoids morin and morin-3-O-β-D-glucopyranoside from *Acridocarpus orientalis*—a wild Arabian medicinal plant,” *Molecules*, vol. 19, no. 11, pp. 17763–17772, 2014.

[42] T. B. Emran, M. A. Rahman, M. M. N. Uddin et al., “Effects of organic extracts and their different fractions of five Bangladeshi plants on *in vitro* thrombolytic,” *BMC Complementary and Alternative Medicine*, vol. 15, no. 1, pp. 1–8, 2015.

[43] N. Aslam, T. Javed, S. Khalid et al., “Antiprotease activity of indigenous medicinal plants against Pakistani *Echis carinatus* venom,” *Pharmacognosy Magazine*, vol. 16, no. 69, pp. 416–421, 2020.

[44] A. Shah, R. Sarvat, S. Shoaib et al., “An ethnobotanical survey of medicinal plants used for the treatment of snakebite and scorpion sting among the people of Namal valley, Mianwali district, Punjab, Pakistan,” *Applied Ecology and Environmental Research*, vol. 16, no. 1, pp. 111–143, 2018.

[45] D. A. Warrell, N. M. Davidson, B. M. Greenwood et al., “Poisoning by bites of the saw-scaled or carpet viper (*Echis carinatus*) in Nigeria,” *QJM: An International Journal of Medicine*, vol. 46, no. 1, pp. 33–62, 1977.

[46] G. D. Dimitrov and R. C. Kankonkar, “Fractionation of _Vipera russelli_ venom by gel filtration – I,” *Toxicon*, vol. 5, no. 3, pp. 213–221, 1968.

[47] R. M. Kini and H. J. Evans, “Effects of snake venom proteins on blood platelets,” *Toxicon*, vol. 28, no. 12, pp. 1387–1422, 1990.

[48] M. C. Boffa and G. A. Boffa, “Correlations between the enzymatic activities and the factors active on blood coagulation and platelet aggregation from the venom of *Vipera aspis*,” *Biochimica et Biophysica Acta (BBA)-General Subjects*, vol. 354, no. 2, pp. 275–290, 1974.

[49] N. J. daSilva and S. D. Aird, “Prey specificity, comparative lethality and compositional differences of coral snake venoms,” *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, vol. 128, no. 3, pp. 425–456, 2001.

[50] P. Giovannini and M. J. R. Howes, “Medicinal plants used to treat snakebite in Central America: review and assessment of scientific evidence,” *Journal of Ethnopharmacology*, vol. 199, pp. 240–256, 2017.

[51] P. J. Houghton and I. M. Osibogun, “Flowering plants used against snakebite,” *Journal of Ethnopharmacology*, vol. 39, no. 1, pp. 1–29, 1993.

[52] S. V. Upasani, V. G. Beldar, A. U. Tatiya, M. S. Upasani, S. J. Surana, and D. S. Patil, “Ethnomedicinal plants used for snakebite in India: a brief overview,” *Integrative Medicine Research*, vol. 6, no. 2, pp. 114–130, 2017.

[53] M. Khalid, M. Bilal, D. Hassani, S. Zaman, and D. Huang, “Characterization of ethno-medicinal plant resources of kara-mar valley Swabi, Pakistan,” *Journal of Radiation Research and Applied Sciences*, vol. 10, no. 2, pp. 152–163, 2019.

[54] A. Gomes, R. Das, S. Sarkhel et al., “Herbs and herbal constituents active against snake bite,” *Indian Journal of Experimental Biology*, vol. 48, no. 9, pp. 865–878, 2010.

[55] J. Félix-Silva, A. A. Silva-Junior, S. M. Zucolotto, and M. D. F. Fernandes-Pedrosa, “Medicinal plants for the treatment of local tissue damage induced by snake venoms: an overview from traditional use to pharmacological evidence,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, Article ID 5748256, 52 pages, 2017.

[56] M. S. Santosh, M. H. Sreekumar, K. Sunita et al., “Snake venom induced local toxicities: plant secondary metabolites as an auxiliary therapy,” *Mini Reviews in Medicinal Chemistry*, vol. 13, no. 1, pp. 106–123, 2013.

[57] M. Ahmed, N. Latif, R. A. Khan et al., “Enzymatic and biochemical characterization of *Bungarus sindanus* snake venom acetylcholinesterase,” *Journal of Venomous Animals and Toxins Including Tropical Diseases*, vol. 18, no. 2, pp. 236–243, 2012.

[58] M. S. Vineetha, J. Bhavya, K. M. Mirjakar, and S. S. More, “In vitro evaluation of active phytochemicals from *Tabernaemontana alternifolia* (Roxb) root against the *Naja naja-and Echis caryatides* venom,” *International Journal of Green Pharmacy*, vol. 16, no. 2, pp. 136–140, 2022.
carinatusIndian snake venom,” Journal of Biologically Active Products from Nature, vol. 4, no. 4, pp. 286–294, 2014.

[59] V. M. Shrikanth, B. Janardhan, and S. S. More, “In vitro neutralization of Echis carinatus and Naja naja venom by Canthium parviflorum and its GC-MS analysis,” vol. 11, no. 4, pp. 920–927, 2016.

[60] O. J. Ode and I. U. Asuzu, “The anti-snake venom activities of the methanolic extract of the bulb of Crinum jagus (Amaryllidaceae),” Toxicon, vol. 48, no. 3, pp. 331–342, 2006.

[61] P. K. Shukla, L. Gautam, M. Sinha, P. Kaur, S. Sharma, and T. P. Singh, “Structures and binding studies of the complexes of phospholipase A2 with five inhibitors,” Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, vol. 1854, no. 4, pp. 269–277, 2015.

[62] M. A. Tomaz, F. C. Patrão-Neto, and P. A. Melo, “Plant compounds with antiophidic activities, their discovery history, and current and proposed applications,” in Plant Toxins, Toxicology, pp. 449–464, Springer, Dordrecht, 2016.

[63] M. A. Strauch, M. A. Tomaz, M. Monteiro-Machado et al., “Antiophidic activity of the extract of the Amazon plant Humirianthera ampla and constituents,” Journal of Ethnopharmacology, vol. 145, no. 1, pp. 50–58, 2013.

[64] Y. Xiong, B. Li, D. Huang, Q. He, and X. Yu, “Anti-Deinagkistrodon acutus venom properties of ethanolic root extract from Cynanchum paniculatum (Bunge) kitag and its GC-MS analysis,” Journal of Ethnopharmacology, vol. 225, pp. 189–197, 2018.

[65] J. Leanpolchareanchai, P. Pithayanukul, R. Bavovada, and P. Saparpakorn, “Molecular docking studies and anti-enzymatic activities of Thai mango seed kernel extract against snake venoms,” Molecules, vol. 14, no. 4, pp. 1404–1422, 2009.

[66] J. Gershenzon and N. Dudareva, “The function of terpene natural products in the natural world,” Nature Chemical Biology, vol. 3, no. 7, pp. 408–414, 2007.

[67] M. H. Borges, D. L. F. Alves, D. S. Raslan et al., “Neutralizing properties of Musa paradisiaca L. (Musaceae) juice on phospholipase A2, myotoxic, hemorrhagic and lethal activities of crotalidae venoms,” Journal of Ethnopharmacology, vol. 98, no. 1–2, pp. 21–29, 2005.

[68] A. M. Torres, F. J. Camargo, G. A. Ricciardi, A. I. Ricciardi, and E. Dellacassa, “Neutralizing effects of Nectandra angustifolia extracts against Bothrops neuwiedi snake venom,” Natural Product Communications, vol. 6, no. 9, pp. 1393–1396, 2011.

[69] H. M. Min, M. Aye, T. Taniguchi et al., “A structure and an absolute configuration of (+)-alternamin, a new coumarin from Murraya alternans having antidote activity against snake venom,” Tetrahedron Letters, vol. 48, no. 35, pp. 6155–6158, 2007.

[70] J. O. da Silva, R. S. Fernandes, F. K. Ticli et al., “Triterpenoid saponins, new metalloprotease snake venom inhibitors isolated from Pentaclethra macroloba,” Toxicon, vol. 50, no. 2, pp. 283–291, 2007.

[71] P. A. Melo, D. A. Pinheiro, H. D. Ricardo et al., “Ability of a synthetic coumestan to antagonize Bothrops snake venom activities,” Toxicon, vol. 55, no. 2-3, pp. 488–496, 2010.