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Preliminary Phytochemical Screening and Antioxidant Profile of *Euphorbia prostrate*

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Abstract: Phytochemicals profiling is important for the isolation of new and novel chemical substances. The current finding is directed to the detection of different classes of chemical constituents responsible for antioxidant properties of *Euphorbia prostrate.*The crude methanolic extract and various solvent isolated fractions of *E. prostrate* showed the presence of Alkaloids, Terpenoids, Saponins, Tannins, Steroids and Glycosides, Carbohydrates, Monosaccharide's, combined reducing sugars and soluble starch. The crude methanolic extract/fractions were investigated for their antioxidant potentials by DPPH free radical assay. The ethyl acetate and chloroform fraction exhibited significance DPPH scavenging effect among the entire fraction.The current investigation, suggest that *E. prostrate* contain potential sources of free radicals scavenging actions.

Key words: *E. prostrate* · Antioxidant · Alkaloids · Terpenoids · Glycosides · Carbohydrates · Starcsh

INDRODUCTION

Plants are a rich source of producing wide number of natural products in a most efficient way and with precise selectivity. Since the middle of the 19th century, different class of bioactive compounds have been isolated and elucidated. Many of these are used as the active ingredients of the modern medicine. The Family Euphorbiaceae comprises of 2000 species [1]. Antioxidants are radical scavenger's properties which provide protection to human body against free radicals by inhibiting various oxidizing chain reactions. When these constituents are present at low concentration in body they stop the oxidation of an oxidizable substrate [2]. These antioxidants play important roles in delaying the development of prolonged diseases such as cardiovascular diseases, cancer, atherosclerosis and inflammatory diseases [3].

The genus *Euphorbia* is the largest genus of medicinal plants widely distributed in most part of the china and Pakistan. The characterized of *Euphorbia* genus is the presence of milky latex. *Euphorbia prostrata* is a medical plant which is used for the treatment of fever, abdominal disorder and as blood purifier in Pakistan [4]. The extracts of *E. prostrata*also possess anti-inflammatory, analgesic, haemostatic and wound healing activates listed in Table 1 [5]. The major secondary metabolite reported from *E. prostrata*are Apigenin, Luteolin, Apigenin-7-glucoside, Luteolin-7-glucoside. gallic acid, ellagic acid and tannins [6].

MATERIALS AND METHODS

Plant Material: *Euphorbia prostrate, Euphorbia hirta* and *Euphorbia helioscopia* was collected from the garden of Institute of Chemical Sciences.

Table 1: Folk use of *E. helioscopia* (Euphorbiaceae) evaluated for antioxidant activity

S.No	Plant name	Family	Folk uses
01	*E. prostrate*,	Euphorbiaceae	Fever, abdominal disorder, blood purifier, anti-inflammatory, analgesic, haemostatic and wound healing.

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The plant was identified by GhulamJelani Department of Botany University of Peshawar Pakistan.

Extraction and Fractionation: Shade dried plant of *E. prostrate* was filled in the flask and extracted successively with methanol solvent in soxhlet extractor for 48h.The solvent extract was concentrated under reduce pressure at 40°C using rotavapor and suspended in water and successively partitioned with n-hexane, chloroform, ethyl acetate and methanolic fraction with the standard method as earlier discuss [7-9].

Phytochemical Profiling: The chemical tests were performed on the crude extract and different solvent extracted fractions of *E. prostrate* using standard procedure [10-15] to recognize the bioactive secondary metabolite.

Test for Alkaloids: About 0.2 g of each of the fractions was warm with 2% H_2SO_4 for two minutes. The reaction were filter and added a few drops of Dragendroff,s reagent to each filtrate. Orange red precipitate indicates the presence of alkaloids moiety.

Test for Tannins: A small quantity of each extract was mixed with Water and heated on water bath and filtered. A few drops of ferric chloride were added to each filtrate. A dark green solution indicates the presence of tannins.

Test for Anthraquinone: About 0.5 g of each extract was boiled with 10 % HCL for few minutes on water bath. The reaction mixture was filter and allows to cool. Equal volume of $CHCl_3$ was added to each filtrate. Few drops of 10 % ammonia was added to each mixture and heated. Rose-pink color formation indicates the presence of anthraquinone.

Test for Glycosides: Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A Few drops of Feelings solution A and B were added to each mixture. Formation of red precipitate indicates the presence of glycosides.

Test for Reducing Sugar: Each extract was shaken with distilled water and filtered. The filtrates were boiled with few drops of Feling, s Solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

Test for Saponins: 0.2 g of each extract was shaken with 5 ml of distilled water and heated to boiling. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins Test for flavonoids: About 0.2 g of each extract was dissolved in diluted NaOHand few drops of HCl were added. A yellow solution that turn colorless indicates the presence of flavonoids.

Test for Phlobatanins: About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

Test for Steroids: Exact 2 ml of acetic anhydride was added to the mixture of o.5 g of each extract and H_2SO_4 (2ml). The color change from violet to blue or green in some samples indicates the presence of steroids.

Test for Terpenoids: About 0.2 g of each extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. The formation of a reddish brown coloration at the interface indicates positive results for presence of terpenoids.

Test for Cardiac Glycoside: To 2ml of plant extract, 1ml of glacial acetic acid and 5% ferric chloride was added. Then few drops of concentrated H_2SO_4 were added. Presence of greenish blue colour indicates the presence of cardiac glycosides.

Test for Caumarine: Exist 3 ml of 10% NaOH was added to 2ml of aqueous extract formation of yellow colour indicates the presence of caumarine.

Test for Emodins: Exact 2ml of NH4oH and 3ml of benzene was added to extract. Appearance of red color indicates the presence of emodins.

Test for Anthocyanin and Betacyanin: To 2ml of plant extract, 1ml of 2N NaOH was added and heated for 5 minutes at 100C. Formation of bluish green color indicates the presence of anthocyanin and formation of yellow color indicates the presence of betacyanin.

Test for Carbohydrates: Few drops of Molisch's reagent were added to each of the portion dissolved in distilled water; this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then

allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Test Monosaccharide's: About 0.5 g each portion was dissolved in distilled water and filtered. 1 ml of the filtrate was then mixed with 1 ml of Barfoed's reagent in a test tube and then heated on a water bath for a period of 2 minutes. Reddish precipitate of cuprous oxide was considered as a positive test

Test for Free Reducing Sugar: About 0.5 g each portion was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

Test for Combined Reducing Sugars: About 0.5 g each portion was hydrolysed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralised with sodium hydroxide solution. To this, few drops of Fehling's solution was added and then heated on a water bath for 2 minutes. Appearance of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars.

Shinoda's Test for Flavonoids: About 0.5 of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.

Lead Ethanoate Test for Flavonoids: Few quantity of the each portion was dissolved in water and filtered. To 5 ml of each of the filtrate, 3 ml of lead ethanoate solution was then added. Appearance of a buff-coloured precipitate indicates the presence of flavonoids.

Test for Soluble Starch: Few quantity of each portion was boiled with 1 ml of 5% KOH, cooled and acidified with H_2SO_4. A yellow coloration was taken as the presence of soluble starch.

DPPH Radical Scavenging Assay: The antioxidant activity was performed by DPPH radical scavenging assay according to standard protocol as earlier discusses [16]. The hydrogen atom or electron donation abilities of the corresponding extracts/fractions and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH.) Experiments were carried out in triplicate. Briefly, a 1mM solution of DPPH radical solution in methanol was prepared and 1ml of this solution was mixed with 3ml of sample (extracts/fractions) solutions in methanol (containing 10-100ug) and control (without sample).The solution was stand for 30 min, in dark the absorbance was measured at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follows.

$$\% \text{ DPPH} = (\text{OD control} - \text{OD sample}) \times 100 / \text{OD control}$$

Where, OD control is the absorbance of the blank sample and OD sample is the absorbance of samples or standard sample.

RESULTS AND DISCUSSION

The phytochemical screening of various solvent extracted fractions is listed in table 2. The *DPPH radical scavenging* potential of various solvent extracted fractions and crude extracts are given in Tables 3.

Euphorbia prostrate was tested at accumulative concentrations i.e. 10, 20, 40, 60, 80 and 100 mg/kg. The maximum antioxidant effect was observed with ethyl acetate fraction followed by methnolic, chloroform, ethanolic extract and *n*-hexane fraction. The percent free radicals scavenging effect of ethyl acetate fraction was 60.44, 80.80, 89.21, 88.60, 91.60 and 97.10 at the tested concentrations of 10, 20, 40, 60, 80 and 100 mg/kg respectively. Testing methanolic fraction against DPPH induced oxidation at the same concentration produced 55.2, 78.43, 84.40, 87.00, 92.54 and 96.88% (Table 3) free radicals scavenging action. Chloroform fraction was also proved good antioxidant and caused maximum inhibition of free radicals (90.55%) at tested dose of 100 mg/kg. The outstanding antioxidant effect (80.55%, at 100 mg/kg) of crude ethanolic extract cannot be ignored as compared to moderate effect of *n*-hexane fraction.

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole

Table 2: Phytochemical assortment of aerial parts of *Euphorbia prostrate*extract/fractions

Chemical components	n-Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	-	+	+
Steroids	+	-	-	-
Terpenoids	+	+	+	+
Flavonides	-	+	+	+
Anthraquinones	-	-	-	-
Tannins	-	-	+	+
Phlobatanins	-	-	-	-
Saponins	-	-	+	+
Glycoside	-	-	-	-
Reducing sugars	-	-	+	+
Carbohydrates	-	+	+	+
Monosaccharide	-	-	-	-
Combined Reducing sugars	-	+	-	-
Soluble starch	-	+	+	+

Key= – absent, + = present

Table 3: DPPH radical scavenging activities of crude extract and various extracts of *Euphorbia prostrate*

	%DPPH				
Conc(µg/ml)	*n*-hexane	Chloroform	Ethyl acetate	methanol	Ethanol
10	5.00	55.21	60.44	55.2	44.55
20	15.12	60.33	80.8	78.43	50.55
40	20.90	69.02	89.21	84.40	60.33
60	25.1	78.4	88.6	87.0	69.22
80	37.9	88.29	91.6	92.54	77.22
100	45.11	90.55	97.1	96.88	80.55

grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in plants has been substantially investigated and reported in the literature. In the present study three selected plant of the same family were scrutinized for their free radical scavenging ability. The maximum scavenging potential of any sample is considered as good antioxidant. The antioxidant potential of ethyl acetatewas higher followed by chloform and methanol. The maximum antioxidant capacity of *E. prostrate* strongly supports the use of this plant in various aliments like fever, blood purifier, anti-inflammatory, analgesic and even antibiotic. It is well recommended that the presence of antioxidant substance protect the living system from hundredth of diseases and this statement is quite clear from our results. The use of *E. prostrate* in number of aliments might be attributed to its outstanding free radical scavenging effect. The antioxidant power of hexane fraction was somewhat lesser than that of ethyl acetateand therefore

its ethno medicinal uses are limited. The difference in free radical scavenging effect of different fractions might be attributed to the difference of their chemical composition because various constituents have variable degree of antioxidant effect. It is clear from our results that highly polar and highly nonpolar solvent fractions of the plants were weaker antioxidant as compared to intermediate polar solvent fractions. Therefore the antioxidant potential of these plants might be due to the presence of chemical constituents of intermediate polarity. Keeping in view these finds it is very strongly recommended that further research work should be conducted *E. prostrate*to find out the exact chemical moieties acting as antioxidant.

CONCLUSION

It is concluded that *E. prostrate* are the rich sources of antioxidant molecules and these plants are recommended to use as antioxidant for the management of various ailments.

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