

Phytochemical screening and biological activities of *Bromus pectinatus* Thunb

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Abstract: The present research study investigates the phytochemical and pharmacological importance of *Bromus pectinatus*. Qualitative phytochemical analysis of this plant was carried out to use standard method for the presence of various bioactive constituents. Results showed the ethanolic extract contain natural product such as steroids, alkaloids, tannins, coumarin, saponins, flavonoids and phenols. These compounds play a key role to reducing various disease and microbial inhibition. The ethanolic extract also showed the antimicrobial and antifungal activity against different pathogenic bacterial strains e.g *Escherichia coli*, *Micrococcus leutus*, *Protus vulgarus*, and *Kelebsela pneumonia* and three fungal strains *Aspergillus fumigatus*, *Aspergillus flavous*, *Aspergillus niger*. The antioxidant assay was performed as % inhibition of DPPH (1, 1-diphenyl-2-picryl-hydrazyl) free radicals. The plant extract has more antioxidant activity as compared to ascorbic acid. The maximum concentration (800µg/ml) is the most effective of all. The plant extract showed the high cytotoxicity activity against Brine shrimp. Moreover, the plant extract exhibited allelopathic effect on different growth parameters of wheat plant mostly at higher concentration. These results indicate that the BPEE have a potential broad-spectrum antimicrobial, cytotoxic, antioxidant and phytotoxic activity due to the presence of bioactive compounds.

Keywords: *Bromus pectinatus*, medicinal plant, antimicrobial activity, phytochemical screening.

INTRODUCTION

The flora of Pakistan due to its diverse climatic and soil conditions and many ecological regions is very rich in medicinal plants. Over the last decades, there has been a growing scientific and commercial interest in Pakistan in medicinal plants, mainly due to their economic potential and the widespread cultural acceptability of plant based medicinal products (Sher *et al.*, 2015a, 2014). In Pakistan, similarly to other developing countries, an estimated 80% of the rural population depends on traditional medicine, especially medicinal plants, for their primary healthcare needs (Khan, 2012). Numerous studies have established that consumption of medicinal plants leads to an important intake of phytochemicals (Torres *et al.*, 2011), which are strongly associated with the reduction in the incidence of cardiovascular diseases (Pounis *et al.*, 2013), various types of cancer (Zamora-Ros *et al.*, 2013), type II diabetes (Anhe *et al.*, 2013), immune disorders and neurodegenerative disorders (Marszalek *et al.*, 2017) and at the gastrointestinal level (Ozidal *et al.*, 2016). Among important bioactive compounds are P-Coumaric acid, which has hepatoprotective (Calil Brondani *et al.*, 2017) and UV-protection qualities (Aguilar-Hernandez *et al.*, 2017). Flavanols like catechin and epicatechin are accredited for their anti-inflammatory activity and cancer preventives for humans (Fujiki *et al.*, 2002) glycosides are reported for antifungal and antibacterial properties.

Bromus pectinatus is an annual grass belonging to Poaceae and used as fodder. This species have moisture

contents (5.5%), ash (10%), fiber (23.5%), fats (5%), proteins (4.18%), carbohydrates (51.81%) and Gross energy is 387.52% (Khan *et al.*, 2018). Similarly, the beneficial effects on human health are well recognized, such as high fiber content, vitamins with an antioxidant function, total polyphenols, vitamins and minerals were reported (Vanzani *et al.*, 2011). *Bromus pectinatus* have the following macro and micro elemental status of this plant was total nitrogen (0.67%), phosphorus (0.23µg/gm), Potassium (8.896%), Calcium (3.055µg/gm), Mg (1.044µg/gm), Fe (2.585µg/gm), Zn (0.500µg/gm), Pb (0.187µg/gm), Cr (0.027µg/gm), Cd (0.003µg/gm) and Ni (0.004µg/gm) (Guarrera *et al.*, 2006). The present project was designed to evaluate the biological activities of *Bromus pectinatus*.

MATERIALS AND METHODS

Plant collection and sample extraction

Botanically classified *Bromus pectinatus* were collected from district Bannu and then dried in shade and the complete laboratory work was conducted in the Department of Botany, University of Science and technology Bannu. The plant was identified by Dr. Ihsan and was given voucher number (BB-12) and placed in Herbarium, Department of Botany University of Science and Technology Bannu, Pakistan in March 2017. The dried powder (30g) was packed into Soxhlet apparatus and extracted with 400ml analytical grade ethanol for 5 hrs. at 60°C. The extract was filtered and concentrated through evaporation in water bath at 40°C. The resulting dried extract was stored for further experimental work (reconstitution in which vehicle for further test).

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Phytochemical testing

The test for the presence of phytochemicals was carried out using the methods of Trease and Evans (1989) with modifications according to our experiment. The screening involves detection of alkaloids, flavonoids, saponins and tannins shown in table 1.

Determination of total phenol and alkaloids contents

The total phenolic content (TPC) of the ethanolic extract was determined by applying the Folin-Ciocalteu method. 200 μ l (1-5 mg/ml in respective solvent) of each fraction was added in 130 μ l of 1:10 folin-ciocalteu reagent. The resulting solution was incubated for 2 h before absorbance readings were taken at 765 nm. Gallic acid (Trihydroxybenzoic acid) a standard chemical (1-5 mg/ml in respective solvent) was used for the calibration curve (table 2).

Determination of the total flavonoids content

Briefly, 0.25 ml of each fraction (1-5 mg/ml in respective solvent) and rutin as standard (15-250 μ g/ml) was mixed with 1250 μ l of distilled water in a test tube, followed by addition of 75 μ l of a 5% (w/v) sodium nitrite solution. After 6 min, of incubation 150 μ l of 10% (w/v) $AlCl_3$ solution was added and then allowed to stand for a further 5 min before 0.5 ml of 1M NaOH was added. Rutin chemical (1-5 mg/ml in respective solvent) was used for the calibration curve (Table 3). The absorbance was measured immediately at 510 nm. Flavonoids were estimated as rutin equivalent mg/g of dried fraction.

Bioassay

Phytotoxicity Assay procedure

The allelopathy/phyto lethal assay was run in petri plate's mode. Autoclaved petri plates were set with filter papers. Different concentrations of extract i.e 100 μ g/ml, 250 μ g/ml, 500 μ g/ml and 1000 μ g/ml were sprayed separately on the labeled petri plates. Five seeds were positioned at equal distance in each plate and were incubated at room temperature. The readings were taken after 7 days by graduated ruler of both shoot and root length with respect to control and mean was taken of each (McLaughlin, Rogers *et al.* 1998).

Antifungal bio-assay

Sabourad Dextrose Agar (SDA), nutrient media was used for fungal growth. The media was dissolved in distilled water, autoclaved at 121°C and cooled to 40 °C. SDA was transferred to each test tube along with extract (67 μ L). The negative control test tubes were treated only with DMSO used as vehicle and positive control received antifungal drug. Terbinafine 1mg/ml solutions were made in DMSO and used as positive control. Then the respective fungal inoculums were uniformly speckled to each agar surface and the tubes were sealed with cotton plugs. All the tubes were positioned slanted and placed in incubator at 30°C with open water in a tub. Inhibition of fungal growth was observed after 7 days.

$$\% \text{ Inhibition} = \text{Inhibition in test} / \text{Inhibition control} * 100$$

Antibacterial activity (agar diffusion method)

Three bacterial strains *Micrococcus luteus* (ATCC® 4698), *Escherichia coli* (ATCC® 25922™), *Klebsiella pneumonia* (ATCC® BAA-1705™) and *Proteus vulgaris* (ATCC® 7829™) were obtained from Khalifa Gulnawaz Hospital Bannu. Bacterial suspensions were prepared by caring 1 CFU of bacterial strain from the maintained slants in 0.9% NaCl solution. These preparations were incubated for 24 hours at 37°C. Similarly, 100 μ l (1mg/ml) of levofloxin (positive control) were added in one hole. Lastly, all the petri dishes were placed in the luminal flow (not to contaminate) and incubated for 24 hours. After 24 hours the area of inhibition was measured in mile meter and compared with the control.

$$\% \text{ Inhibition} = \text{Inhibition in test} / \text{Inhibition control} * 100$$

Determination of antioxidant activity

DPPH radical scavenging activity

Standard method of free radical scavenging was used to screen *Bromus pectinatus* phytochemicals potential to scavenge DPPH (1, 1-diphenyl-2-picrylhydrazyl). Stock solution of DPPH was prepared in methanol, measured its absorbance through spectrophotometer at 517nm which was 0.708nm (<1). Ascorbic acid (Vitamin c) dissolved in ethanol was used a standard antioxidant and diluted to 50, 100, 150, 200, 250 and 500 μ g/mL. Plant extract was diluted similarly.

The potential of extract scavenging free radicals was expressed as IC₅₀. DPPH radical scavenging activity was calculated using the following formula:

$$\text{Scavenging effect (\%)} = [(\text{Control absorbance}) - (\text{sample absorbance}) / (\text{control absorbance})] \times 100$$

ABTS⁺ radical cation (potassium per sulfate) bioassay

The basic theme of ABTS bioassay was the capability of crude to scavenge 2, 2'- azino-bis (ethylbenzthiazoline-6-sulfonic acid radical cation. Briefly 7mM ABTS were mixed with 2.45mM potassium per sulfate and incubated 30 minutes. Next 0.2mL extract was added to 1.8mL ABTS⁺ potassium per sulfate solution. The absorbance (ABTS⁺ Potassium per sulfate) were taken in comparison to control (ascorbic acid) at 745nm. The scavenging potential of extract was calculated at various concentrations;

$$\% \text{ Antitoxicity} = 1 - \frac{\text{Abs of test sample}}{\text{Abs of ascorbic acid}} \times 100$$

Cytotoxicity

Brine shrimps were used to check out any lethal or inhibitory effect of *B. pectinatus* extract. Necessary quantity of sea salt was dissolved in distilled water. Eggs of shrimps were placed in sea salt solution under proper light. After 48 hours successful hatching was observed.

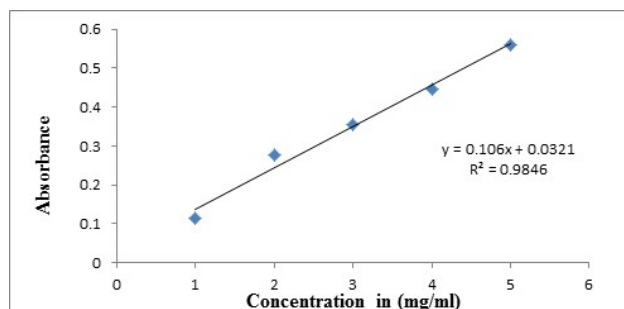


Fig. 1a: Standard calibration curve of Rutin

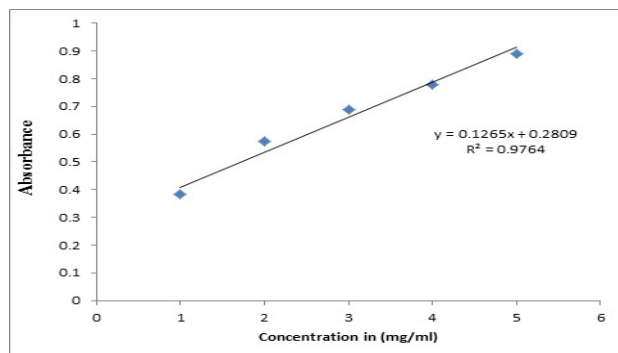


Fig. 1b: Standard calibration curve of Gallic acid

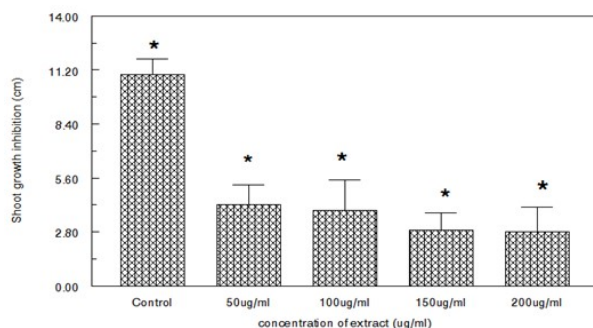


Fig. 2: Seven days treatment of shoot growth with crude extract of *B. pectinatus*.

*shows that results are statistically significant.

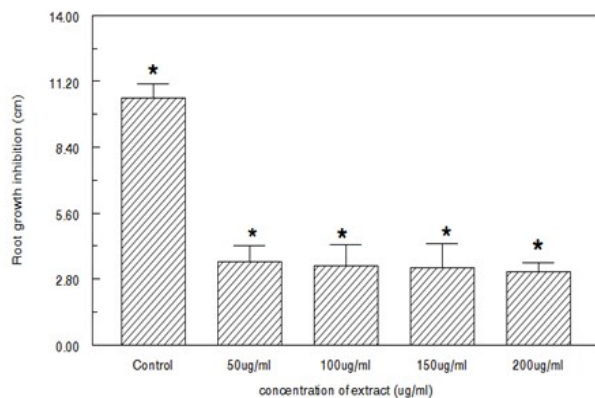


Fig. 3: Seven days treatment of root growth with crude extract of *B. pectinatus*.

* shows that results are statistically significant.

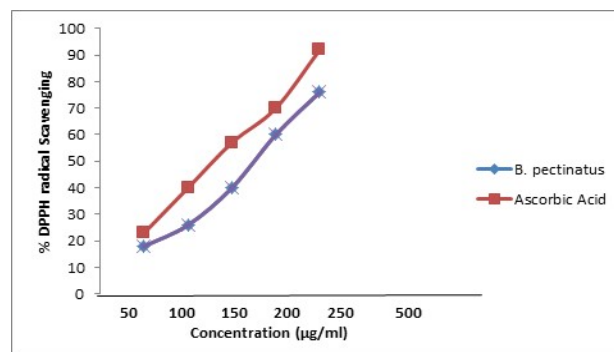


Fig. 4a: Antioxidant activities of *B. pectinatus* crude extract against DPPH radical

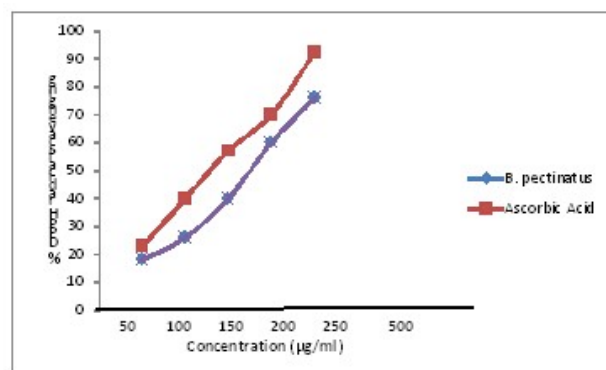


Fig. 4b: Antioxidant activities of *B. pectinatus* crude extract against ABTS radical.

In experimental test tubes 100, 250, 500 and 1000µg/ml of ethanolic crude, sea salt and shrimps were poured. Control test tubes contained only shrimps. Now to every glass vials 10 brine shrimps larvae were shifted by dropper both in the controlled sample solution glass vials and volume of every vial raised up to 5ml with brine solution. The assembly was kept at room temperature and screened after 24 hours using magnifying glass.

STATISTICAL ANALYSIS

Percentages of inhibition were expressed as Mean \pm Standard deviation from three observations in each case. All samples were run in triplicate. All Calculations and Table representation was performed in Slide Write Plus and Microsoft Excel 2010 respectively.

RESULTS

Different bioactive ingredients were found in *B. pectinatus* crude extract (table 1). The total flavonoids contents of the plant was 7.24mg RUT/g and the total phenols contents was 4.1mg GA/g shown in the fig. 1a & 1b. The crude extract of *B. pectinatus* showed lower shoot and root growth as compared to control (fig. 2 & 3). In this study different concentrations of *B. pectinatus* were used to scavenge DPPH and ABTS free radical and

Table 1: Results of Phytochemical Screening

Observation	Phytochemical						
	Tannin	Saponins	Flavonoids	Steroids	Alkaloids	Quinones	Coumarins
	Blue-black coloration	Formation of stable foam	Yellow coloration	Violet to blue or green	Reddish brown colored ppt	coloration Blue green or red	Yellow colour
Result	+	+	+	+	+	+	+

it was found that *B. pectinatus* show good scavenging activity (fig. 4a & 4b). *Bromus pectinatus* ethanolic crude extract showed good antibacterial activities at various doses (table 4a). It has been found that *Bromus pectinatus* crude extract strongly inhibited growth of tested fungal strains except *Aspergillus niger* (table 4b) and proved a good valuable antifungal source. In the current study, cytotoxic effect of *B. pectinatus* was measured against brine shrimps growth (table 5).

Table 2: Absorbance of standard compound of Gallic acid

Gallic acid concentration in (mg/ml)	Absorbance (mean value) at λ_{max} =765nm
1	0.383
2	0.573
3	0.69
4	0.78
5	0.89

Table 3: Absorbance of standard compound of Rutin

Rutin concentration in (mg/ml)	Absorbance (mean value) at λ_{max} =510nm
1	0.114
2	0.276
3	0.356
4	0.445
5	0.5595

DISCUSSION

Different bioactive ingredients were found in *B. pectinatus* crude extract. Alkaloids influence the central nervous system, decreases hunger and carry on as diuretic (Washington D.C., 2010). Steroids help in managing the immune response (Shah *et al.*, 2009). Coumarins can be recommended to be useful for anti-inflammatory and antimicrobial effects (Lerdau 2003). Moreover, terpenoids can be utilized as defensive substances in keeping agriculture items as they are referred to have insecticidal properties too (Sultana and Ata 2008). The present research significantly identified these phytochemicals and will be useful in the coping different diseases. Free radicals can cause damage to the cell by damaging component of the cell and associated with pathological conditions such as atherosclerosis and carcinogenesis, as well as with aging (Valko *et al.*, 2007).

An imbalance between defense system and free radicals generation results in oxidative stress causing the damage to the tissue, cell or cell component including lipid, protein and most important DNA. Free radical scavenging activity of the *B. pectinatus* was determined using different free radicals like 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino bis (3-ethylbenzothiazolidine-6-sulphuric acid) (ABTS) using spectrophotometer.

Secondary metabolites from plants used as a model to develop less toxic and more efficient medicines in eradicating the growth of pathogens. These compounds like alkaloids, tannins, saponins, flavonoids, steroids, quinones and coumarin compounds have a potential for many biological activities which include antimicrobial activity (Compean and Ynalvez, 2014). The problems with available antimicrobial drugs is high price, none availability at the right place and right time, besides these the bacterial resistance is another changing problem. Keeping in view these problems associated with antibacterial drugs, the search for novel, efficient, safe and less toxic natural products is a big challenge for researchers. To cope with this problem, the current work sought to explore antibacterial activities of *B. pectinatus* with the hope of finding effective, safe and cheap antibacterial medicine.

Numerous researchers found plant extract inhibitory potential against microorganisms due to occurrence of saponins, which have antifungal properties, polyphenolic compounds like catechin and tannins (Sahreem S, MR Khan and RA Khan 2011). The antifungal activities of *B. pectinatus* represent a potential source of saponins and may be of economic importance as a source of natural antifungal plant products.

Cytotoxicity is the quality of being toxic to cells. There is increasing demands for anticancer therapy. *In vitro* cytotoxicity testing procedures reduces the use of laboratory animals. The outcomes revealed that the brine shrimp survival is inversely proportional to the concentration of the extract. Mentor *et al.*, (2014) examined that ethanolic extract of *Artemia salina* L. showed 100% cytotoxicity for brine shrimp at high dose which are similar to our results. The toxicity of the plants may due to the different phytochemicals that are part of the plant (Mentor *et al.*, 2014). Allelopathy is the negative impact of chemicals discharged by plant species on development of different organisms (Rice, 1984). Comparable outcomes were discovered by (Lakic *et al.*, 2010) strongly justify our results.

Table 4a: Antibacterial activity

Tested Bacteria	Concentration (mg/ml)			
	10mg/ml	20 mg/ml	30 mg/ml	1 mg/ml
	Zone of inhibition in mm ± (SD)			
<i>M. luteus</i>	08±0.27	8±0.11	15±0.35	18
<i>K. pneumoniae</i>	–	–	–	08
<i>P. vulgaris</i>	06±0.17	09±1.0	10±0.20	14
<i>E. coli</i>	10±0.30	13±0.32	15±0.18	20

Table 4b: Antifungal activity

Tested Fungal Strains	Concentration (mg/ml) Terbinafine			
	10 mg/ml	20 mg/ml	30 mg/ml	1mg/ml
	Zone of inhibition in mm ± (SD)			
<i>A. niger</i>	–	–	–	10
<i>A. flavus</i>	0.3±2.1	05±1.3	07±0.9	10
<i>A. fumigatus</i>	01±1.4	07 ± 1.7	08 ± 2.1	10

Table 5: Cytotoxic effect of plant extract on brine shrimps

	Concentration (µg/ml)				
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml
Brine shrimps died	15±2	30±2.3	50±3.1	65±3.7	85±4.1

CONCLUSION

The current study was carried out to investigate antioxidant, phytochemical and antimicrobial potential of ethanolic extract of *Bromus pectinatus*. The extract samples were used against *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris* and *Klebsiella pneumoniae*. Phytochemical results showed that major classes of natural product detected were tannins, Saponins, flavonoids, steroids, alkaloids, quinine and Coumarin. Antioxidant potential was highest against DPPH with 78%, followed by ABTS with 77%. The extract at 30 µg/ml inhibited growth of *Escherichia coli*, *Micrococcus luteus* and *Proteus vulgaris* at the rate of 15±0.18, 15±0.35 and 10±0.20mm respectively while showing no inhibition against *Klebsiella pneumoniae*. Similarly, fungal strains like *Aspergillus fumigatus* and *Aspergillus flavus* growth was inhibited to 08±2.1 and 07±0.9 cm respectively. These results show that *Bromus pectinatus* have antioxidant and antimicrobial activity. The existence of diverse phytochemicals in the plant is the promising answer for its active antimicrobial profile. In future, work will be done to isolate bioactive constituents of *Bromus pectinatus* extract to find promising pharmacological agents.

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