

Full Length Research Paper

Nutritional investigation and biological activities of *Parthenium hysterophorus*

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Parthenium hysterophorus is used as a vegetable and in the treatment of various human ailments traditionally in Pakistan. The current study was arranged to investigate the nutritional importance, microbial inhibition and its potency against free radicals. The results revealed that extract possesses macro elements Ca, K, Na, Mg, Fe, Zn, as well as micro elements including Ni, Li, Pb, Co, Mn, Cu, Cr, and Cd. Scavenging of free radicals revealed that methanolic extract of *P. hysterophorus* (PM) possesses lowest IC₅₀ ($2.5 \pm 0.05 \mu\text{g/ml}$) comparatively to ascorbic acid (3.7 ± 0.03) and n-hexane extract of *P. hysterophorus* (PH) ($5.1 \pm 0.07 \mu\text{g/ml}$). The extracts also showed maximum antibacterial potential in various concentrations of n-hexane and methanolic extract of *P. hysterophorus*. The results revealed that *P. hysterophorus* contain a remarkable fungal inhibition due to the presence of bioactive constituents. Based on this screening various fractions of *P. hysterophorus* are recommended for future bioassay guided isolation of bioactive constituents.

Key words: *Parthenium hysterophorus*, nutritional analysis, antimicrobial activity, 1,1-diphenyl 1-2-picrylhydrazyl (DPPH).

INTRODUCTION

Plants possess a number of bioactive compounds which are used as medicine or in preparation of new drugs. Nowadays 30% of worldwide drugs are based on natural products isolated from medicinal plants (Grabley and Thiericke, 1999; Sahreen et al., 2010; Sahreen et al., 2011a, b). Many efforts have been made to extract new antimicrobial bioactive compounds from various kinds of sources. One of such resource is fractions of folk medicinal plants. In ancient times people used spices and herbs in their food as flavoring agents (Beuchat, 1994; Cutler, 1995; Khan et al., 2009; Khan et al., 2011). These can be used locally as food preservatives and in folk medicine also. Many herbs and spices also possessed free radical scavenging, antioxidant and antimicrobial activities like bactericidal and bacteriostatic (Khan et al.,

2010a, b). Today, worldwide, many medicinal plants, spices and herbs, exert antimicrobial activities. A large number of these spices and herbs are in regularly used as alternative medicines (Lis-Balchin et al., 1996). Reactive oxygen species (ROS) are major free radicals generated in many redox processes, which may induce oxidative damage to biomolecules, including carbohydrates, proteins, lipids and DNA. Reactive oxygen species affect living cells, which mediate the pathogenesis of many chronic diseases, such as atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancers, and other degenerative diseases (Halliwell and Grootveld, 1987; McDermott, 2000). The action of ROS is opposed by a balanced system of antioxidant compounds produced *in vivo* (Halliwell and Gutteridge, 1999; Khan et al., 2011a, b). Endogenous antioxidants are insufficient, and dietary antioxidants are required to countermeasure excess ROS (Lim and Murtijaya, 2007). Significant attention has been paid to antioxidants in food as well as

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additives to cosmetic and pharmaceutical products.

Parthenium hysterophorus L. (Asteraceae) is a common weed distributed worldwide. Its decoction has been used in traditional medicine to treat fever, diarrhoea, neurologic disorders, urinary infections, dysentery and malaria and as emmenagogue (Ramos et al., 2001; Kalsi et al., 1995; Surib-Fakim et al., 1996). The present study was therefore undertaken to investigate the free radical scavenging and antimicrobial efficacy of *P. hysterophorus*.

MATERIALS AND METHODS

Biochemical analysis of plants

Pharmacological activities of plants are rendered by the occurrence of bioactive compounds. *P. hysterophorus* is medicinally used in the treatment of different disorders as folk medicines and also as pot herb. On account of ethno pharmacological use of these plants, evaluation for the biochemical composition is utmost important.

Plant collection

Collection of *P. hysterophorus* was carried out from Bannu (Pakistan) in June and September 2010. The identification of the plants was carried out at Herbarium of botany Department University of Science and Technology Bannu, KPK, Pakistan and a specimen was submitted vide voucher R-97 (*P. hysterophorus*). *P. hysterophorus* were shade dried at 25°C for two weeks, chopped and powdered.

Extract preparation

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

Elemental analysis

100 ppm stock solution of the magnesium (Mg), calcium (Ca), potassium (K), ferrous (Fe), sodium (Na), cobalt (Co), manganese (Mn), copper (Cu), chromium (Cr), zinc (Zn), nickel (Ni), lithium (Li), lead (Pb), and cadmium (Cd) were formed by mixing required quantity of salts in distilled water for elemental analysis of *P. hysterophorus* in whole plant powders. Perchloric acid digestion method was used for elemental analysis (Allen, 1974). 0.25 g powder was immersed in 6.5 ml of mixed acid solution, that is nitric acid, sulfuric acid and perchloric acid (5:1:0.1) and digested in a flask (50 ml) in fume hood on hot plate till the digestion was completed which was indicated by white fumes coming out from the flasks. Digested samples were allowed to cool and transferred in 50 ml volumetric flask, by rising volume with distilled water. Filtrate (Whatmann No. 42) was collected and concentration of each element was determined on Shimadzu AA-670 atomic absorption spectrophotometer. Quantity of each element was calculated by using the formula as follows:

Nutrient cation in plants = (ppm in extract – blank) × A/W × dilution factor

where A is the total volume of extract (ml) and W is the weight of dry plant.

Nutritional investigation of *P. hysterophorus*

Estimation of leaf protein

Lowry et al. (1951) method was used for determination of leaf protein content. Monobasic sodium phosphate (16 ml) and dibasic sodium phosphate (84 ml) was mixed to get the desired pH (7.5) of phosphate buffer. More so, the following reagents were used:

1. Reagent A: 2 g sodium carbonate (Na₂CO₃), 0.4 g NaOH (0.1 N) and 1 g Na-K tartrate was dissolved in 100 ml of distilled water.
2. Reagent B: CuSO₄.5H₂O (0.5 g) was dissolved in 100 ml of distilled water.
3. Reagent C: Solution A (50 ml) and Solution B (1 ml) were mixed.
4. Reagent D: Folin phenol reagent was diluted with distilled water in 1:1 ratio.

Fresh leaves 0.1 g was homogenized in 1 ml of buffer solution (phosphate buffer pH 7.5). The homogenate was centrifuged for 10 min (3000 rpm). The supernatant (0.1 ml) was poured in the test tubes and finally 1 ml volume was made by distilled water. Reagent C (1 ml) was added to it. 0.1 ml of reagent D was added after shaking for 10 min. The absorbance of each sample was recorded at 650 nm after 30 min of incubation and protein content was calculated with help of standard bovine serum albumin (BSA).

Sugar estimation

Sugar was estimated using fresh plant materials, homogenized and treated with concentrated sulphuric acid. The incubation of sample was carried out for 4 h at 25°C. Optical density (OD) of each sample was measured at 420 nm. The concentration of glucose in both fractions was calculated using standard curve of glucose.

In vitro evaluation of fractions

DPPH radical scavenging activity

Procedure of Gyamfi et al. (1999) was used for determination of DPPH scavenging capacity of various fractions. 2.4 mg DPPH were dissolved in 100 ml methanol to prepare stock solution. The stock solution was further diluted with methanol until attaining an absorbance less than 1.00 using the spectrophotometer at 517 nm. 3 ml solution was mixed with 100 µl sample solution (1 to 100 µg/ml) and measured absorbance at 517 nm. % inhibition was calculated as:

Scavenging effect (%) = [(OD of control - OD of sample) / (OD of control)] × 100

While IC₅₀ value was obtained by using graph prism pad software.

Antibacterial assay

The antibacterial potency of various fractions of *P. hysterophorus* was carried out through protocol (Bagamboula et al., 2003).

Preparation of samples: 100 mg of each fraction was dissolved in DMSO (10 ml) to prepare stock solution.

Table 1. Elemental analysis of *P. hysterophorus*.

Nutrient ($\mu\text{g/g}$)	<i>P. hysterophorus</i>
Ca	75.14 \pm 0.45
K	29.09 \pm 0.21
Na	11.37 \pm 0.14
Mg	6.161 \pm 0.31
Fe	7.36 \pm 0.01
Zn	4.63 \pm 0.009
Ni	0.056 \pm 0.0005
Li	0.081 \pm 0.004
Pb	0.45 \pm 0.002
Co	0.69 \pm 0.003
Mn	0.089 \pm 0.003
Cu	0.096 \pm 0.002
Cr	0.098 \pm 0.002
Cd	0.54 \pm 0.005

Mean \pm SE (n = 3).

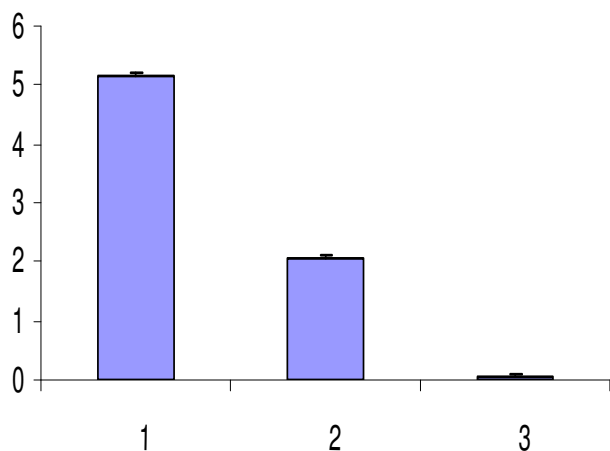


Figure 1. Nutritional values (mg/g fresh weight) of *P. hysterophorus* various constituents; 1 (protein), 2 (sugar) and 3(chlorophyll).

Media for bacteria: It was composed of peptone (5 g/L) and meat extract (3 g/L), while nutrient agar medium (MERCK) was composed of peptone (5 g/L), meat extract (3 g/L) and agar-agar (12 g/L). Nutrient broth medium was prepared by dissolving 0.8 g/100 ml nutrient broth in distilled water, while nutrient agar medium by dissolving 2 g of nutrient agar in 100 ml of distilled water (pH 7.0) and was autoclaved.

Microorganisms: Six strains of bacteria were used; which were *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (ATCC6633), *Micrococcus luteus* (ATCC10240), *Escherichia coli* (ATCC15224), *Klebsiella pneumoniae* (MTCC618) and *Enterobacter aerogenes* (ATCC13048). 24 h old culture in nutrient broth (MERCK) of selected bacterial strain was mixed with physiological saline (0.9% NaCl w/v.) and turbidity was corrected by adding sterile physiological saline until a McFarland 0.5 BaSO₄ turbidity standard

[10⁶ colony forming unit (CFU) per ml density was obtained]. These inoculums were used for seeding the nutrient agar.

Assay procedure (agar diffusion method): 2 g nutrient agar was dissolved in 100 ml distilled water (pH 7.0) and autoclaved. It was allowed to cool up to 45°C. Then, it was seeded with 1 ml of prepared inoculums to have 10⁶ CFU/ml. 14 cm Petri plate was used. Eleven wells per plate were made with sterile cork borer (8 mm). Using micropipette, 100 μl of test solutions were poured in to respective wells. Eight samples of two solutions for positive control (Roxithromycin (Rox) 1 mg/ml and Cefixime-USP (Cefix) 1 mg/ml, one for each) and one for negative control (DMSO) were applied to each petri plate. Finally, the petri plates were incubated for 24 h at 37°C. Antibacterial activities were measured as a mean of 3 replicates.

Antifungal assay

Protocol of Duraipandiyan and Ignacimuthu (2009) was used for antifungal activity, using *Fusarium solani* (0300), *Aspergillus niger* (0198), *Aspergillus flavus* (0064) and *Aspergillus fumigatus* (66).

Determination of total chlorophylls: Methanolic solutions of plant extracts of the appropriate concentration (1.0 to 4.0 mg/ml) were analyzed through UV/VIS spectrophotometer at 470, 653 and 666 nm. The concentrations of chlorophylls α and β were determined according to the equations reported by Lichtenthaler and Wellburn (1985) as follows:

$$\text{Chlorophyll } \alpha \text{ (mg/L)} = 15.65 \text{ Abs}_{666} - 7.340 \text{ Abs}_{653}$$

$$\text{Chlorophyll } \beta \text{ (mg/L)} = 27.05 \text{ Abs}_{653} - 11.21 \text{ Abs}_{666}$$

$$\text{Total Chlorophyll (mg/L)} = \text{Chlorophyll } \alpha + \text{Chlorophyll } \beta$$

Statistical analysis

Statistical Processor System Support (SPSS) 13 was used for determination of Mean \pm SE using replicate data of various experiments.

RESULTS AND DISCUSSION

Elemental and nutritional analysis

Mineral analysis of *P. hysterophorus* showed that potassium, calcium, magnesium, sodium, iron, zinc, copper, molybdenum, lead, lithium, nickel, cadmium, chromium and manganese contents were found as presented in Table 1. The digestion method revealed that *P. hysterophorus* possessed highest concentration of macro elements in $\mu\text{g/g}$ of Ca (75.14 \pm 0.45), K (29.09 \pm 0.21), Na (11.37 \pm 0.14), Mg (6.161 \pm 0.31), Fe (7.36 \pm 0.01) and Zn (4.63 \pm 0.009), as well as micro elements including Ni (0.056 \pm 0.0005), Li (0.081 \pm 0.004), Pb (0.45 \pm 0.002), Co (0.69 \pm 0.003), Mn (0.089 \pm 0.003), Cu (0.096 \pm 0.002), Cr (0.098 \pm 0.002) and Cd (0.54 \pm 0.005) dry powder. The nutritional analysis of *P. hysterophorus* revealed the presence of protein (5.15 \pm 0.32 mg/g weight) and sugar (2.07 \pm 0.04 mg/g weight), respectively (Figure 1). Wild growing leafy vegetables play crucial role

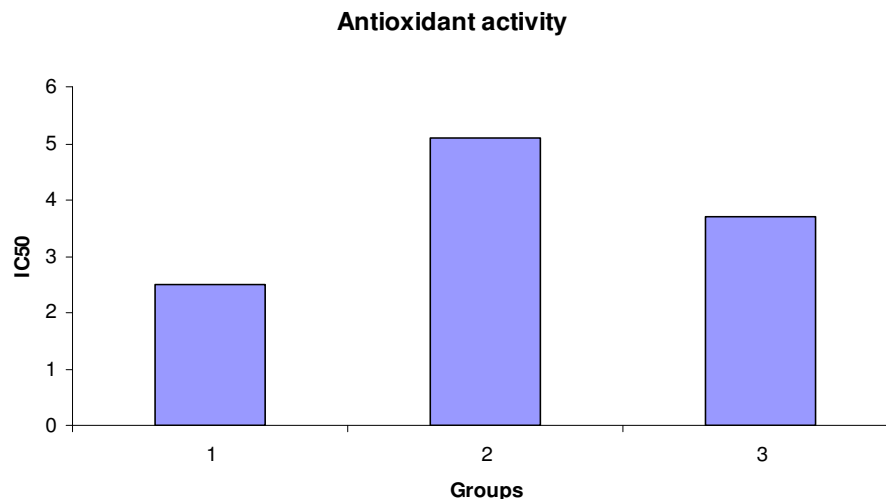


Figure 2. Antioxidant activity of *P. hysterothorus* (L.); 1; PM, 2; PH and 3; ascorbic acid.

as an integral component in the diet of inhabitants of different parts of the world. The proximate analysis of *P. hysterothorus* shows that their leaves are a good source of metallic elements, protein and sugar. Due to the presence of these constituents, consumers are more benefited when they use them as a substitute of leafy vegetable in various food preparations. These inorganic elemental constituents have profound effects in the maintenance of normal glucose-tolerance and in the release of insulin from β -cells islets of Langerhans through amelioration of oxidative stress (Choudhary and Bandyopadhyay, 1999; Khan et al., 2010c). Similar investigation was reported by Tadhani and Subhash (2006) during the preliminary studies on *Stevia rebaudiana* leaves for phytochemical and mineral screening. Study of Garg et al. (2009) reported the presence of the same metallic elements in brahmi (*Bacopa monnieri*) extract comparable to our results.

DPPH free radical scavenging antioxidant assay

Oxidation is a necessary process of living organisms for energy production; however, during normal metabolism oxygen consumption, through many enzymatic systems, reactive free radicals (RFR) are produced. In small amounts, these ROS are beneficial in signal transduction and growth regulation. However, large amount of ROS produce oxidative stress, attack many molecules, such as protein, DNA and lipids (Halliwell and Gutteridge, 1999; Ahmad et al., 2011). 1,1-diphenyl 1-2-picryl-hydrazyl (DPPH) a free radical possess the ability to steal electron from antioxidant, therefore, it is widely used for estimation of *in vitro* antioxidant scavenging activities of medicinal plants. Figure 2 shows the scavenging activity of different fractions of *P. hysterothorus* for free radicals of DPPH.

Lowest IC₅₀ has been recorded with PM (2.5 ± 0.05 μ g/ml) and compared to ascorbic acid (3.7 ± 0.03) and PH (5.1 ± 0.07 μ g/ml). Our result shows similarity with the investigation of Hagerman et al. (1998) and Falleh et al. (2008) who have reported that medicinal plants markedly scavenge free radicals. The antioxidant potential of various fractions of both fractions could be due to the presence of plant bioactive phenolic and polyphenolic compounds which significantly reduce the free radicals which cause oxidative stress.

Antibacterial assay (MIC) and antifungal assay

Due to emergence of antibiotic resistant strains as well as side effects of synthetic drugs, investigation of potent antimicrobial drugs obtained from natural resources has been an objective of researchers and investigators. The minimum inhibitory concentrations (MIC) of both fractions of *P. hysterothorus* were evaluated for antibacterial activity and are tabulated in Table 2. Growth of the Gram negative bacteria, such as *S. aureus* was inhibited with a MIC value of 1.5 mg/ml for PM and 3.8 mg/ml for PH. MIC for *E. coli* was PM (2.5 mg/ml), PH (5 mg/ml); however, MIC for *K. pneumoniae* growth was 1 mg/ml for PM, 5 mg/ml for PH. In case of Gram positive bacteria the observed MIC was PM (5 mg/ml), PH (2.5 mg/ml) against *M. luteus*, while *B. subtilis* was inhibited by 3 mg/ml of PM, 4.5 mg/ml PH. PM and PH inhibited the growth of *E. aerogenes* with MIC is 2.3 and 3.6 mg/ml, respectively. Data of the antifungal assay are shown in Table 3. Growth of *A. niger* was inhibited more than 50% by the PM (56.0 ± 2.0%), while, PH showed less than 25% inhibition (12.6 ± 0.04%). In case of *F. solani* PM (70.7 ± 8.0%) and PH showed no inhibition of the growth of the said fungus. *A. flavus* was inhibited by liquid-phase

Table 2. Effects of various fractions of *P. hysterothorus* on MIC.

Treatment	Minimum inhibitory concentration (MIC) (mg/ml)					
	<i>B. subtilis</i>	<i>E. aerogenes</i>	<i>M. luteus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>S. aureus</i>
Cefix	0.60 ± 0.002	0.51 ± 0.001	0.1 ± 0.001	0.25 ± 0.002	0.30 ± 0.001	0.25 ± 0.001
Rox	1.0 ± 0.004	0.75 ± 0.002	1.0 ± 0.001	0.55 ± 0.001	0.25 ± 0.005	0.45 ± 0.024
PM	3.0 ± 0.004	2.3 ± 0.008	5 ± 0.002	1.0 ± 0.007	2.5 ± 0.005	1.5 ± 0.004
PH	4.5 ± 0.04	3.6 ± 0.05	2.5 ± 0.06	5.0 ± 0.04	5.0 ± 0.01	3.8 ± 0.09

Mean ± SE (n = 3).

Table 3. Antifungal activity of various fractions of *P. hysterothorus*.

Treatment	% inhibition of various fungal species at 200 µg/ml concentration of tested fractions			
	<i>F. solani</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. fumigates</i>
Terbinafine	80.8 ± 5.6	75 ± 1.05	92.2 ± 5.0	80.2 ± 3.4
PM	70.7 ± 8.0	56.0 ± 2.0	53.0 ± 3.0	60.0 ± 2.9
PH	No activity	12.6 ± 0.04	41.05 ± 3.5	38.5 ± 2.7

Mean ± SE (n = 3).

microextraction (LPME) (53.0 ± 3.0%), and PH (41.05 ± 3.9%); however, PM (60.0 ± 2.9%) and PH (38.5 ± 2.7%) inhibited the growth of *A. fumigates*. Our results agreed with results reported by Ndhlala et al. (2009) during antimicrobial characterization of the South African tree aloe (*Aloe barberae*) due to the presence of bioactive polyphenolic constituents. Narod et al. (2004) studied the antimicrobial effects of *Toddalia asiatica* extracts against Gram negative and Gram positive bacteria which strongly justify our results.

Conclusion

Methanolic plant extracts of *P. hysterothorus* showed significant antimicrobial and antioxidant activity. This might be due to the presence of bioactive elements and polyphenolic constituents present in the extract. Based on the significant results obtained in this study, further work is in progress towards isolation and purification.

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