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Full Length Research Paper

Phytochemical and cytotoxic analysis of *Pharthenium hysterophosis* selected from District Bannu, Pakistan

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***Parthenium hysterophorus* is a well known medicinal plant widely used traditionally in the treatment of various diseases and as a constituent of various drugs, and in phytotherapy. The current study was designed to investigate the phytochemical screening and cytotoxic capacity of methanolic and n-hexane extract of *P. hysterophorus*. Quantitative analysis of *P. hysterophorus* showed maximum quantity of flavonoids in methanolic extract of *P. hysterophorus* which turned down gradually in n-hexane extract of *P. hysterophorus* due to the decrease in organic solvents polarity. Similar results were also observed for saponins and tannins during this investigation. The highest quantity of alkaloids was recorded in the methanolic extract of *P. hysterophorus* when compared to n-hexane extract. The extracts also showed maximum cytotoxic potential in various concentrations of n-hexane and methanolic extract of *P. hysterophorus*. The results revealed that *P. hysterophorus* contains a remarkable cytotoxic activity due to the presence of bioactive constituents.**

Key words: *Parthenium hysterophorus*, cytotoxic, phytochemical screening.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999; Sahreen et al., 2010, 2011). Numerous studies have revealed that medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Mothana et al., 2004). Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (Khan et al., 2010). The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Scientific experiments since the late 19th century have documented the antioxidant properties of some spices, herbs, and their components. Many studies reported the activities of spices and herbs on food borne pathogenic microorganisms (Khan et al., 2009, 2011).

Parthenium hysterophorus, Inula aucherana, Fumaria officinalis, Crocus sativus, Vicum album, Tribulus terestris, Polygonatum multiflorum, Alkanna tinctoria and *Taraxacum officinale* has been widely used in traditional medicine as spice or herb so long a time. Among them, particularly *P. hysterophorus* has special importance for medicinal use. The present investigation was arranged for

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Table 1. Phytochemical composition of *P. hysterophorus.*

Sample	Flavonoids	Alkaloids	Terpenoids	Coumarins	Saponins	Cardiac glycosides	Phlobatannins
PM	+	+	+	+	+	+	+
PH	+	+	-	+	+	-	-

+, presence; -, absence; PM, *P. hysterophorus* methanolic; PH, *P. hysterophorus* n-hexane.

phytochemical and cytotoxic characterization.

MATERIALS AND METHODS

Plant collection

Collection of *P. hysterophorus* was carried out from District Bannu (Pakistan) in June 2010, identified and a specimen was submitted through voucher R-110 at Herbarium of Bannu, University of Science and Technology Bannu, Pakistan. It was shade dried at 25°C for two weeks, chopped and powdered mechanically up to mesh size 0.5 mm.

Extract preparation

3 kg powder of *P. hysterophorus* was extracted two times in 5 L of methanol, after a week of socking, filtration were conducted with whatmann filter paper # 45 and evaporated through rotary evaporator to obtain crude methanolic extract. The extract was fractionated with n-hexane. Both the fractions were stored further for phytochemical and *in vitro* investigations at 4°C.

Phytochemical studies

Presence of various chemicals in each fraction was carried out by using standard procedures. Qualitative studies for flavonoids, alkaloids, terpenoids and saponins were carried out according to Harborne (1973), while tannins, coumarins, cardiac glycosides, anthraquinones and phlobatanins by were determined by Trease and Evans (1989). Quantitative determination of flavonoids (Boham and Kocipai, 1974), alkaloids (Harborne, 1973), tannins and saponins (Obadoni and Ochuko, 2001) were done.

Cytotoxic brine shrimp bioassay

Cytotoxic activities of methanolic crude extract of *P. hysterophorus* was carried out according to the standard procedure of Meyer-Alber et al. (1992). Sample was prepared by dissolving 5 mg of crude plant extract in respective solvent (methanol) to form stock solution of 5 mg/ml in methanol and further diluted into 500, 1000 and 1500 µg/ml. 28 g sea commercial sea salt (Sigma) was dissolved in one liter of dH_2O with constant stirring for 2 h. Brine shrimps were hatched in two compartment rectangular tray having sea salt saline. 0.5 ml of each dilution was put in vials and evaporated then 2 ml of saline was added. Eight shrimps were transferred to each vial and the volume was increased up to 5 ml and incubated at 25 to 28°C. After 24 h of incubation, survivors were counted with the help of 3× magnifying glass and calculation was done using Abbot's formula;

% Death = (Sample-control/control) × 100

LD_{50} was determined through prism graph pad software.

Statistical analysis

To determine LD_{50}, Graph prism pad software was used while SPSS 13 was used for mean analysis.

RESULTS

Phytochemical analysis of *P. hysterophorus*

Phytochemical screening provides basic information about the medicinal importance of the plant extracts. *P. hysterophorus* methanolic (PM) extract showed the presence of alkaloids, flavonoids, saponins, terpenoids, coumarins, phlobatannins and cardiac glycosides; whereas cardiac glycosides, terpenoids, and phlobatannins were absent in *P. hysterophorus* n-hexane (PH) extract as shown in Table 1. Quantitative analysis of *P. hysterophorus* showed maximum quantity of flavonoids in PM which turned down gradually in pH due to the decrease in organic solvents polarity. Similar results were also observed for saponins and tannins during this investigation. Highest quantity of alkaloids was recorded in PM as compared to pH (Figures 1 to 3). Similar results were reported by various studies which show that methanolic fraction possess the highest amount of phenolic and poly phenolic compounds (Mustafa et al., 2010). A number of previous studies also reported the same results which are in close association to our study (Aruoma, 2003; Coruh et al., 2007).

Determination of cytotoxic activity through brine shrimp assay

Preliminary screening of plant extracts through cytotoxicity provides helpful information about the anticancer activity of the extracts. Cytotoxic effect of various fractions of *P. hysterophorus* was measured against brine shrimps growth under controlled condition using normal control (Table 2). After complete hatching, shrimps were transferred into glass bottles with saline of sea salt and extracts of various fractions. After 24 h, the effects of fraction were noted and it was found that the brine shrimp survival was inversely proportional to the concentration of the various fractions of *P. hysterophorus. P. hysterophorus* showed maximum death which might be due to the presence of bioactive constituent. Our results show that the brine shrimp survival was inversely proportional to the concentration of

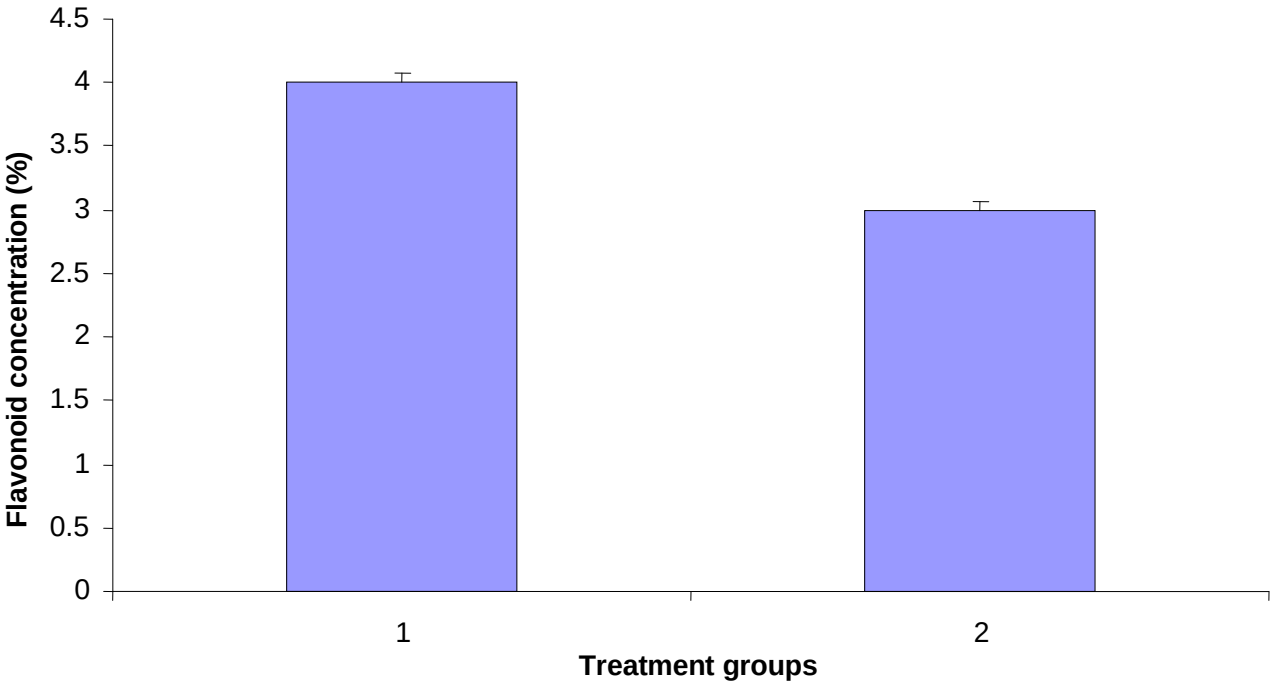

Figure 1. Percent flavonoid of various fractions of *P. hysterophorus.* 1, PM; 2, PH.

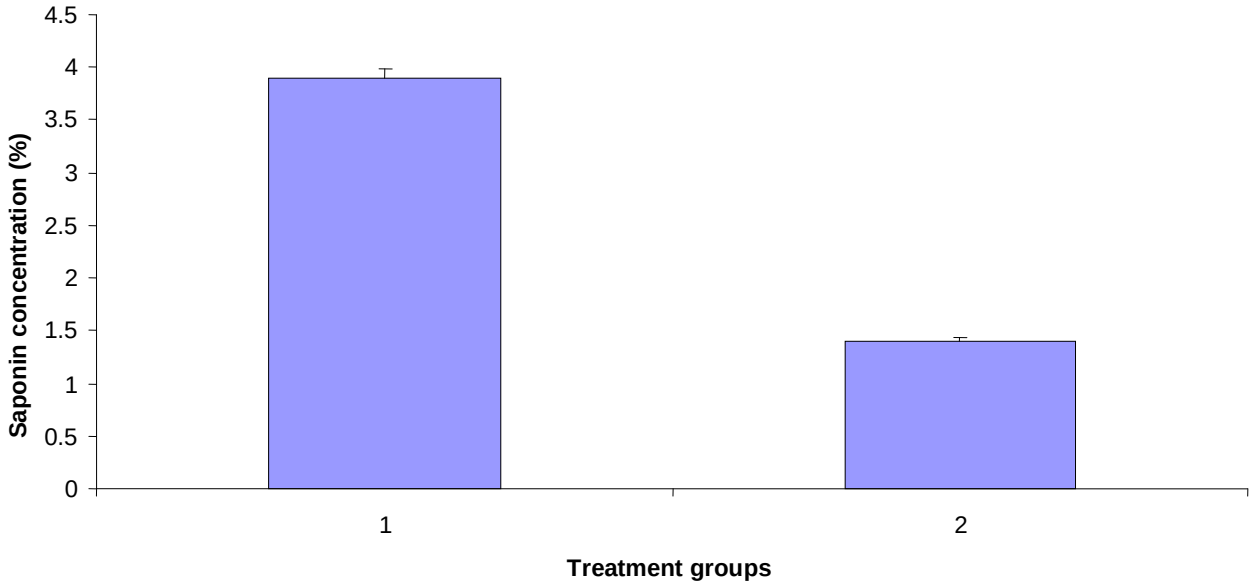

Figure 2. Percent saponin of various fractions of *P. hysterophorus.* 1, PM; 2, PH.

the extract used. Zaidi et al. (2006) showed that methanolic fraction of *Arceuthobium oxycedri* exhibited 100% cytotoxicity for brine shrimps at high dose which are in accordance to our results. The results of the present study evaluated the folk use of these medicinal plants and suggest that methanolic fraction possess some bioactive constituents having anticancer activities that can be the focal point of new drugs having anticancer and protective role against different pathogens.

Conclusion

Methanolic plant extracts of *P. hysterophorus* showed significant activity, and this might be the presence of bioactive phenolic and polyphenolic constituents in the

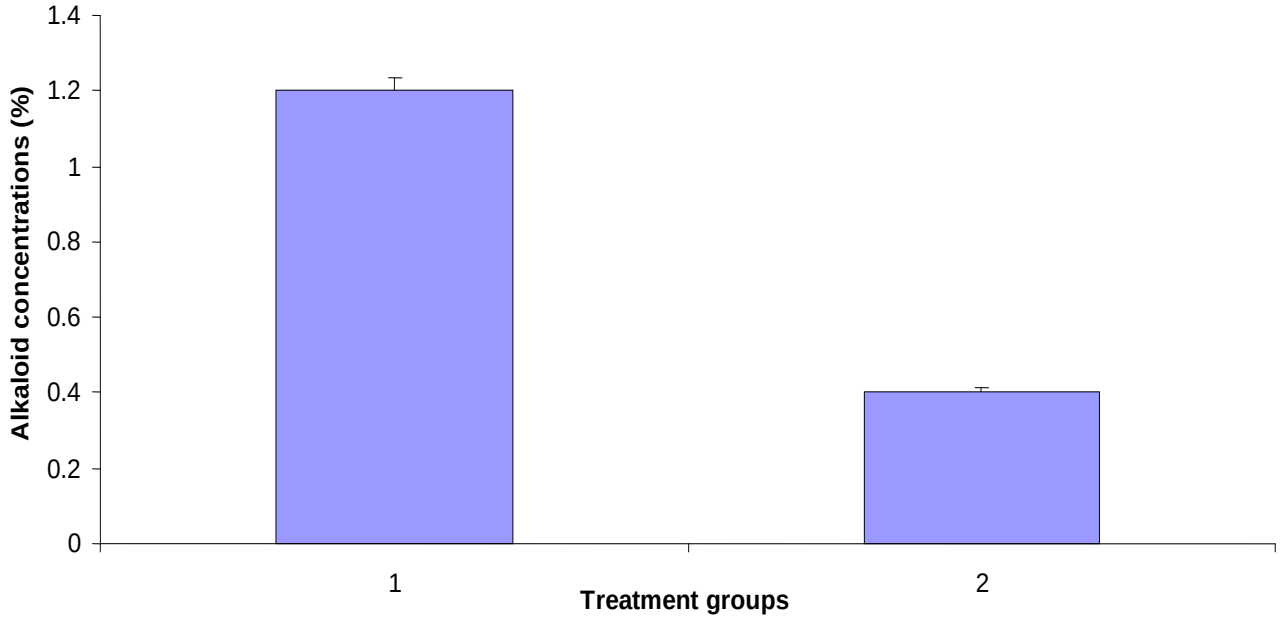

Figure 3. Percent alkaloids of various fractions of *P. hysterophorus.*1, PM; 2, PH.

Table 2. Cytotoxicity brine shrimp assay of fractions of *Parthenium hysterophosis*

Treatment	% inhibition of *P. hysterophorus* against brine shrimps cytotoxicity after 24 h			LD_{50}
	10 (µg/ml)	100 (µg/ml)	1000 (µg/ml)	(µg/ml)
PM	50.5±0.28	56±0.6	62.7±1.3	25±0.5
PH	49.3±2.3	83±2.1	86±2.05	14±0.95

Values are mean ± SE (n= 3).

extract.

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