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Full Length Research Paper

Antioxidant and antibacterial activity of crude methanolic extract of *Euphorbia prostrata* collected from District Bannu (Pakistan)

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Medicinal plants help in improving the human health that is primarily due to bioactive constituents such as flavonoids, alkaloids, saponins, cardiac glycosides and tannins. These compounds play important role in minimizing oxidative stress, cancer, impotency, cardiac dysfunction and microbial inhibition. *Euphorbia prostrata (L)* is a small prostrate, annual herb found all over the world including Pakistan. This study was designed to investigate the protective effects of *E. prostrata* against oxidative stress and antibacterial potential. Methanolic extract of *E. prostrata* were used in three different concentrations of 1, 3 and 5 mg/ml. The maximum inhibition was shown at highest concentration of 5 and at 3 mg/ml the moderate inhibition while at 1 mg/ml the minimum inhibition was shown. Similarly concentration of methnolic extract of *E. prostrata* exhibited excellent scavenging activity. The % scavenging of *E. prostrata is* directly proportional to the concentration. The order of % scavenging activity is 50 < 100 < 150 < 200 < 250 µg/ml. Ascorbic acid is used as negative control

Key words: Antioxidant, antibacterial, *Euphorbia prostrata,* dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid.

INTRODUCTION

The imbalance between antioxidant and reactive oxygen species, such as superoxide radical (O_2^-), hydroxide radical (^-OH), peroxide radical (ROO^-), and nitric oxide radical produced due to excessive metabolism in the cell and its ability to detoxify these reactive intermediates is called oxidative stress. These reactive oxygen species damage the biological system and causes different chronic diseases like cancer and heart diseases (Aruoma and Cuppette, 1997; Prakash et al., 2007). The modern research claims that oxidative stress is the cause of various disorders and diseases, therefore, the researcher focus on the role of antioxidants in the maintenance of biological system (human health), its remedy and treatment (Etsuo, 2010).

The sources of naturally occurring antioxidants are primarily plant phenolic compounds which are present in all parts of plants (Pratt and Hudson, 1990). The phenolic compounds, especially flavonoids, import the antioxidant and pharmacological properties to plants and herbs (Dawidowicza et al., 2006). The biological, pharmacological and medicinal properties of this group of compounds have been extensively reviewed (Marchand, 2002). Medicinal plants play important role in recovery of various diseases (Khan et al., 2009, 2010a, b, 2011a, b; Sahreen et al., 2010, 2011), microbial inhibition (Khan et al., 2010c) and cardio protection (Khan et al., 2011).

Medicinal plants are the best source to obtain different drugs, about 80% of people in industrialized countries uses traditional medicines, which are derived from medicinal plants, so the properties and efficiency of plants must be, investigated (Ellof, 1998). In developing countries, more than 40% of the people are put to death due to infection of the microorganism, besides this is also spoiliage of food materials due its pathogenicity (Marino et al., 2001). The food materials can be protected by use

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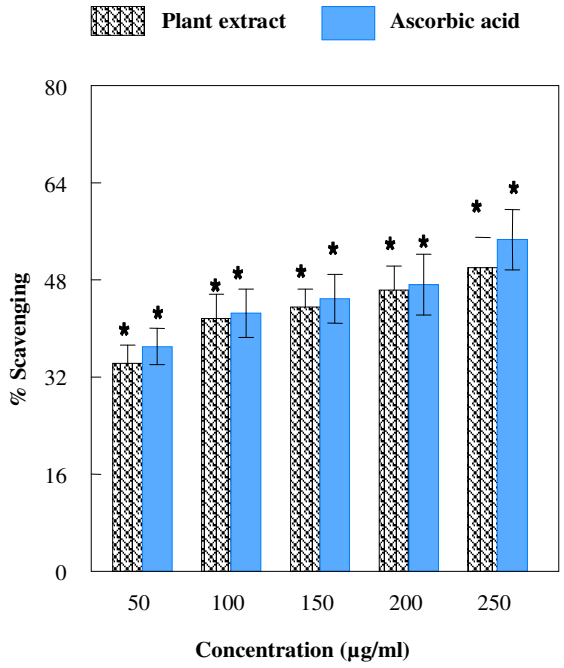

Figure 1. DPPH scavenging activity of *E. prostrate*.

of phenolic compounds (essential oils) as antibacterial agents because some of the antimicrobial compounds produced by plants are effective against pathogens (Mitscher et al., 1987). Antimicrobial agents, including food preservatives, inhibit food burn bacteria and preserve the food. Many naturally occurring extract from herbs, medicinal plants, are known to possess antimicrobial activities and can serve as antimicrobial agents, against food spoilage (Bagambula et al., 2003).

MATERIALS AND METHODS

Plant collection

Plant of *Euphorbia prostrata* (L) was collected from District Bannu at its maturity during the month of December 2010, identified by associate Professor Mr. Abdur Rehman, Govt. Post Graduate College, Bannu. Shade dried plant was then selected for biological study and powdered into very fine powder.

Plant extraction

700 g of plant powder of *E. prostrata* plant were socked in 3 L of 80% methanol, with random shaking for a period of 7 days. The plant is extracted and filtered by using Whatman filter paper and concentrated with the help of rotary evaporator at 37°C to obtained crude extract which was 40 g.

Antioxidant bioassay

Solution of extract and ascorbic acid prepared

In the proceeding of antioxidant activity, three extract solutions were prepared as stock solution. From the stock solution, another type of solution was prepared with different concentrations. The total volume of this solution was 500 µl. Another five solutions of different concentrations were prepared as discussed previously; these five solutions of 50, 100, 150, 200 and 250 µg/ml was also reused as a stock solution. Ascorbic acid is used as a control which inhibits the free radicals. Solution of ascorbic acid was prepared by the same method of extract.

Solution of DPPH (1-1 diphenyl 1-2 picryl- hydrazyl)

1.5 mg of DPPH is weighted and dissolved in 5 ml/5 ml methanol. Since DPPH is light sensitive so the solution is covered in aluminum sheet. After checking its absorbance at 517 nm, it was found to be less than one (1). 900 µl of DPPH was added to 100 µl extract and ascorbic acid, and incubated at 25°C for 30 min and its absorbance at 517 nm was checked with the UV Spectra Photometer. Scavenging activity was determined based on the formula as % scavenging.

$(DPPH_{ab} – Sample_{ab}/DPPH_{ab}) \times 100$

Antibacterial activity

Media and bacterial strains

Nutrient broth medium (MERCK) was used for the growth of bacteria for the preparation of inoculums. Nutrient broth medium was prepared by dissolving 0.8 g/ 100 ml nutrient broth in distilled water while nutrient agar medium medium was prepared by dissolving 2 g of nutrient agar in 100 ml of dH_2O. Antibacterial activity was tested against four strains of bacteria which were *Micrococcus luteus* (ATCC10240), *Staphylococcus aureus* (ATCC6538), *Escherichia coli* (ATCC15224) and *Pseudomonas* aeruginosa.

Assay procedure (agar diffusion method)

Nutrient agar medium was prepared by suspending nutrient agar 2 g in 100 ml of distilled water and was autoclaved and allowed to cool. Petri plates (14 cm) were papered by pouring 75 ml of seeded nutrient agar and then allowed to solidify. Five wells per plate were made with cork borer (8 mm). Using micropipette 100 µl of test solution was poured in each well. Samples was applied to each Petri plate and incubated at 37°C for 24 h. Then clear inhibition zone were detected around each hole. DMSO was used as negative control. The diameter of clear zone showing no bacterial growth around each well was measured in mm.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

DPPH (1-1 diphenyl 1-2 picryl- hydrazyl) is a free radical and has strong electron attracting ability from antioxidant. The scavenging activity of the methanolic extract of *E. prostratra* is shown in Figure 1. The graph showed that the concentration of methanolic extract is directly proportional to the % scavenging of *E. prostrata*. The minimum scavenging activity is shown at lowest concentration of 50 µg/ml which is 34.25%. The order of

Table 1. Antimicrobial activity of *E. prostrata* against different strains of bacteria.

Group	Concentration	Zone(mm)			
		Micrococcus luteus	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
EPME	1	20	13	16	21
	3	24	14	18	34
	5	25	24	20	26
DMSO		1.2	0.9	1	1.4

EPME, *E. prostrata* methanolic extrac; DMSO, negative control. Concentration expressed in microgram per ml in respective solvent- methanol.

% scavenging activity is 50 < 100 < 150 < 200 < 250 µg/ml. Ascorbic acid is used as control.

Information of the present study indicated that the methanolic extract of *E. prostrate* (L) possesses marked scavenging properties and scavenged the DPPH. The result obtained from the present work is very closely related to that obtained by the investigations of Hagerman et al. (1998). The antioxidant potential of the plants is due to the presence of components of phenolic and polyphenolic species which are significantly inhibited by the free radicals that cause oxidative stress. The results obtained by Duenas et al. (2006) and Kilani et al. (2008) are also similar to the present work.

Antibacterial assay

Result of antibacterial assay showed that the methanolic extracts of *E. prostrata* possessed antibacterial activity tested against *M. luteus*, *S. aureus, E. coli* and *P. aeruginosa*. In this activity, methanolic extract of *E. prostrata* were used in three different concentrations, that is, 1, 3 and 5 mg/ml. Table 1 showed that the concentration of extract is directly proportional to the inhibition. The maximum inhibition for each bacterium was shown at highest concentration of 5 mg/ml, while 3 mg/ml show moderate inhibition and lowest inhibition was shown by 1 mg/ml. Control DMSO showed negligible inhibition.

The result showed that methanolic extract of *E. prostrata* plant had significant antibacterial activity. The result obtained from the present work is closely related to that of Ndhlala et al. (2009) who reported antimicrobial potential of South African plant *Aloe barberae* (L). This has strongly supported our work.

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