Metal analysis, phytotoxic, insecticidal and cytotoxic activities of selected medicinal plants of Khyber Pakhtunkhwa

Fazli Khuda[1]*, Zafar Iqbal[1], Zakiullah[1], Ayub Khan[2], Fazli Nasir[1], Naveed Muhammad[1], Jamshaid Ali Khan[1] and Muhammad Shafiq Khan[3]

[1]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan
[2]Department of Chemistry, Kohat University of Science and Technology, Kohat, Pakistan
[3]Department of Pharmacy, Hazara University, Mansehra, Pakistan

Abstract: In the present study four medicinal plants traditionally used in Pakistan for treatment of various ailments were evaluated for their heavy metals content, insecticidal, cytotoxic and phytotoxic actions. The metals like Cr, Cu, Zn, Mn, Ni, Pb, Fe and Co were determined in crude extract and various fractions. Soil samples were also tested for heavy metals to determine assimilation of any metal by the plant. Lead, Chromium, copper, nickel and cobalt exceeded the permissible limit in most of the tested samples while the concentration of zinc, manganese and iron was within the permissible limit. Chloroform fraction from *Achyranthes aspera* and ethyl acetate fraction from *Duchesnea indica* showed significant phytotoxic activities. Crude extract and chloroform fraction from *Xanthium strumarium* showed insecticidal activity comparable to that of permethrin and thus could be a significant source of natural insecticide. The butanol fraction from *X. strumarium* showed significant cytotoxicity with LC_{50} 1.9306 µg/ml, having mortality rate 93% at highest dose, while the crude extract from *Valeriana wallichii* showed 90 % mortality rate (LC_{50} 4.9730 µg/ml) at highest dose. However, the extracts from other plants were not effective against the brine shrimps tested.

Keywords: Medicinal plants, Hazardous heavy metals, phytotoxicity, insecticidal activity, cytotoxicity.

INTRODUCTION

Despite the development of large number of clinically effective compounds, indigenous phytotherapy is still practiced in many rural areas of developing countries. World wide about 85% of all medications for primary health care are derived from natural sources (Abbasia *et al*., 2010).

Possessing diverse climate and edaphic conditions, Pakistan occupies a unique position among developing countries having considerable potential within the variety of medicinal plants (Khan and Gul, 2007). Among the 6000 flowering plants reportedly present in Pakistan, about 400–600 are considered to be of medicinal value.

The practice of herbal treatment is well established in Pakistan like most other developing countries of the world. Large numbers of Hakims are involved in this practice especially in the rural areas of the country. It is interesting that over 50% of the population in Pakistan is being treated with traditional medicines by almost 50,000 traditional herbal practitioners *Hakims*. More than 350 regular items (as whole herbs or specific parts) have been enlisted, which are used in unani herbal preparations by various Herbal Drugs Manufacturing Laboratories (*Dawakhanas*) in Pakistan. As for herbal medicine, Pakistan is among the eight leading exporters of medicinal plants. Traditional health care systems using medicinal plants can be recognized and used as a starting point for the development of novelties in drugs (Khan *et al*., 2008; Ahmad *et al*., 2008; Shaikh and Hatcher, 2005).

Valeriana wallichii (Valerianaceae) is one of the most important and most recognized species of *Valeriana* genus, comprises of nearly 250 species with important pharmacological effects and has been used as sedative, anxiolytic (Marder *et al*., 2003; Bos *et al*., 1998; Fernandez *et al*., 2004), and as a diuretic in traditional herbal medicine. It has received wide attention in the last few decades due to its remarkable pharmacological effects, particularly as tranquilizer (Gilani *et al*., 2005), anti-epileptic, insomnia and habitual constipation (Nadkarni, 1986; Rehman, 2006).

Xanthium strumarium (Compositae), an annual herb, mainly distributed in China and Europe. In traditional Chinese medicine, *X. strumarium* has been used for sinusitis, headache, urticaria, emphysema, and arthritis (Qin *et al*., 2006; Han *et al*., 2007; Yoon *et al*., 2003). The whole plant was found to posses diaphoretic, sedative and diuretic property. Literature data indicate its efficacy in alleviating longstanding cases of malarial fever (Sharma, 2003). The genuses Xanthium also possess antiviral, antibacterial, insecticidal, herbicidal and antitrypanosomal (Tsankova *et al*., 1994; Talakal, *et al*., 1995; Kamboj and Saluja 2010), antimalarial (Joshi *et al*., 1997), fungicidal (Ginesta-Peris *et al*., 1994), and cytotoxic activities against cancer cell lines (Kinghorn *et al*., 1999). It has been found to possess hypoglycemic activity in normoglycaemic rats (Talakal *et al*., 1995).

**Corresponding author:* fazli_khuda2008@yahoo.com

Achyranthes aspera (Amaranthaceae) an erect herb, used in Ayurveda medicine as an abortifacient (Vasudeva and Sharma, 2006). The roots of *A. aspera* are used for contraceptive purposes, inflammatory disorders, malarial fever, hypertension, leprosy, dyspepsia, renal and vesical calculi, and general debility (Paul *et al.*, 2006; Chakraborty *et al*., 2002; Rao *et al.*, 2006; Gokhale *et al*., 2002). The hypoglycemic effect of *A. aspera* has been reported in diabetic rabbits (Akhtar and Iqbal, 1991), and in rats (Tahiliani and Kar, 2000**)**.

Duchesnea indica (Rosaceae)**,** a perennial herb, commonly occur on shady, grassy slopes (up to 2400 meters) in India, Pakistan and China (Qiao *et al.,* 2009)**.** Ethnobotanical studies indicate that the plant is widely used in the treatment of inflammatory disorders, cough, diarrhea and pharyngitis, (Lee *et al.*, 2008; Zuoa *et al.*, 2008; Peng *et al.,* 2008). The anticancer properties of *D.indica* have also been reported (Graham *et al.,* 2000; Choudhary *et al.*, 1995; Purohit and Vyas 2004).

Although plant based medicines are considered to be relatively free from side effects as compared to allopathic medicine but the presence of toxic metals in these products is a health issue (Hussain *et al*., 2006). In this context the present study was conducted to explore the biological properties and metal profile of the above mentioned plants which are extensively employed in traditional medicines.

MATERIAL AND METHODS

Plant material

Leaves of *X. strumarium* and *A. aspera* were collected from Charsadda (Peshawar Division), Khyber Pakhtunkhwa, Pakistan. The leaves of *V. wallichii* and roots of *Duchesnea indica* were collected from Bara Gali (Hazara Division), Khyber Pakhtunkhwa, Pakistan. Authentication of the plant material was done by Prof. Dr. Muhammad Ibrar, Department of Botany, University of Peshawar for *V. wallichii, X. strumarium, A. aspera and D. indica* and a specimen with catalogue No: 9526 (BOT), 8708 (BOT), 8708-1 (BOT) and 10708 (BOT) were deposited, respectively in the herbarium of the same department. Soil was collected for each plant from the place of collection.

Plant extraction and fractionation

The powdered plant material (1 kg each plant) was extracted with methanol at room temperature. The methanolic extract was filtered through filter paper and the marc obtained was again macerated with methanol. The same process of extraction was repeated three times and the combined filtrates were concentrated under vacuum at low temperature (40°C) using rotary evaporator (Khan *et al*., 2007), until 50 g of the crude extracts of each plant were obtained. The crude extract was dissolved in distilled water and sequentially partitioned with various solvents to obtained *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fractions.

Metals analysis

Two grams (2 g) of each fraction were taken in a small beaker. 10 ml of concentrated Nitric acid was added and allowed to stand overnight. Each beaker was heated carefully on a hot plate until the production of red NO_2 fumes had ceased. They were then allowed to cool down and 2-4 ml perchloric was added and were again heated and allowed to evaporate to a small volume. They were cooled again and 10 ml of aqua regia was added and each was heated until evaporate to a small volume. The samples were transferred to a 50 ml volumetric flask and diluted to volume with distilled water (Saeed *et al.*, 2010). The stock solutions, appropriately labeled were analyzed through the Atomic Absorption Spectrotometer (Parkin Elmer AAS 700) for quantitative detection of Cr, Cu, Zn, Mn, Ni, Pb, Fe, Co.

In vitro Phytotoxicity assay

In vitro Phytotoxicity assay was carried out for the crude extract and subsequent solvent fractions against *Lemna minor* (Zaidi *et al*., 2006). The medium was prepared by mixing various inorganic components in 100 ml of distilled water and KOH solution was added for the adjustment of pH at 6.0-7.0. The medium was autoclaved at 121°C for 15 min. Test samples (30 mg) dissolved in ethanol (1.5 ml), served as stock solution. Nine flasks (three for each dilution) were inoculated with 1000, 100 and 10 µl of the stock solution for 500, 50 and 5 ppm and were then completely evaporated at room temperature. Working E-medium (20 ml) along with a rosette of two to three fronds (Total 20) of Lemna *minor* were then added to each flask. Flasks supplemented with E-Medium and plant served, as negative control while flasks containing medium, reference drug (Paraquat) was used as positive control. All flasks were then incubated in growth chamber at 30^{0} C for seven days with relative humidity 56 ± 10 % and 9000 lux light intensity, and were daily examined during this period. At the end of incubation period number of fronds per flask were counted, recorded and the growth regulation (in percent) was then determined with reference to negative control.

In vitro insecticidal activity

In vitro insecticidal assay was carried out for the crude extract and its various fractions of each plant against *Sitophilus oryzea, Tribolium castaneum, Callosobruchus analis* and *Rhyzopertha dominica* following method available in literature (Atta-ur-Rahman *et al*., 2001). The test sample was prepared (200 mg of crude extract was dissolved in 3 ml of methanol and served as stock solution). The sample (1572.7 µg/ cm^2) was loaded over the filter paper of appropriate size (9 cm or 90 mm) on

Petri plate using micropipette. The plate was left overnight (24 h) to evaporate the solvent. Next morning, 10 healthy and active insects of each species of same size and age were added to each plate including control (methanol) and standard drug (Permethrin, 235.71 µg/cm^2). There after the plates were incubated in growth chamber at 27°C for 24 h with 50% relative humidity. For calculation, the number of survived insects was counted and the mortality (%) was determined using formula. Results were the mean of three different experiments.

Brine shrimp lethality assay

Brine shrimp (*Artemia saline* Leach) eggs were placed in a hatching tank containing sea water for 48 h (Mohtasheem *et al*., 2001). Crude extract and its subsequent fractions (20 mg) were dissolved in 2ml of DMSO. From this solution, 10, 100 and 1000 µl/ml concentration solutions were prepared in three separate vials. The solvent was then evaporated and 30 shrimps were added in each vial and the final volume (5 ml) was made with simulated sea water (Sea salt 38 g/L of distilled water, pH 7.4). The vials were then incubated under illumination at 25-27 ^{0}C for 24 hours, and the survived brine shrimps were counted in each vial. LC_{50} values (95% confidence interval) were then determined using "Finney" computer software. Two vials, supplemented with reference cytotoxic drug etoposide (Lianyungang Guiyuan Chem Pharm Co, China) and solvent served as positive and negative controls, respectively.

RESULTS

Lead, one of the toxic metals, has no known beneficial function in the body, but its accumulation causes a number of adverse effects on various body systems. The permissible limit of lead according to Word Health Organization for medicinal plants is 10 ppm (WHO, 1998). Excluding the Chloroform and *n*-butanol fractions of *X. strumarium* all samples have high concentration of lead. The soil samples of all plants also showed the outstanding concentration of lead. The highest concentration was observed in Chloroform (421.68 ppm) and *n*-hexane (154.98 ppm) fractions of *D. indica.*

Chromium is one of the abundant elements on the earth (Emsley and John, 2001). It is found as trivalent and hexavalent. The trivalent is safe for human and the hexavalent is carcinogenic (Food and Nutrition Board, 2001). Chromium was found in concentration rage of 7.08-278.16 ppm as shown in the table 1. The permissible limit of Cr in plants is 1.5 ppm (Markert, 1994). The results have shown that almost all samples exceeding the permissible limit. The highest concentration was observed in the soil sample of *A. aspera* (278.16 ppm) and in crude extract (266.28 ppm) of *V. wallichii.* Copper was detected in the concentration rage of 0.11-228.96 ppm. The permissible limit of Cu is 10 ppm in plants (Markert, 1994). The Cu concentration was with in limit in chloroform, ethyl acetate, Aqueous, and *n*-butanol fractions of the *V. wallichii*, ethyl acetate faction of *A. aspera* and Aqueous and *n*-butanol factions of *D.indica.* While the remaining all fractions showed high concentration of Cu. Zinc was present in the concentration of 6.12-483.8 ppm as shown in table 1. Zinc is one of the essential trace metals for all living organisms. The recommended dietary allowance (RDA) of *Zn* for children is 3-8 mg/day and for adults it is 11mg/day (Saeed *et al*., 2010). The permissible limit of Zinc in medicinal plants is 50 ppm. Almost all fractions were found within normal zinc concentrations however, all the soil samples have high Zn content. The highest Zn concentration was found in aqueous faction of *D. indica* (398.76 ppm). In the present study manganese was found in the range of 0.24-5994 ppm showing that the plant does not possess metal accumulation. WHO has no permissible limits for Mn, however in the literature it is 200 ppm for medicinal plants (Srivastava *et al*., 2006). The soil samples of all plants were found with high concentrations of Mn while the entire plants fractions have Mn within permissible limit. Nickel was present in concentration range of 3.96–243 ppm. Taking in larger quantities, Ni can cause cancer of different organs such as nose, lungs and prostate. The most common side effects from Ni are allergic reactions in hypersensitive persons produced at the site of contact (ATSDR, 2008). The permissible limit of Ni in plants is 1.5 ppm which shows that all the tested samples exceed the permissible limit. Iron was found in the range of 140.76-1412.28 ppm but this concentration is not toxic. Cobalt naturally occurs in plants, animals, rocks, and water. The concentration range of Co was 0.24–95.88 ppm. The permissible limit of Co in medicinal plants is 0.2 ppm so almost all samples exceed the permissible limit.

In order to determine the assimilation of heavy metals by a certain fraction, all the groups were compared using *ANOVA*. No significantly different group as regards the presence of heavy metals were observed (P>0.05), showing that the heavy metals are randomly distributed and there is no assimilation of them by a certain fraction.

Crud extracts as well as various solvent fractions were also analyzed for their phytotoxic activities (Table 2). Significant dose dependent phytotoxicity was observed for ethyl acetate fraction of *D. indica* (70 % inhibition at 500 µg/ml) for *L. minor,* the activity increasing with high concentration. This activity is comparable to that of herbicide Paraquat showing the presence of some herbicidal principle in the extract. The chloroform fraction from *A .aspera* showed 20, 35 and 60% growth inhibition at 5, 50 and 500 µg/ml, respectively. Further, the highest phytotoxic activity was observed for aqueous and *n*- butanol fractions from *X. strumarium*. Contrary to

Table 1: Concentration (ppm) of various toxic heavy metals in crude extract and solvent fractions

Plants name	Fractions and soil	Cr	Cu	Zn	Mn	Ni	Pb	Fe	Co
Valeriana wallichii	*n*-hexane	204.12 ± 0.05	34.68 ± 0.01	44.52 ± 0.11	6 ± 0.20	27.96 ± 0.06	19.68 ± 0.09	483.48 ± 0.12	4.2 ± 0.20
	Chloroform	209.54 ± 0.06	0.11 ± 0.05	31.32 ± 0.03	0.76 ± 0.11	13.98 ± 0.30	17.65 ± 0.05	132.43 ± 0.08	2.65 ± 0.12
	Ethyl acetate	226.68 ± 0.20	0.12 ± 0.02	21.6 ± 0.11	0.84 ± 0.05	18.96 ± 0.13	21 ± 0.02	185.76 ± 0.17	0.24 ± 0.05
	n-butanol	208.08 ± 0.11	6.36 ± 0.20	27.36 ± 0.07	1.08 ± 0.11	21.12 ± 0.12	96.36 ± 0.13	127.56 ± 0.17	4.2 ± 0.12
	Aqueous	212.88 ± 0.05	0.24 ± 0.05	36.36 ± 0.12	4.56 ± 0.20	10.92 ± 0.08	30.84 ± 0.60	239.28 ± 0.11	1.8 ± 0.20
	Crude	266.28 ± 0.12	11.28 ± 0.06	11.64 ± 0.11	6 ± 0.05	20.4 ± 0.04	46.2 ± 0.06	140.76 ± 0.17	3.84 ± 0.05
	Soil	86.9 ± 0.09	101.4 ± 0.08	372.72 ± 0.20	294 ± 0.11	79.56 ± 0.03	130.56 ± 0.17	130 ± 0.13	52.44 ± 0.17
Xanthium strumarium	*n*-hexane	65.4 ± 0.11	22.2 ± 0.13	23.16 ± 0.19	1.08 ± 0.05	10.8 ± 0.20	54.96 ± 0.11	130.8 ± 0.05	11.4 ± 0.13
	Chloroform	43 ± 0.12	10.56 ± 0.11	31.54 ± 0.12	15.43 ± 0.09	22.54 ± 0.06	8.9 ± 0.17	131.54 ± 0.08	5.6 ± 0.19
	Ethyl acetate	42 ± 0.13	32.4 ± 0.13	29.52 ± 0.20	29.4 ± 0.13	29.76 ± 0.19	20.28 ± 0.12	1412.28 ± 0.17	6.36 ± 0.11
	Chloroform	43.0 ±	10.56 ±	31.54 ±	15.43 ±	22.54 ±	8.9 ±	131.54	5.6 ±
	n-butanol	60.12 ± 0.05	11.4 ± 0.20	92.04 ± 0.06	16.32 ± 0.17	5.16 ± 0.06	7.32 ± 0.12	273.36 ± 0.19	1.56 ± 0.13
	Aqueous	62.52 ± 0.11	12.72 ± 0.02	14.4 ± 0.05	6.96 ± 0.20	19.08 ± 0.07	41.88 ± 0.05	163.8 ± 0.13	2.28 ± 0.06
	Soil	142.56 ± 0.05	107.16 ± 0.05	483.8 ± 0.20	599 ± 0.07	153.24 ± 0.19	160.56 ± 0.11	ND	72.96 ± 0.05
Achyranthes aspera	*n*-hexane	30.24 ± 0.06	41.04 ± 0.13	22.68 ± 0.07	0.24 ± 0.13	9.36 ± 0.13	ND	129.72 ± 0.06	6.72 ± 0.06
	Chloroform	57.96 ± 0.05	47.4 ± 0.07	33.72 ± 0.13	8.76 ± 0.17	16.2 ± 0.05	29.52 ± 0.13	147.12 ± 0.04	2.16 ± 0.13
	n-butanol	64.23 ± 0.06	54.67 ± 0.17	11.65 ± 0.05	7.98 ± 0.13	16.84 ± 0.13	23.16 ± 0.05	167.96 ± 0.06	3.51 ± 0.17
	Ethyl acetate	74.88 ± 0.08	8.28 ± 0.05	15.12 ± 0.19	4.56 ± 0.05	13.28 ± 0.13	11.52 ± 0.19	558.96 ± 0.07	6.96 ± 0.19
	Aqueous	61.72 ± 0.13	13.43 ± 0.11	17.54 ± 0.13	3.78 ± 0.17	14.41 ± 0.11	23.15 ± 0.19	154.87 ± 0.06	4.32 ± 0.17
	Crude	87.48 ± 0.08	15.6 ± 0.13	14.76 ± 0.08	5.88 ± 0.13	28.44 ± 0.19	53.04 ± 0.11	190.32 ± 0.04	6.72 ± 0.08
	Soil	278.16 ± 0.11	228.96 ± 0.19	372.6 ± 0.06	3393 ± 0.17	243 ± 0.08	96.84 ± 0.19	ND	95.88 ± 0.11
Duchesnea indica	*n*-hexane	42.52 ±	16.74 ±	24.63 ±	0.54 ±	18.72 ±	154.98	176.74	ND
	Chloroform	61.8 ± 0.1	24.6 ± 0.06	120 ± 0.07	10.92 ± 0.06	42.24 ± 0.11	421.68 ± 0.08	458.88 ± 0.17	6.72 ± 0.11
	Ethyl acetate	12.36 ± 0.06	12.72 ± 0.06	15.84 ± 0.08	0.36 ± 0.08	6.24 ± 0.11	ND	125.76 ± 0.08	ND
	n-butanol	24.72 ±	7.8 ±	6.12 ±	0.5 ±	3.96 ±	120.12	114.6 ±	ND
	Aqueous	103.08	8.68 ±	398.76	17.76 ±	28.2 ±	33.96 ±	481.56	ND
	Crude	7.08 ± 0.20	11.28 ± 0.08	14.76 ± 0.11	1.2 ± 0.06	4.8 ± 0.20	ND	228 ± 0.30	2.4 ± 0.11
	Soil	231.48 ± 0.20	186 ± 0.30	282.12 ± 0.05	1207.5 ± 0.5	198.24 ± 0.20	59.04 ± 0.05	ND	66.84 ± 0.20

ND=Not detected. Data are expressed as the mean ± SD (n=3)

that, crude extract and various fractions from *V. wallichii* failed to exhibit any significant phytotoxic activity even at highest dose levels.

All the four plants were also screened for their insecticidal activity. Permethrin was used as standard, showing 100% insecticidal activity at concentration 235.71 µg cm^2. Only crude extract and chloroform fractions from *X. strumarium* showed activity comparable to that of standard, where as the rest possess only weak or no activity (40% each against *R. dominica* by hexane fraction from *A. aspera* and *D. indica*).

Table 2: Phytotoxic activity of the crude extract and subsequent fractions against the *Lemna minor*

Plant species	Extracts	Control	No. of fronds survived					
			5µg		50 µg		500 µg	
			Sample	GI (%)	Sample	GI (%)	Sample	GI (%)
Valeriana wallichii	*n*-hexane	20	19	05	18	10	17	15
	Chloroform	20	18	10	17	15	16	20
	Ethyl acetate	20	20	-	20	-	19	05
	n-butanol	20	19	05	18	10	17	15
	Aqueous	20	20	-	19	05	18	10
	Crude	20	20	-	19	05	19	05
Xanthium strumarium	*n*-hexane	20	20	-	19	05	18	10
	Chloroform	20	19	05	18	10	17	15
	Ethyl acetate	20	20	-	19	05	18	10
	n-butanol	20	18	10	17	15	12	40
	Aqueous	20	18	10	17	15	12	40
	Crude	20	18	10	18	10	18	10
Achyranthes aspera	*n*-hexane	20	20	-	20	-	20	-
	Chloroform	20	16	20	13	35	08	60
	Ethyl acetate	20	20	-	19	05	18	10
	n-butanol	20	20	-	19	05	18	10
	Aqueous	20	20	-	20	-	20	-
	Crude	20	15	25	12	30	10	50
Duchesnea indica	*n*-hexane	20	20	-	17	15	15	25
	Chloroform	20	20	-	20	-	20	-
	Ethyl acetate	20	10	50	08	60	06	70
	n-butanol	20	14	30	12	40	10	50
	Aqueous	20	14	30	12	40	10	50
	Crude	20	18	10	17	15	15	25

GI = % Growth Inhibition, Standard drug = Paraquat (3.142µg/ml).

Table 3: Insecticidal activity displayed by crude extract and various fractions of selected medicinal plants

Plant species	Extract	[1]Mortality (%)				
		Insects[a]	R.d.	T.c.	C.a.	S.o.
Valeriana wallichii	*n*-hexane	-	20	20	-	-
	Chloroform	-	-	-	-	-
	Ethyl acetate	-	-	-	-	-
	n-butanol	-	-	-	-	-
	Aqueous	-	-	-	20	-
	Crude	-	-	-	-	-
Xanthium strumarium	*n*-hexane	-	-	-	-	-
	Chloroform	-	40	20	100	-
	Ethyl acetate	-	-	-	-	-
	n-butanol	-	-	-	-	-
	Aqueous	-	-	-	-	-
	Crude	-	40	40	100	-
Achyranthes aspera	*n*-hexane	-	40	20	-	-
	Chloroform	-	40	-	-	-
	Ethyl acetate	-	-	-	-	-
	n-butanol	-	-	-	-	-
	Aqueous	-	-	-	-	-
	Crude	-	-	-		-
Duchesnea indica	*n*-hexane	-	40	-	-	-
	Chloroform	-	-	-	-	-
	Ethyl acetate	-	-	-	-	-
	n-butanol	-	-	-	-	-
	Aqueous	-	-	-	-	-
	Crude	-	-	20	-	-

[1]The mortality is expressed relative to standard which is taken as 100%

Insects[a]: R.d., *Rhyzopertha dominica*; T.c., *Tribolium castaneium*; C.a., *Callosbruchus analis*; S.o., *Sitophilus oryzae.*

Table 4: Cytotoxic activity of the crude extract and subsequent fractions at concentration of 10, 100 and 1000 µg/ml using Brine shrimp lethality test.

Plant species	Extracts	Control	No. of survivors			
			10µg/ml	100 µg/ml	1000 µg/ml	LC_{50} (µg/ml)
Valeriana wallichii	*n*-hexane	30	26	25	24	-
	Chloroform	30	27	25	16	1750.4020
	Ethyl acetate	30	28	28	32	-
	n-butanol	30	26	25	21	-
	Aqueous	30	24	24	15	1833.0190
	Crude	30	13	07	03	4.9730
Xanthium strumarium	*n*-hexane	30	29	24	28	-
	Chloroform	30	23	27	16	6864.1820
	Ethyl acetate	30	27	26	22	-
	n-butanol	30	09	13	02	1.9306
	Aqueous	30	25	24	21	-
	Crude	30	15	15	04	21.5204
Achyranthes aspera	*n*-hexane	30	27	26	24	-
	Chloroform	30	27	26	24	-
	Ethyl acetate	30	26	22	19	-
	n-butanol	30	30	28	24	-
	Aqueous	30	28	27	25	-
	Crude	30	28	22	22	-
Duchesnea indica	*n*-hexane	30	26	24	25	-
	Chloroform	30	30	30	29	-
	Ethyl acetate	30	27	27	25	-
	n-butanol	30	29	30	28	-
	Aqueous	30	30	30	20	-
	Crude	30	29	30	28	-

Std. Drug; Etoposide, LC_{50} (7.4625 µg/ml)

The cytotoxic properties of the crude extract and various fractions were investigated at concentration of 10,100, and 1000 µg/ml, using etoposide as standard (LC_{50} 7.4625 µg/ml). Crude extract from *V. wallichii* showed significant cytotoxic activity (LC_{50} 4.9730 µg/ml), where as all other fractions were similar, exhibiting weak cytotoxic activity (LC_{50} more than 1750.4020 µg/ml). Similarly crude extract and *n*- Butanol fraction from *X. strumarium* showed significant activity (LC_{50} 21.5204 µg/ml and 1.9306 µg/ml respectively), the later being more potent than etoposide. All the other fractions were similar, having no significant activity. Similarity among groups was determined using *ANOVA* technique showing no significant difference among groups ($P>0.05$ for *V. wallichii* and *X. strumarium* respectively). *A. aspera* and *D. indica* showed no cytotoxicity at all.

DISCUSSION

Weeds are among the most important factors responsible for low yield of crops. In Sindh and Punjab about 40 different weed species have been reported from wheat crop which account for about 40 percent yield losses on the average. Various chemical herbicides are effectively used for controlling these weeds. However, their use is restricted by certain factors like environmental pollution, carcinogenic effects, residual toxicity, and high cost associated with the use of these herbicides (Saeed *et al*., 2010). Therefore, alternative herbicides which are safe and cost effective are to be investigated. Natural herbicides may be one of these alternatives. It is therefore, assumed on the basis of results that the phytotoxic principle of the ethyl acetate fraction from *D.indica* could be a significant source of natural herbicides for weeds control in a sustainable manner to increase per acre yield.

Reportedly, about 20% of total world production is deteriorated by microbes and other insects annually. These are effectively controlled by the continuous application of various insecticides such as phosphine and methyl bromide, but their use has adversely affected the biological control systems. Plants possessing insecticidal principles, which are often active against specific insects, may be used as potential alternatives to these synthetic insecticides. This could lead to the development of more safe and effective compounds. Our results showed significant insecticidal activity against *R. dominica, C. analis and T. castaneum* which confirm the usefulness of *X. strumarium* as potent insect control agent.

Recently, wide number of plants has been investigated for their antitumor properties. We select Brine shrimps larvae to determine the preliminary cytotoxicity screening of selected plant extracts in different solvents. The butanol

extract from X. *strumarium* exhibited maximum cytotoxicity for brine shrimps as 93% of the shrimps were killed at high dose level. Further investigations are going on in our laboratory to isolate and characterize the active components of the plant extract.

CONCLUSION

Our screening demonstrated strong insecticidal, cytotoxic as well phytotoxic activities in a dose dependent manner, however the absence of cytotoxic effects in *Achyranthes aspera* and *Duchesnea indica* suggests that the plants are probably safe to be used in traditional medicine, though further tests for safety may be appropriate.

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