Evaluation of different Pakistani medicinal plants for inhibitory potential against *Echis carinatus* induced Phospholipase A_2 toxicity

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Abstract: Medicinal plants of Pakistan are known for their curative properties against snake bite as rural people have been using natural herbs for such injuries for hundreds to thousands of years. People of rural areas of Pakistan are prone to snakebite, and on the whole death due to snakebite has been increasing worldwide. The objective of this study was to test the neutralizing potential of 17 Pakistani medicinal plant extracts against phospholipase A_2 activity in *Echis carinatus* venom. Plant material was extracted by simple maceration and fractionation of active plant extracts. Venom was collected by manual massage of the venom glands. The PLA_2 enzymatic assay was performed to map out the venomous activity of *Echis carinatus* envenomation. Snake venom released fatty acids at different concentrations (0.1-5 mg/ml) of venom in a dose-dependent manner. Reduction of pH by 01 correlated with 133 µmol of fatty acids released at 5mg/ml of venom. All plants extract inhibited PLA_2 activity, however, *Curcuma longa*, *Citrullus colocynthis* and *Rubia cordifolia* inhibited maximum of PLA_2 activity (Σ78%) comparable to the standard antidote ($p>0.5$). Medicinal plants possess secondary metabolites and many active compounds that may have neutralizing or inhibiting properties against the PLA_2 activity of *Echis* venom. Further studies such as compound analysis could provide an alternative against snakebites injuries resulting from *Echis carinatus* venom.

Keywords: *Echis carinatus*, Pakistan, medicinal plants, anti-PLA_2

INTRODUCTION

Snakebite is a frequently overlooked cause of mortality and morbidity worldwide (Longbottom *et al.,* 2018) that affects over 2.5 million people and causes around 125,000 deaths annually (Warrell and Gutérrez, 2013; Nagaraju and Kannappan, 2015). It has been reported that 40,000 snakebites occur in Pakistan, particularly in rural areas, which results in 8,200 deaths annually (Parveen *et al.,* 2017). Venomous snakes include more than two hundred species (WHO, 2007) divided among four major families Elapidae, Viperidae, Colubridae, Lamprophidae (Tu, 1996). Snake bite envenomation resulted in severe complications in humans including hypotension, necrosis, cardiac arrest, edema, pain, paralysis, mucus discharge, bleeding gums, bleeding wounds, hematuria and eventually death (Asad *et al.,* 2012; Urs *et al.,* 2015).

Most venomous snakes found in Pakistan are from the families Elapidae and Viperidae. The black cobra (*Naja naja karachiensis*) and common krait (*Bungarus caeruleus*) are from the family Elapidae while the saw-scaled viper ***(****Echis carinatus****)*** and Russell's viper ***(****Daboia russelli****)*** belong to the family Viperidae (Chatterjee *et al.,* 2006). Out of these, *Echis carinatus* is found in the deserts of Thar and Cholistan, and the Island of Makran in Baluchistan, Pakistan. Its adult length is around 0.4 to 0.6 m, and it has a flattened body and a pointed tail (Warrell, 1995). Envenomation by *Echis carinatus* is known for inducing local tissue damage by the collective action of hyaluronidases, phospholipases A_2, and proteases which are also known as hydrolytic enzymes (Urs *et al.,* 2015). Venom from *Echis carinatus* induces chronic toxic effects that continue even after treatment with anti-venom (Parveen *et al.,* 2017).

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Snake venom is a complex mixture of various enzymatic, non-enzymatic, inorganic and organic molecules. Out of all these components enzymes and polypeptides affect the body of humans in a multisystem manner. Snake venom phospholipases A_2 (PLA_2) are a group of enzymes that act both systemically and locally to induce a wide range of physical and biochemical functional abnormalities in the victim. The lysophospholipids released by the action of PLA_2 can act as surfactants, which in turn rupture the erythrocyte membrane causing indirect hemolysis. Snake venom PLA_2 also affects neurons (pre- or post-synaptically) and skeletal muscle to cause neurotoxicity and myotoxicity, respectively. Snake venom PLA_2s also cause hemostatic disturbances by hydrolyzing pro-coagulant phospholipids (Amog *et al.,* 2016).

Ideally, treatment for snake bite is use of antiserum which is prepared from antibodies (IgG) of venom-immunized animals (usually horses) (Forks, 1994). Antiserum has many limitations including difficult storage, high cost, administration issues, short expiry, unavailability and low efficiency in the protection against haemorrhage, necrosis, nephrotoxicity, and local tissue damage. It can also cause allergic reactions (Cannon *et al.,* 2008). Finding alternative approaches is important and effective inhibitors from medicinal plant would be extremely useful (Rita *et al.,* 2011).

Various ethnobotanical studies have indicated that many medicinal plants have been used for decades as herbal antidotes, but limited scientific data is available for specific medicinal plants used against particular snake species. Pakistan has abundant medicinal plants with natural chemicals with potential anti-venom properties (Asad *et al.,* 2011). The historical mode of plant application for treatment of snakebites includes external routes. The most common type of preparation used is paste (*Achyranthes aspera*) (Samy *et al.,* 2008) followed by juice (*Albizia procera*) (Butt *et al.,* 2015) and powder (*Arisaema jacquemontii*) (Butt *et al.,* 2015) of plant parts or the whole plant. The highest percentage of treatments utilizing plant parts included leaves followed by roots, the whole plant, flowers, wood, and milky exudates (Asad *et al.,* 2011). Therefore, it is important to investigate these traditional medicinal plants by conducting pharmacological studies in order to determine whether plants have natural inhibiting properties that could enhance the action of antiserum by neutralizing venom-induced local tissue damage (Soares *et al.,* 2005), This study was performed to investigate the inhibition potential (through *in vitro* experiments) of medicinal plants extract against the PLA_2 activity of *Echis carinatus* venom.

MATERIALS AND METHODS

Collection of Echis carinatus snakes

Echis carinatus snakes were collected from the Thar (Sind province) and Cholistan (Punjab province) deserts Pakistan, with the help of local snake charmers and were identified by expert zoologist Dr. Muhammad Latif at the University of Education, Faisalabad Campus, Pakistan.

Echis carinatus venom extraction

Snake venom was obtained when their fangs over the cup by pressing the glands under their eyes. Venom was transferred into a sterilized light-resistant bottle and stored in an air-tight box at 4°C. Venom was dissolved in phosphate buffered saline (PBS) in terms of its dry weight (Razi *et al*., 2011).

Chemical reagents

Calcium chloride, sodium deoxycholate and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. All reagents were of analytical grade.

Plants collection

Medicinal plants with purported curative properties against snakebite were collected from different areas of Pakistan. After collection, they were identified by expert botanist Dr. Zafar Ullah Zafar and voucher specimens were deposited in the herbarium of the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan. A list of medicinal plants is summarized in table 1.

Reference standard antidote (anti-venom)

The standard antidote used was snake anti-venom serum (polyvalent equine immunoglobulin) which was purchased from the Biological Production Division, National institute of Health, Islamabad, Pakistan. It was used as a positive control to compare with plant extract results.

Plant material extraction and fractionation

Shade-dried plant material was chopped and subjected to a simple maceration process. For this process methanol was used as solvent, and dried powder of the desired part(s) of the plant (1 kg) was soaked in solvent (5 L). All soaked plants were kept at ambient temperature for about a month. Subsequently, filtration was carried out in two steps: 1) initially with a Whatman filter paper no 1 and later on via no 41. The solvent was evaporated and remaining extracts were stored for later use (Hussain *et al.,* 2007). For fractionation of active plants, a modified method of Kupchan (Muhit *et al*., 2010) was followed. Crude methanol extract (5 g) was suspended in distilled water and mixed thoroughly to make a solution that was then partitioned by the four solvents *n*-hexane, chloroform, dichloromethane and ethyl acetate using a separating funnel. All fractions were evaporated using a rotary evaporator (under reduced pressure), and dried extracts were stored in air tight containers in a refrigerator to be used in further research (Asad *et al*., 2014).

Phospholipase A_2 assay

Enzymatic assay for PLA_2 was followed by using method of Tan and Tan, 1988 with slight modification. Briefly, an egg yolk solution was prepared by mixing $CaCl_2$ (18 mM), deoxycholic acid (8.1 mM) and egg yolk for about 10 minutes. NaOH (1 M) was used to adjust the pH of the solution to 8.0. *Echis carinatus* venom (0.1-5mg/0.1mL) was added to 15 mL of the egg yolk solution, and NaCl was used in place of venom as a negative control. After two minutes, the pH change was measured with a pH meter. Drop in 01 pH correlated with 133 µmoles of fatty acid released in the egg yolk mixture. Anti-venom properties of medicinal plants were tested by pre-incubating snake venom (0.1mg) with their extracts (0.1 mg/mL) prior to adding them to egg yolk solution and measuring PLA_2 activity (Yap *et al.,* 2011).

Phytochemical screening

Phytochemical screening of secondary metabolites present in the bioactive plant extracts of medicinal plants under study was carried out according to standard procedures given in table 2.

STATISTICAL ANALYSIS

Results of experiments were presented as mean while inhibition of enzymatic activity was expressed in percentage. Student *t* test was used to compare the results with the standard drug and level of significance was set at $p>0.5$.

RESULTS

Echis carinatus venom released fatty acids during the acidimetric assay, indicating PLA_2 activity. Venom at a concentration of 0.1mg/mL liberated 21.28 µmol/min of free fatty acids, and the amount of liberated fatty acid increased with the concentration of venom. The change in pH of 1 unit corresponds to 133 µmol of fatty acids and was observed when pH decreased from 8 to 7 at a concentration of 5 mg/mL of venom. PLA_2 enzyme activity (units/mg) was found to increase with venom dose. Details of enzymatic activity are shown in table 3. Eighteen medicinal plant species from Pakistan were tested for their ability to inhibit/neutralize snake venom PLA_2 activity. Of these species, *Curcuma longa* L, *Citrullus colocynthis* (L.) Schrad and *Rubia cordifolia* Thumb were found to inhibit 70% of venom PLA_2 activity whereas *Fagonia cretica* Linn and *Adiantum capillus-veneris* L. inhibited 50% of PLA_2 activity. Neutralizing potentials of medicinal plants against venom PLA_2 activity has been summarized (table 4).

Fractionation of the most active plant extracts (*Curcuma longa, Citrullus colocynthis* and *Rubia cordifolia* using four solvents showed that the *n*-hexane fraction of *Curcuma longa* L. inhibited 78% PLA_2 activity, the *n*-hexane fraction of *Citrullus colocynthis* (L.) Scrad inhibited 77% and the ethyl acetate fraction of *Rubia cordifolia* Thumb inhibited 78.6% of PLA_2 activity (table

Table 1: List of Pakistani medicinal plants having ethnobotanical evidences to neutralize snake venom activity

Scientific name of medicinal plants	Family	Parts used	Voucher number of medicinal plants	References
Adiantum capillus-veneris L.	Adiantaceae	Whole plant	R.R. Stewart F.W. Pak. 4(2)	Butt *et al.*, 2015
Albizia lebbeck (L.) Benth.	Mimosaceae	Seeds	R.R. Stewart F.W. Pak. 381(9)	Butt *et al.*, 2015
Althaea officinalis L.	Malvaceae	Roots	R.R. Stewart F.W. Pak. 477(6)	Chopra *et al.*, 2008
Calotropis procera W. T. Aiton	Asclepiadaceae	Flowers	R.R. Stewart F.W. Pak. 566(6)	Basha, 2012
Citrullus colocynthis (L.) Schrad.	Cucurbitaceae	Fruits	R.R. Stewart F.W. Pak. 702(10)	Butt *et al.*, 2015
Curcuma longa L.	Zingiberaceae	Rhizome	R.R. Stewart F.W. Pak. 66(3)	Samy *et al.*, 2008
Eclipta prostrata L.	Asteraceae	Whole plants	R.R. Stewart F.W. Pak. 743(5)	Samy *et al.*, 2008; Naidu *et al.*, 2013
Fagonia cretica L.	Zygophyllaceae	Leaves and twigs	R.R. Stewart F.W. Pak. 433(2)	Razi *et al.*, 2011
Matthiloa incana (L.) R.Br.	Brassicaceae	Seeds	R.R. Stewart F.W. Pak. 322(2)	Asad *et al.*, 2014
Momordica charantia L.	Cucurbitaceae	Fruits	R.R. Stewart F.W. Pak. 706(1)	Grover & Yadav *et al.*, 2004; Kumar and Bhowmik, 2010
Trichodesma indicum (L.) R. Br.	Boraginaceae	Leaves	R.R. Stewart F.W. Pak. 604(3)	Dey & De, 2012
Psoralea corylifolia L.	Fabaceae	Seeds	R.R. Stewart F.W. Pak. 418(1)	Baquar, 1989
Rubia cordifolia Thumb.	Rubiaceae	Roots	R.R. Stewart F.W. Pak. 689(4)	Butt *et al.*, 2015
Sapindus mukorossi Gaertn.	Sapindaceae	Fruits	R.R. Stewart F.W. Pak. 463(3)	Butt *et al.*, 2015
Swertia chirayita (Roxb. ex Flem.) Karst.	Gentianaceae	Stems	R.R. Stewart F.W. Pak. 561(4)	Kumar *et al.*, 2010
Terminalia arjuna (DC) Wight & Arn	Combretaceae	Bark	R.R. Stewart F.W. Pak. 502(4)	Asad *et al.*, 2014
Lepidium sativum L.	Brassicaceae	whole plant	R.R. Stewart F.W. Pak. 319(4)	Jabeen *et al.*, 2017

Table 2: Standard chemical tests for confirmation of secondary metabolites in plants extract/fractions.

Secondary metabolite	Recipe/Procedure	Confirmation	References
Alkaloids	Plant extract + 1% HCl + steam. Add 6 drops of Wagner's reagent.	Red-brown precipitate	Maria *et al*., 2018
Carbohydrates	2ml extract + 5 drops of iodine solution	Blue color	Godghate *et al*., 2012
Fatty acids	Extract + ether + evaporate	Appearance of transparency on filter paper	Savithramma *et al.*, 2011
Flavonoids	Extract + dilute NaOH + dilute HCl	Intense yellow color which turns colorless with dilute HCl	Alabri *et al.*, 2014
Glycosides	Plant ext+ CH_3COOH(2ml) +few drops $FeCl_3$+1ml H_2SO_4	Formation of ring (brown)	Ayoola *et al.*, 2008
Phenols	Plant extract + 5% ferric chloride solution	Blue color	Jaradat *et al.*, 2015
Proteins	2ml extract + 1ml 40% NaOH + few drops of 1 % $CuSO_4$	Violet color	Ismail *et al.*, 2016
Tannins	Plant extract + 2% ferric chloride solution	Black or blue-green color	Jaradat *et al.*, 2015
Terpenoids	plant extract+0.5ml chloroform +few drops of sulfuric acid	Reddish brown color of interface	Ayoola *et al.*, 2008
Saponin	Extract+ deionized H_2O(5ml) shake thoroughly	frothing remains for a good one minute	Alabri *et al.*, 2014
Steroids	1 ml extract + 10ml of chloroform + 10 ml of sulphuric acid	$CHCl_3$ layer (red) and H_2SO_4 layer (yellow/greenish fluorescence)	Ayoola *et al.*, 2008

Table 3: Phospholipases A_2 activity present in *Echis carinatus* venom.

Concentration (mg/ml)	Change in pH	Fatty acid released/min (µmoles)	Enzyme activity of venom (unit/mg)
Control (saline)	8	0	0
0.1	7.84	21.28	133
0.2	7.7	39.9	249.3
0.4	7.42	77.007	481.29
0.8	7.31	91.371	571
1.6	7.26	98.42	615.1
3.2	7.16	111.72	698
5	7	133	831.25

Table 4: Neutralizing potential of medicinal plants of Pakistan against phospholipase A_2 present in *Echis carinatus* venom.

Evaluated sample	Change in pH	Percentage protection against PLA_2 (%)
Calotropis procera W. T. Aiton	7.75	17
Terminalia arjuna (DC) Wight & Arn	7.75	17
Psoralea corylifolia	7.8	33.33
Eclipta prostrate L.	7.71	3.33
Swertia chirayita (Roxb. ex Flem.) Karst.	7.81	37
Fagonia Cretica Linn.	7.86	53.33
Trichodesma indicum (L.) R. Br.	7.74	13.33
Eclipta prostrata L.	7.92	73.33
Syzygium cumini L.	7.85	50
Momordica charantia L.	7.75	17
Albizia lebbek Benth.	7.8	33.33
Citrullus colocynthis (L.)Schrad.	7.92	73
Rubia cordifolia Thumb.	7.909	70
Adiantum capillus-veneris L.	7.86	56
Sapindus mukorossi Gaertn.	7.793	33
Althaea officinalis Linn.	7.8	33
Lepidium iberis Linn.	7.781	27
Saline (negative control)	7.7	0
Anti-sera (standard anti-venom)	7.924	74

Table 5: Fractionation of active plants extract via different solvents inhibiting phospholipase A_2 activity present in *Echis carinatus* venom.

Name of active plant	Inhibition (%) of fractionated active plants extract in the solvents			
	n-hexane	Chloroform	Dichloromethane	Ethyl acetate
Curcuma longa L	78.33	63.33	77.33	43.33
Citrullus colocynthis (L.) Schrad	77	74	68.66	69
Rubia cordifolia Thumb	67	77.33	74.66	78.66

Table 6: Qualitative phytochemical tests for three active plants crude extract.

Phytochemicals	*Curcuma longa* (L.)	*Citrullus colocynthis* (L.) Scrad	*Rubia cordifolia* Thumb
Alkaloids	+		+
Flavonoids	+	+	+
Fatty acids	-	-	-
Steroids	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Glycosides	-	+	+
Saponins	+	-	+
Proteins	-	+	+
Terpenoids	+	+	+
Carbohydrate	-	-	-

Table 7: Qualitative phytochemical tests for active fractions of *Curcuma longa* (L.) crude extract.

Phytochemicals	*Curcuma longa* (L.)		
	n-hexane	Chloroform	Dichloromethane
Alkaloids	-	+	+
Flavonoids	+	+	+
Fatty acids	-	-	-
Steroids	-	+	+
Phenols	-	-	+
Tannins	-	-	+
Glycosides	-	+	+
Saponins	+	+	+
Proteins	-	-	-
Terpenoids	-	+	+
Carbohydrate	-	-	-

5). Tables 6 to 9 summarized the presence/absence of different phytochemicals in all active plants as well as their active fractions.

DISCUSSION

In Pakistan, due to lack of hospitals and unavailability of anti-venom (because it is expensive and requires specific storage conditions), people of rural areas use local medicinal plant speciess collected in the area or get them from traditional healers (Davidson *et al.*, 1995; Asad *et al.*, 2012). Medicinal plant extracts are purported to play an important role and have been used for decades to neutralize harmful effects caused by snake envenomation. Natural inhibitors are important because anti-venom serum, apart from unavailability, has certain limitations which include serum sickness, edema, hemorrhage and local effects which could lead to permanent scars and deformities. Anti-venom neutralizes snake venom by stopping further damage but cannot reverse the damage already done. It is important to search for different venom inhibitors, both synthetic and natural, that could complement the action of anti-venom (Makhija and Khamar, 2010).

PLA_2 enzymes are known for their local and systematic effects on victims due to their hydrolyzing nature (Ushanandini *et al.*, 2006). PLA_2s are a group of enzymes primarily used by snakes for prey incapacitation and digestion but in many species it shows a range of pharmacological activities like cardiotoxicity, neurotoxicity and myotoxicity (Shashidharamurthy and

Table 8: Qualitative phytochemical tests for active fractions of *Citrullus colocynthis* (L.) Scrad crude extract.

Phytochemicals	*Citrullus colocynthis* (L.) Scrad			
	n-hexane	Chloroform	Dichloromethane	Ethyl acetate
Alkaloids	+	+	+	-
Flavonoids	+	-	-	+
Fatty acids	-	-	-	-
Steroids	+	+	+	+
Phenols	+	-	-	-
Tannins	+	-	-	-
Glycosides	+	-	-	-
Saponins	-	-	-	-
Proteins	+	+	+	-
Terpenoids	+	-	-	+
Carbohydrate	-	-	-	-

Table 9: Qualitative phytochemical tests for active fractions of *Rubia cordifolia* Thumb crude extract.

Phytochemicals	*Rubia cordifolia* Thumb			
	n-hexane	Chloroform	Dichloromethane	Ethyl acetate
Alkaloids	+	+	+	+
Flavonoids	+	-	-	-
Fatty acids	-	-	-	-
Steroids	+	+	+	+
Phenols	-	+	-	+
Tannins	-	+	-	+
Glycosides	-	-	-	+
Saponins	-	-	-	-
Proteins	-	+	+	+
Terpenoids	+	+	+	+

Kempa-raju, 2006; Higuchi et al., 2007). The interaction of PLA_2s may include specific or non-specific phospholipase actions based on protein-phospholipid interaction along with covalent, non-covalent interactions (Asp 49) which could lead to release of free fatty acids (Kini, 2003). This is likely the reason that in present study when the venom amount was increased, the amount of liberated fatty acids also increased (in the presence of deoxycholic acid).

In previous studies, phospholipase A_2 activity was inhibited by methanolic extracts of different plants including *Hemidesmus indicus*, *Euphorbia hirta*, *Azadirachta indica*, and *Camellina sinensis* L. (Mukherjee *et al.,* 2008; Pithayanukul *et al.,* 2010; Gopi *et al.,* 2015). Aqueous extracts of *Albizia lebbeck* seeds inhibited edema and PLA2 activity in *Echis carinatus* venom (Amog *et al.,* 2016). In the present study *Curcuma longa* L., *Citrullus colocynthis* (L.) Schrad and *Rubia cordifolia* Thumb inhibited more than 74% PLA_2 activity. As in this study, Asad *et al.,* (2014) conducted research on medicinal plants from Pakistan against PLA_2 from *Naja naja karachiensis* venom. *Citrullus colocynthis* (L.) Schrad inhibited 100% of PLA_2 activity, whereas *Rubia cordifolia* Thumb inhibited 50%. Medicinal plants inhibit venom toxins owing to the presence of secondary metabolites as documented previously for tannins, flavonoids, alkaloids, glycosides xanthenes, terpenoids and steroids (Asad *et al.,* 2011). Phytochemical analysis indicates that plants contain many secondary metabolites along with phenols and that this could be the reason for inhibition of venom activity. Melo *et al.,* (2005) reported that *Curcuma longa*, due to the presence of the natural phenolic compound curcumine inhibited PLA_2-induced activity from *Bothrops alternatus* venom. Fractionation of all active plants showed that the highest inhibition (79%) was observed with the ethyl acetate fraction of *Rubia cordifolia* Thumb as observed previously with *Azima tetracantha* Lam against toxic PLA_2 activity from *Bungarus caeruleus* and *Vipera russelli* venoms (Janardhan *et al.,* 2014).

CONCLUSIONS

Indigenous medicinal plants (*Curcuma longa*, *Citrullus colocynthis* and *Rubia cordifolia*) were found beneficial to neutralize (78%) PLA_2 activity posed by Pakistani *Echis carinatus* venom. Based on present evaluation, they could be used as a first aid treatment if toxicity and other parameters addressed properly in future.

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REFERENCES

Afzal S, Afzal N, Awan MR, Khan TS, Gilani A, Khanum R and Tariq S (2009). Ethno-botanical studies from Northern Pakistan. *J. Ayub. Med. Coll. Abbottabad.*, **21**(1): 52-57.

Alabri THA, Al Musalami AHS, Hossain MA, Weli AM and Al-Riyami Q (2014). Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. *J. King. Saud. Univ. Sci.,* **26**(3): 237-243.

Amog PU, Manjuprasanna VN, Yariswamy M, Nanjaraj Urs AN, Joshi V, Suvilesh KN and Gowda TV (2016). Albizia lebbeck seed methanolic extract as a complementary therapy to manage local toxicity of *Echis carinatus* venom in a murine model. *Pharm. Biol.,* **54**(11): 2568-2574.

Asad MHHB, Murtaza G and Hussain I (2014). A Remedial Approach for *Naja naja karachiensis* Envenomation: Enzymatic Assay for Alkaline Phosphatase Activity in Extracts of Local Plants of Pakistan. *Pakistan. J. Zool*., **46**(6): 1775-1781.

Asad MHHB, Murtaza G, Siraj S, Khan SA, Azhar S, Sik M and Hussain I (2011). Enlisting the scientifically unnoticed medicinal plants of Pakistan as a source of novel therapeutic agents showing anti-venom activity. *Afr. J. Pharm. Pharmacol.,* **5**(20): 2292-2305.

Asad MHHB, Razi MT, Khan T, Saqib QNU, Murtaza G, Hussain MS and Hussain I (2012). Coagulopathies in *Naja naja karachiensis* (black Pakistan cobra) bites and its effect on coagulation tests upon storage of platelet poor plasma. *Acta. Pol. Pharm. Drug. Research*, **69**: 1031-1034.

Asad MHHB, Durr-e-Sabih, Choudary BA, Asad AF, Muratza G and Izhar Hussain I (2014). Compensatory effects of medicinal plants of Pakistan upon prolongation of coagulation assays induced by *Naja naja karachiensis* bite. *Curr. Sci.,* **106**(6): 870-873.

Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC and Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Trop. J. Pharm. Res.,* **7**(3): 1019-1024.

Ayyanar M and Subash-Babu P (2012). *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian. Pac. J. Trop. Biomed.,* **2**(3): 240-246.

Bansode TS and Salalkar D (2015). Phytochemical analysis of some selected Indian medicinal plants. *Int. J. Pharma. Bio. Sci.,* **6**(1): 550-556.

Baquar SR (1989). Medicinal and poisonous plants of Pakistan. 1st Ed. Printas Karachi Pakistan, pp.118-121.

Basha SK (2012). Traditional use of plants against snakebite in Sugali tribes of Yerramalais of Kurnool district, Andhra Pradesh, India. *Asian. Pac. J. Trop. Biomed.,* **2**(2): S575-S579.

Butt MA, Ahmad M, Fatima A, Sultana S, Zafar M, Yaseen G and Kayani S (2015). Ethnomedicinal uses of plants for the treatment of snake and scorpion bite in Northern Pakistan. *J. Ethnopharmacol.,* **168**: 164-181.

Cannon R, Ruha AM and Kashani J (2008). Acute hypersensitivity reactions associated with administration of crotalidae polyvalent immune Fab antivenom. *Ann. Emerg. Med.,* **51**(4): 407-411.

Chatterjee I, Chakravarty AK and Gomes A (2006). *Daboia russellii* and *Naja kaouthia* venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus* R. Br. *J. Ethnopharmacol.,* **106**(1): 38-43.

Chopra RN, Nayar SL and Chopra IC (1956). Glossary of Indian medicinal plants CSIR publication. *New Delhi*, India, pp.379-381.

Condrea E, De Vries A and Mager J (1964). Hemolysis and splitting of human erythrocyte phospholipids by snake venoms. *Biochim. Biophys. Acta*., **84**(1): 60-73.

Davidson TM, Schafer S and Killfoil J (1995). Cobras. *Wilderness. Environ. Med.,* **6**(2): 203-219.

Dey A and De JN (2012). Traditional use of plants against snakebite in Indian subcontinent: A review of the recent literature. *Afr. J. Tradit. Complement. Altern. Med.,* **9**(1): 153-174.

Forks TP (1994). Evaluation and treatment of poisonous snakebites. *Am. Fam. Physician.,* **50**(1): 123-30.

Godghate A, Sawant R and Sutar A (2012). Phytochemical analysis of ethanolic extract of roots of *Carrisa carandus* Linn. *Rasayan J. Chem*., **5**(4): 456-459.

Grover JK and Yadav SP (2004). Pharmacological actions and potential uses *of Momordica charantia:* A review. *J. Ethnopharmacol.,* **93**(1): 123-132.

Higuchi DA, Barbosa CMV, Bincoletto C, Chagas JR, Magalhaes A, Richardson M and Pesquero JL (2007). Purification and partial characterization of two phospholipases A_2 from *Bothrops leucurus* (white-tailed-jararaca) snake venom. *Biochimie*., **89**(3): 319-328.

Husain SZ, Malik RN, Javaid M and Bibi S (2008). Ethonobotanical properties and uses of medicinal plants of Morgah biodiversity park, Rawalpindi. *Pak. J. Bot.* **40**(5): 1897-1911.

Hussain A, Zia M and Mirza B (2007). Cytotoxic and Antitumor Potential of *Fagonia cretica* L. *Turkish. J. Biol.* **31**(1): 19-24.

Ismail AM, Mohamed EA, Marghany MR, Abdel-Motaal FF, Abdel-Farid IB and El-Sayed MA (2016). Preliminary phytochemical screening, plant growth inhibition and antimicrobial activity studies of *Faidherbia albida* legume extracts. *J. Saudi. Soci. Agr. Sci*. **15**(2): 112-117.

Jabeen A, Rani S, Ibrahim M and Mohammad AS (2017). A review *on Lepidium sativum*. *Indo. Am. J. Pharm. Sci.* **4**(8): 2223-2227.

Janardhan B, Shrikanth VM and Mirajkar KK (2014). In vitro screening and evaluation of antivenom phytochemicals from *Azima tetracantha* Lam. leaves against *Bungarus caeruleus* and *Vipera russelli*. *J. Venom. Anim. Toxins. Incl. Trop. Dis.* **20**(1): 12.

Jaradat N, Hussen F and Al Ali A (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata* Decne. *J. Mater. Environ. Sci.,* **6**(6): 1771-1778.

Kini RM (2003). Excitement ahead: structure, function and mechanism of snake venom phospholipase A_2 enzymes. *Toxicon*., **42**(8): 827-840.

Kumar KS and Bhowmik D (2010). Traditional medicinal uses and therapeutic benefits of *Momordica charantia* Linn. *Int. J. Pharm. Sci. Rev. Res.,* **4**(3): 23-28.

Kumar KS, Debjit B and Margret C (2010). *Swertia chirayita*: A traditional herb and its medicinal uses. *J. Chem. Pharm. Res.*, **2**(1): 262-266.

Kunjam SR, Jadhav SK and Tiwari KL (2013). Traditional herbal medicines for the treatment of snake bite and scorpion sting by the tribes of South Surguja, Chhattisgarh, India. *Med. Aromat. Plants*, **2**(1): 120.

Longbottom J, Shearer FM, Devine M, Alcoba G, Chappuis F, Weiss DJ and Williams DJ (2018). Vulnerability to snakebite envenoming: A global mapping of hotspots. *The Lancet*, **392**(10148): 673-684.

Makhija IK and Khamar D (2010). Anti-snake venom properties of medicinal plants. *Pharm. Lett.* **2**(5): 399-411.

María R, Shirley M, Xavier C, Jaime S, David V, Rosa S and Jodie D (2018). Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. *J. King. Saud. Univ. Sci.*, **30**(4): 500-505.

Melo MM, Habermehl GG, Oliveira NJF, Nascimento EF, Santos MMB and Lucia M (2005). Treatment of *Bothrops alternatus* envenomation by *Curcuma longa* and *Calendula officinalis* extracts and ar-turmerone. *Arq. Bras. Med. Vet. Zoo*, **57**(1): 7-17.

Muhit MA Tareq SM, Apu AS, Basak D and Islam MS (2010). Isolation and Identification of Compounds from the Leaf Extract of *Dillenia indica* Linn. *Bangladesh. Pharm. J.*, **13**(1): 49-53.

Mukherjee AK, Doley R and Saikia D (2008). Isolation of a snake venoms phospholipase A_2 (PLA_2) inhibitor (AIPLAI) from leaves of *Azadirachta indica* (Neem): mechanism of PLA_2 inhibition by AIPLAI *in vitro* condition. *Toxicon*., **51**: 1548-1553.

Nagaraju K, Kannappan N (2015) Survey on patern of snake bite cases admitted in South Indian Tertary Care Hospitals. *Int. J. Pharm. Sci. Rev. Res.*, **6**: 4362.

Naidu MT, Babu NC and Venkaiah M (2013). Ethnic remedies against snake bite from Kotia hills of Vizianagaram district, Andhra Pradesh, India. *Indian. J. Nat. Prod. Resour.*, **4**(2): 194-196.

Panhwar AQ and Abro H (2007). Ethnobotanical studies of Mahal Kohistan (Khirthar National Park). *Pak. J. Bot.*, **39**(7): 2301-2315.

Parveen G, Khan MF, Ali H, Ibrahim T and Shah R (2017). Determination of Lethal Dose (LD_{50}) of Venom of four Different Poisonous Snakes found in Pakistan. *Biochem. Mol. Biol. J.*, **3**(18): 1-5.

Pithayanukul P, Leanpolchareanchai J and Bavovada R (2010). Inhibitory effect of tea polyphenols on local tissue damage induced by snake venoms. *Phytother Res.*, **24**(S1): S56-S62.

Razi MT, Asad MHHB, Khan T, Chaudhary MZ, Ansari MT, Arshad MA and Najam-us Saqib Q (2011). Antihaemorrhagic (Antivenom) potentials of *Fagonia cretica* against Pakistani Cobra venom (*Naja naja karachiensis*). *Nat. Prod. Res.*, **25**(20): 1902-1907.

Rita P, Animesh DK, Aninda M, Benoy GK, Sandip H and Datta K (2011). Snake bite, snake venom, anti-venom and herbal antidote: A review. *Int. J. Res. Ayurveda. Pharm.*, **2**(4): 1060-1067.

Samy RP, Thwin MM, Gopalakrishnakone P and Ignacimuthu S (2008). Ethnobotanical survey of folk plants for the treatment of snakebites in Southern part of Tamilnadu, India. *J. Ethnopharmacol.*, **115**(2): 302-312.

Savithramma N, Linga Rao M and Suhrulatha D (2011). Screening of Medicinal Plants for Secondary Metabolites. *Middle-East J. Sci. Res.*, **8**(3): 579-584.

Shashidharamurthy R and Kemparaju K (2006). A neurotoxic phospholipase A_2 variant: Isolation and characterization from eastern regional Indian cobra (*Naja naja*) venom. *Toxicon.*, **47**(7): 727-733.

Soares AM, Ticli FK, Marcussi S, Lourenco MV, Januario AH, Sampaio SV and Pereira PS (2005). Medicinal plants with inhibitory properties against snake venoms. *Curr. Med. Chem.*, **12**(22):2625-2641.

Tan NH and Tan CS (1988). Acidimetric assay for phospholipase A_2 using egg yolk suspension as substrate. *Anal. Biochem.*, **170**(2): 282-288.

Tetik F, Civelek S and Cakilcioglu U (2013). Traditional uses of some medicinal plants in Malatya (Turkey). *J. Ethnopharmacol.*, **146**: 331-346.

Thirumalai T, Elumalai EK, Therasa SV, Senthilkumar B and David E (2010). Ethnobotanical survey of folklore plants for the treatment of jaundice and snakebites in Vellore districts of Tamilnadu, India. *Ethnobot. Leaflets*. **4**: 15.

Tu AT (1996). Overview of snake venom chemistry. *Adv. Exp. Med. Biol.*, **391**: 37-62.

Urs AN, Yariswamy M, JoshiV, Suvilesh KN, Sumanth MS, Das D and Vishwanath BS (2015). Local and systemic toxicity of *Echis carinatus* venom: neutralization by *Cassia auriculata* L. leaf methanol extract. *J. Nat. Med.* **69**(1): 111-122.

Ushanandini S, Nagaraju S, Harish Kumar K, Vedavathi M, Machiah DK, Kemparaju K and Girish KS (2006). The anti-snake venom properties of *Tamarindus indica* (leguminosae) seed extract. *Phytother. Res.*, **20**(10): 851-858.

Van-Wagenen BC, Larsen R, Cardellina JH, Randazzo D, Lidert ZC and Swithenbank C (1993). Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. *J. Org. Chem.*, **58**(2): 335-337.

Walter CY, Shinwari ZK, Afzal IM and Malik RN (2011). Antibacterial activity in herbal products used in Pakistan. *Pak. J. Bot.*, **43**: 155-62.

Warrell D (1995). Clinical toxicology of snakebite in Asia. Handbook of clinical toxicology of animal venoms and poisons. CRC Press, Boca Raton, USA.

Warrell DA and Guterrez JM (2013). New approaches and technologies of venomics to meet the challenge of human envenoming by snakebites in India. *Indian. J. Med. Res.*, **138**(1): 38.

World Health Organisation. Rabies and envenomings: A neglected public health issue. Geneva, 2007.

Yap MKK, Tan NH and Fung SY (2011). Biochemical and toxicological characterization of *Naja sumatrana* (Equatorial spitting cobra) venom. *J. Venom. Anim. Toxins. Incl. Trop. Dis.*, **17**(4): 451-459.