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Pharmacological evaluation of *Euphorbia helioscopia* L. and *Euphorbia hirta* L.

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Abstract

Medicinal plants are a natural source for the curing of various diseases and free from side effects. In the current study the cytotoxic and phytotoxic potential of *E. helioscopia* and *E. hirta* have been investigated. In cytotoxic bioassay at 1000 µg/ml *E. helioscopia* showed 100% death rate which indicated the cytotoxic potential of *E. helioscopia*, while in phytotoxic bioassay *E. hirta* showed good phytotoxic potential. Both the plants were highly toxic at high concentration, as the concentration increased mortality rate was also increased, so it is evaluated that both the plants are dose dependent.

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Introduction

The pharmacognosy has been derived from two Greek words i.e "pharmakon" means "a drug" and "gignosco" means to "acquire a knowledge of" it is closely related to botany, chemistry and history entitles it to be the parent of both sciences (Trease and Evans, 1992). Humans have relied on nature throughout the ages for their basic necessities like the food production, shelter, clothing, means of transportation, fertilizers, flavors, fragrances, and not the least medicine" (Fakim, 2006). Medicinal plants are moving from fringe to a major stream, used by greater number of people who seek remedies for health with the belief that it will be free from side effects which is caused by synthetic chemicals. In recent past, considerable attention had been gained by the utilization of ecofriendly and biofriendly plant based products for curing and prevention of various human diseases. Considering the high degree of side effects of synthetic medicines, the global population is searching for natural remedies, which are relatively safe and effective (Dubey, 2004). Crops have always been suffered by the presence of weeds for which synthetic herbicides are intensively used for eradication. These synthetic herbicides have been proved to be harmful to the environment due to the contamination of soil, water and foodstuffs which in turn affect human health (Wahab, 2009 and, Mancini *et al.,* 2008). These facts had provoked the researchers towards finding out the alternative source for weed management, from natural sources, which can be biodegrable and act by their allelopathy (Petroski & Stanley, 2009). Plants extract containing various secondary metabolites as bioactive compounds. Extract of these plants are often toxic to *Artemia salina* (leach) shrimp larvae (Kivack *et al.,* 2001, Carballo, 2002). The toxic effect of plants on shrimps larvae is related to their antitumor potential, as shrimps larvae show similar response as the mammalian carcinoma do (Mclaughlin, 1991; Solis, 1993). The brine shrimps bioassay is an easy and conventional preliminary model for selection of bioactive plant extract possess cytotoxic potential which in turn speaks about the antitumor properties of plants (Fatimi *et al.* 2007). Alkaloidal fractions of phytocomponents are known to be responsible for the cytotoxicity (Lagnika, 2011), similarly among the phytochemicals, flavonoids, saponins, terpines were also reported with pronounced antitumor/cytotoxic potentials (Wang, 2000). Various other researchers like Tolulope (2007) evaluated hydro-methanolic extracts of *Hibiscus sadbariffa*, Khan & Khan (2007) evaluated various fractions of crude extracts of *Rhazya stricta,* Muthiah (2008) analysed *Hopea utilis* for cytotoxic potential, similarly Many workers like Ali *et al.* (2009) *Euphorbia wallichii*; Khan *et al.* (2011) *Euphorbia prostrate* carried out biological screening of plants for phytotoxic bioassays**.**

Materials and methods

Plant collection

The fresh plant specimens of *Euphorbia helioscopia* L. and *Euphorbia hirta* L. were collected at their fruiting stages, from various localities of Peshawar including campus of Peshawar University, Pakistan.

Extracts preparation

The dried plant specimens were ground to 60 mesh diameter through an electric grinder. Hundred grams (100 g) of powdered plant samples were soaked in 500 ml of 70 % ethanol for 72 hours and there after filtered through Whatman filter paper. To the residue again 500 ml of 70% ethanol was added and filter after 72 hours and this process was repeated three times. The three filtrates were mixed and concentrated by evaporating through rotary evaporator at 40° C. These concentrated extracts were stored at 4° C in refrigerator priors to use. The extracts were dissolved in dimethylsulphoxide (DMSO) to which distilled water was added to prepare stock solutions, from which the required concentrations were prepared for different biological activities.

Cytotoxic bioassay

The cytotoxic activity of the crude ethanolic extracts of the plants were carried out by using Brine shrimps lethality assay following the standard method of

McLaughlin *et al.* 1991, Zakaria *et al.* 2007 and Atta Ur Rahman *et al.,* 2001.

Bioassay requirement

Brine shrimps eggs (*Artemia salina*), Sea Salt solution (38 g/L, pH 7.4), A small tank with perforated partition, Light source to attract shrimp larvae, Magnifying glass, Aluminum foil, Micro pipettes (10, 100 and 1000µl), and glass vials.

Brine shrimps eggs hatching.

The hatching tray (rectangular tray) was half filled with filtered brine solution (38 g/L). The tank was divided into two unequal halves with a perforated partition. About 50 mg of brine shrimps eggs were sprinkle in the small half, which was covered with aluminium foil to make it dark. A bulb was hanged over the tray, which served to illuminate the large half of the tray. After 24-48 hours, the eggs hatched into larvae which actively swim and passed through the perforations of partition into the large illuminated partition of the hatching tray. These larvae were used for cytotoxicity test of the plant extract.

Sample preparation

The ethanolic extracts (100mg) were dissolved in 10 ml of ethanol with a gentle heating on water bath. This solution acted as a stock, and from it, 5, 50 and 500 µl solutions were transfer to glass vials (3 vials for each concentration). The vials were left uncovered over night, so that the solvent from the vials evaporated. To each vial 10 brine shrimps larvae were transferred with the help of long tipped dropper and the volume of each vial was made to 5ml with the brine solution, making the concentration to 10, 100 and 1000 µg/ml (ppm) respectively.

The other three vials were added with brine solution to serve as negative control, and a reference cytotoxic drug (3 vials) as positive control.

The number of dead larvae in each vial was recorded after 24 hours and the data was analyze with BIOSTATE statistical software to determine Finney LD_{50} values with a fitted least square regression line and 95% confidence levels.

Phytotoxic Bioassay

Phytotoxic bioassay of the crude 70% ethanolic extracts was carried out using *Lemna minor* toxicity assay following the standard procedure after McLaughlin *et al.* 1991. The growth medium for *Lemna* was prepared by dissolving various constituent salts in distilled water (1L) and pH was of the medium was adjusted to pH 5.5-5.6 by adding potassium hydroxide (KOH) pellets. The medium was autoclaved at high temperature (121° C) for fifteen minutes prior to use. The ethanolic extracts (100 mg) of both plants were dissolved in ethanol (10 ml) to prepare a stock solution. Three concentrations were prepared as 10, 100 and 1000µg/ml by taking 10, 100 and 1000µl of the stock in Petri dish. The solvent was allowed to evaporate overnight under sterile conditions. After evaporation of solvent 10 ml of growth medium was added to each petri plate. Three Petri plates were taken for each concentration. To each Petri dish *Lemna minor* plants were added (10 individual plants), each of which consist of a rosette of 3 fronds. Three other plates each were provided with growth media and a standard herbicide, each serving as negative and positive controls respectively. Total number of fronds per petri plate was counted on third and sixth day. Percent growth inhibition for each treatment was calculated as follow. % Growth Regulation = $100 - \frac{\text{Number of fronds in test samlpe}}{\text{Number of fronds in possitive control}} \times 100$.

Result and discussion

Cytotoxicity

The plants extracts having secondary metabolites as bioactive compounds, are often toxic to *Artemia salina* (leach) shrimp larvae (Kivack *et al.* 2001, Carballo *et al.* 2002). The toxic effect of plants on shrimps larvae is related to their antitumor potential, because shrimps show similar response as the mammalian tissues (Mclaughlin *et al.* 1991; Solis *et al.* 1993) This cytotoxic screening is a preliminary model for selection of bioactive plant extracts against cancer (Fatimi *et al* . 2007).

In the present study, *Artimia salina* toxicity bioassay was conducted to evaluate the toxic potential of *E. helioscopia* and *E. hirta*. The 70% ethanolic extracts of both the plants were applied at three different concentrations (10, 100 and 1000µg/ml). The experimental data obtained for bioassay (Table 1) showed a dose dependent cytotoxicity was exhibited by *E. helioscopia* towards the shrimp's larvae. A positive linear correlation was observed between dose concentration and percent mortality, at the highest experimental dose (1000 µg/ml) 100 % mortality was observed as compared to 10 µg/ml concentration where the shrimp's mortality percentage was comparatively low (70%). The LD_{50} was found to 3.08µg/ml. The 95% confidence limits and fitted regression line are given (Table 1).Similar results were obtained in case of *Euphorbia hirta,* where the toxicity was also dose dependent. The cytotoxicity was highly significant with LD_{50} of 7.60µg/ml. The percent mortality at various doses, 95% confidence intervals of LD_{50}, chi square values and fitted regression lines are given (Table 1).

Table 1. Cytotoxic potential of *E. helioscopia* and *E. hirta*.

Extract Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD_{50}	LD_{50} S.E	95% CL		Least square line	χ^2 (p)
						LCL	UCL		
	Euphorbia helioscopia								
0	30	28	6.67	3.087	5.287	0.06	9.51	Y= 4.54+0.95X	0.027 (0.87)
10	30	9	70.00						
100	30	3	90.00						
1000	30	0	100.00						
	Euphorbia hirta								
0	30	28	6.67	7.60	4.78	1.52	15.66	Y= 4.92+1.22X	0.011 (0.82)
10	30	13	56.67						
100	30	3	90.00						
1000	30	1	96.67						

Alkaloidal fraction of phytocomponents is known to be responsible for the cytotoxicity (Lagnika *et al,* 2011), similarly among the phytochemicals, flavonoids, saponins, terpines were also reported with pronounced antitumor/cytotoxic potentials (Wang *et al.* 2000). The promising cytotoxicity of both *E. helioscopia* and *E. hirta* was according to their phytochemical architect, where most of these cytotoxic phytochemical showed positive detective tests.

Phytotoxicity

The crops have always been suffered by the presence of weeds. Synthetic herbicides intensively used for eradication of weeds have proved to be harmful to the environment due to the contamination of soil, water and foodstuffs which in turn affecting human health (Wahab, 2009, Mancini *et al,* 2008). These facts had provoked the researchers towards finding out the alternative source for weed management, which should act by allelopathy mechanism (Petroski and Stanley, 2009). For the last few decades extensive research work has been carried out on the plants having potent allelochemicals with phytotoxic activity and hence could be used as eco-friendly herbicides (Dayan *et al.,* 2000). *Lemna* bioassay is an easy method for detection of biodegradable herbicides, and plant growth promoter substances which may to some extent can fill the commercial need for natural herbicides (Atta-ur-Rehman *et al*, 1991).

To ascertain the phytotoxic potential of *E. helioscopia* and *E. hirta,* ethanolic extracts of both these plants tested for their possible phytotoxicity. The phytotoxic potential was generally found to be dose and time dependent (Table 2). Three doses of *E. helioscopia* (10, 100 and 1000µg/ml) were used, exhibited low to moderate level of toxicity towards Lemna fronds proliferation with respect to the following criterion.

30- 40% inhibition= low toxicity
50% inhibition= moderate toxicity

60-70% inhibition= Good toxicity

Above 70% inhibition= significant toxicity.

The data obtained after 3 days of *E. helioscopia* extract treatment showed 29.73, 40.54 and 54.05 % inhibition respectively at 10, 100 and 1000µg/ml doses whereas after 6 days of extract application the percent inhibition was 32.56, 48.84 and 67.44 % at 10, 100 and 1000µg/ml concentration respectively (Table 2).

Table 2. Phytotoxic potential of *E. helioscopia* and *E. hirta*.

Plant	Dose µg/ml	NO. of fronds in test	% inhibition	Estimate of α (intercept)	Estimate of β (Slope)	FI_{50}
Euphorbia helioscopia	3 days					
	0 (Control)	37	0	5.86	-0.32	505.80
	10	26	29.73			
	100	22	40.54			
	1000	17	54.05			
	6 days					
	0 (Control)	43	0	5.91	-0.45	105.33
	10	29	32.56			
	100	22	48.84			
	1000	14	67.44			
Euphorbia hirta	3 days					
	0 (Control)	37	0	5.84	-0.55	33.10
	10	22	40.54			
	100	16	56.76			
	1000	7	81.08			
	6 days					
	0 (Control)	43	0	5.59	-1.16	3.20
	10	12	72.09			
	100	2	95.35			
	1000	0	100			

On the other hand *E. hirta* showed significant activity causing 40.54, 56.76 and 81.08% inhibition at 10, 100 and 1000µg/ml after 3 days of extract application while the percent inhibition after 6 days of extract application was 72.09, 95.35 and 100% at 10, 100 and 1000µg/ml doses respectively (Table 2). This indicates that *E. hirta* can be used as a useful bioherbicide.

The FI_{50} values for *E. helioscopia* were 505.80 and 105.33µg/ml at 3rd and 6th day of extract application respectively, while for *E. hirta* FI_{50} values at 3rd and 6th day of treatments applications were 33.10 and 3.20µg/ml respectively (Table 2).

Many workers carried out phytotoxicological studies of various members of family euphorbiaceae e.g. Ali *et al,* (2009) carried the phytotoxic activity of *Euphorbia wallichii* root extract obtained from chloroform, n-hexane, n-butanol and ethyl acetate. All of these exhibited a high degree of Phytotoxicity (60-100%) at high concentration (1000 µg/ ml) while at low concentration (10 µg /ml) they exhibited 30-80% Phytotoxicity. Ayatollahi *et al.* (2010) reported significant phytotoxicity of ethyl acetate extract of aerial part of *Euphorbia Aellenii*. Shanee *et al.* (2011) tested the phytotoxic effects for aqueous extract of different parts of *Euphorbia dracunculoides* at two different concentrations on germination and seedling growth of *Cicer arietinum* (chickpea). Onocha *et al.* (2011) reported significant phytotoxicity for the methanolic extract of leaves and stems of *Acalypha torta, A. hispida*, and *A. wilkesiana* (Euphorbiaceae) *lemna minor*. Similarly Khan *et al.* (2012) observed *Euphorbia prostrata* with significant and dose dependent inhibitory potential on the germination and seedling growth of wheat. The results of these researchers are strongly agreed with our current findings.

References

Ali A, Naz R, Khan WN, Gul RMI, Choudhary MI. 2009. Biological screening of different root extracts of *Euphorbia wallichii*. Pakakistan Journal of Botany **41(4),** 1737-1741.

Atta-ur-Rehman. 1991. Studies in Natural Product Chemistry, Netherlands, Elsevier Science Publishers **9,** 383 – 409.

Atta-ur-Rhman, Choudhary MI, Thomsen WJ. 2001. Bioassay technique for drug development. Harwood Academic Publishers.

Ayatollahi AM, Ghanadian M, Afsharypuor S, Siddiq S, Pour-Hosseini M. 2010. Biological Screening of *Euphorbia Aellenii*. Iranian journal of pharmaceutical research (IJPR) **9,** 429-436.

Carballo LJ, Hernandez-inda LZ, Perzer P, Gravalos MD. 2002. A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. BioMed Central **2(1),** 1.

Dayan FE, Romagni JG, Duke SO. 2000. Journal of Chemical Ecology **26,** 2079–2094.

Dubey NK, Kumar R, Tripathi P. 2004. Global promotion of herbal medicine: India's opportunity, *Current Science* **86(1),** 37-41.

Fakim AG. 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspect of Medicine **27(1),** 1-93.

Fatimi M, Wurster M, Schroder G, Lindequist U. 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. Journal of Ethnopharmacol **11,** 657-666. http://dx.doi.org/10.1691/ph.2013.2729

Khan RA, Shah AS, Ahmad M, Khan FU, Aslam N, Khan MR, Shah MS. 2012. Phytotoxic activity of crude methanolic extract of *Euphorbia prostrata* collected from Bannu District (Pakistan). African Journal of Biotechnology **11(10),** 2513-2517. http://dx.doi.org/10.5897/AJB11.154

Khan RA, Shah AS, Ahmad M, Khan FU, Aslam N, Khan MR, Shah MS. 2011. Phytotoxic activity of crude methanolic extract of *Euphorbia prostrata* collected from Bannu District (Pakistan). African Journal of Biotechnology **11(10),** 2513-2517.

Kivack B, Mert T, Tansel H. 2001. Antimicrobial and cytotoxic activities of Ceratonia siliqua L. extracts. Turkish Journal of Biology **26,** 197-200.

Khan S, Khan GM. 2007. In Vitro Antifungal Activity of Rhazya stricta Decne Pakistan Journal of Pharmaceutical Science **20(4),** 274-279.

Lagnika l, Aanago E, Sanni A. 2011. Screening for antibacterial, antioxidant activity and toxicity of some medicinal plants used in Benin flkloric medicine. Journal of Medicinal Plant research **5(5),** 773-777.

Mancini F, Termorshuizen AJ, Jiggins JLS, Bruggen AHC. 2008. Increasing the environmental and social sustainability of cotton farming trough farmer education in Andhra Pradesh. Indian Agriculture Systems **96,** 16-25.

Mclaughing Jl, Chang CJ, Smith DL. 1991. Bench top bioassays for the discovery of bioactive natural products: an update. In: studies in natural products chemistry, Atta ur Rehman, (Ed) vol., Elsevier, Amsterdam 383-409.

McLaughlin JL. 1991. Crown-gall tumours in potato discs and brine shrimp lethality: Two simple bioassays for higher plant screening and fractionation. In: Hostett-mann K, ed. Methods in Plant Biochemistry. Acad. Press, London 1-31.

Muthiah M. (2008). Antimicrobial and cytotoxic Activities of *Hopea utilis* Fruits. *Ethnobotanical Leaflets* **1(75),** 1-1.

Onocha PA, Oloyede GK, Dosumu OO, Ali MS. 2011. Antileishmaniasis and Phytotoxicity of three Nigerian *Acalypha* species. Archives of Applied Science Research **3(6),** 1-5.

Petroski RJ, Stanley DW. 2009. Natural compounds for pest and weed control. Journal of Agricylture and Food Chemistry **57(18),** 8171-8179. http://dx.doi.org/10.1021/jf803828w

Shanee S, Tanveer A, Javaid MM, Chaudhry KM, Aziz A, Khaliq A, Chaudhry MN, Pervez MA, Awan IU. 2011. Phytotoxic effects of *Euphorbia dracunculoides*: a weed of rainfed chickpea-chickpea cropping system. Spanish Journal of Agricultural Research **9(20),** 580- 588.

Solis PN, Wright CW, Andreson MM, Gupta MP, Phillopson JD. 1993. A microwell cytotoxicity assay using *Artemia salina*. Planta Medica **59,** 250-252.

Tolulope OM. 2007. Cytotoxicity and antibacterial activity of Methanolic extract of Hibiscus sabdariffa. Journal of Medicinal Plants Research **1(9),** 13.

Trease GE, Evans WC. 1992. Pharmacognosy. 13th ed., English Language Book, Society, Baillere Tindall, Oxford University Press 3-5.

Wahab V. 2009. Biotechnological approaches for crop pest and disease management. Journal of Biopesticides **2(2),** 115-134.

Wang X, Wang J, Wong SC, Chow LS, Nicholls JM, Wong YC, Liu Y, Kwong DL, Sham JS, Tsa SW. 2000. Cytotoxic effect of gossypol on colon carcinoma cells. Life Science **67,** 2663-2671.

Zakaria HM, Mainen JM, Pax JM, Modest CK, Ramadhani SON. 2007. Antimicrobial Activity and Brine Shrimp Toxicity of Extracts of *Terminalia brownii* roots and stem**.** BMC Complementary and Alternative Medicine **7(9),** 1-5.