

Full Length Research Paper

Antioxidant studies of various solvent fractions and chemical constituents of *Potentilla evestita* Th. Wolf

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In the present research work acacetin (1), chrysin (2) and umbelliferone (3) were isolated for the first time from the chloroform fraction of *Potentilla evestita*. The isolated compounds as well as various solvent fractions of the crude ethanolic extract were assessed for their antioxidant potentials. A significant DPPH free radical scavenging effect was observed in the methanolic and chloroform fractions. The acacetic and chrysin demonstrated significant free radical scavenging activity. Likewise compound 3 showed moderate activity as comparative. It was concluded that the therapeutic potential of this plant is due to its antioxidant potential and the antioxidant effect of *P. evestita* is due to the presence of these three isolated antioxidant compounds.

Key words: *Potentilla evestita*, acacetin, chrysin, and umbelliferone, antioxidant activity.

INTRODUCTION

Antioxidant property of any natural products or chemical constituent provides protection to human body against free radicals by inhibiting various oxidizing chain reactions. When these constituents are present at low concentration in the body they stop the oxidation of an oxidizable substrate (Chun et al., 2005). These antioxidants play important roles in delaying the development of prolonged diseases such as cardiovascular diseases, cancer, atherosclerosis and inflammatory diseases (Velioglu et al., 1998). Plants are the best natural antioxidant products (Muhammad et al., 2011).

Potentilla evestita belongs to the family Rosaceae (Tomczyk et al., 2009). It is distributed in the Eastern Himalayan range from Indus to Kumaon (Anonymous., 1998), in the Arctic, Alpine and temperate regions of the Northern hemisphere. In Pakistan, it is well distributed in

Gilgit. Several medicinal uses of *P. evestita* has been reported such as antimicrobial, anti-inflammatory, anti-diarrheal, anti-diabetic, hepatoprotective, anticancer, anti-spasmodic and ulcerative colitis (Tomczyk et al., 2009).

Phytochemically, approximately 43 compounds have been isolated from *P. evestita*. The rhizomes of *P. evestita* contains rich amount of tannins that is 3.5% hydrolysable tannins and 15-20% condensed tannins, pregnane derivative, 2,6 beta,7beta-trihydroxy-4-methyl-19-norpregna-1,3,5(10)-trien-17-one, and pregnane derivative, 11alpha,17alpha,21-trihydroxypregna-4,16(22)-diene-3,20-dione, have also been isolated from *P. evestita* (Khan et al., 2011). In continuation of our research work on medicinal plants (Uddin et al., 2011a; 2012b; Rauf et al., 2012a) herein we are reporting the isolation of three compounds using chromatographic

methods and characterization by spectroscopic techniques followed by their test for antioxidant activities.

MATERIALS AND METHODS

Materials

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical and vitamin C were obtained from Sigma-Aldrich. All other chemicals and reagents used were of analytical grade.

The whole plant of *P. evestita* (15 kg) was collected from Gilgit, Pakistan. The plant was identified by the Taxonomist Department of Botany, University of Karachi. Voucher specimen (voucher No. 707) has been deposited at the herbarium (Herbarium No. 71212) of the Department of Botany, University of Karachi, Pakistan.

Extraction and Isolation

Shade dried whole plant (15 kg) of *P. evestita* was ground into fine powder and soaked in 25 L ethanol for 10 days at room temperature. The resulting extract was filtered and the filtrate evaporated under reduced pressure at 45°C to yield 300 g dark brown residue. The residue was suspended in water and subsequently extracted with solvents of increasing polarity, namely *n*-hexane (3×10 L), chloroform (3×14 L), ethyl acetate (3×12 L) and methanol (1×3 L). Each extract evaporated under reduced pressure to afford *n*-hexane fraction (70 g), chloroform (75 g) EtOAc extract (8 g), and methanol (40 g). The chloroform fraction (60 g) was subjected to column chromatography on silica gel (Merck Silica gel 60 (0.063 - 0.200 mm), 5 × 60 cm). The column was first eluted with hexane-ethyl acetate (100:0 → 0:100) as solvent system. A total of 100 fractions, RF-1 to RF-100 were obtained based on TLC profiles. Elution of the chromatogram with hexane-ethyl acetate (100:0 → 100:0) gave the isolation of three known compounds, acacetin **1** (80 mg), chrysin **2** (99 mg) and umbelliferone **3** (60 mg). The structures (Figure 1) of compounds were confirmed by comparing their NMR and physical data with the reported data in literature (Dawson et al., 2006; Hemila et al., 1992; Battelli et al., 2001).

DPPH radical scavenging assay

The antioxidant activity was performed by DPPH radical scavenging assay according to standard protocol as earlier discussed (Rauf et al., 2012b; Uddin et al., 2012c; 2012d; 2012e). The positive control used was vitamin C. 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 1 mM solution of DPPH radical solution in methanol was prepared and 1 ml of this solution was mixed with 3 ml of the sample (extracts/fractions) solutions in methanol (containing 10 – 100 ug) for various fraction while (containing 5 – 100 ug) for pure compounds and control (without sample). The solution was allowed to 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.

Statistical analysis

Data were presented as mean and standard errors of mean. The statistical analysis was performed using Prism Graphed. ANOVA followed by Dunnet test for multiple comparison of groups were employed for assessing the statistical significance of the differences observed among the groups.

RESULTS AND DISCUSSION

The results of antioxidant potential of crude ethanolic extract and its various solvent fractions are presented in Table 1. The maximum free radical scavenging effect was demonstrated by methanolic fraction having 96.88, 92.54, 87, 84.40, 78.43 and 55.20% at the tested dose of 100, 80, 60, 40, 20 and 10 ppm, respectively. When the sub fraction (chloroform) was tested for antioxidant effect at accumulative concentration, a dose dependant manner was observed. The percent antioxidant effect at 100 ppm was 91.22% and at lowest concentration the effect was 30.22%. The effect of crude ethanolic extract was lower than methanolic and chloroform fraction but was significant and exhibited 18.55, 28.52, 35.33, 60.11, 70.99 and 78.54% at tested concentrations of 10, 20, 40, 60, 80 and 100 ppm. Ethyl acetate and *n*-hexane fractions were proved comparatively to be weak antioxidants.

The antioxidant activity of isolated compounds is presented in Table 2. When these isolated bioactive constituents were tested at cumulative concentrations (5, 10, 15, 20, 40, 60, 80 and 100 ppm) a dose dependant antioxidant effect was observed. The maximum free radical scavenging effect was observed with compound **2**. The percent protection of DPPH free radical by compound **2** was 90.51, 81.31, 72.23, 50.21, 35.51, 18.31, 10.33, and 6.58% at the tested concentrations of 100, 80, 60, 40, 20, 10 and 5 ppm respectively. The effect of compound **1** was somewhat lesser than compound **2**. The percent activity of compound **1** was 70.55, 66.52, 54.21, 40.83, 30.89, 20.26, 10.22 and 4.22% at the same tested concentrations. When compound **3** was tested against DPPH induced free radicals, a weak antioxidant effect was observed.

The multiple use of this valuable plant in different diseases is directly attributed to the antioxidant effect of various solvent fractions and the isolated chemical constituents are responsible for this radical scavenging effect. It is the antioxidant effect which protects the human body from different diseases.

The discovery or synthesis of new, effective and easily available antioxidant is a big challenge to the researcher in the modern era. It is common perception among the public that natural products are safe due to their minimal

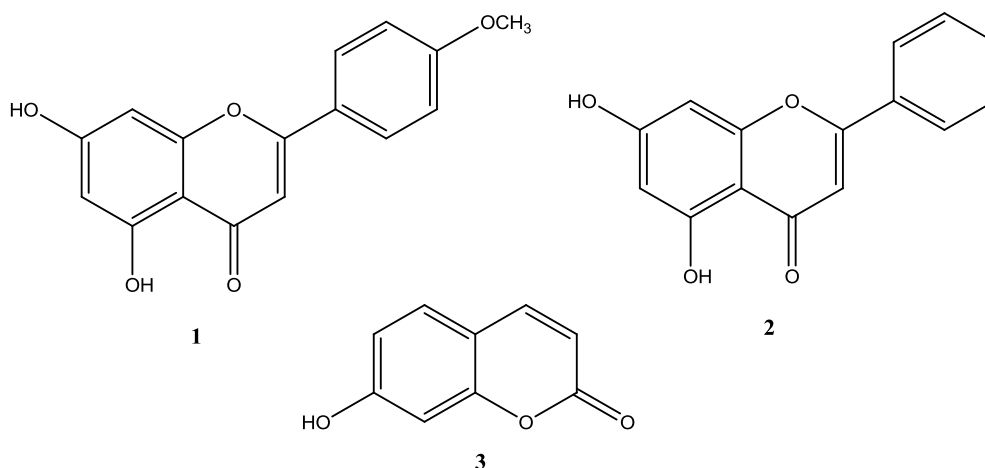


Figure 1. Chemical structures of compound 1, 2 and 3.

Table 1. Antioxidant effect of various solvent fractions.

Sample	Various tested concentration (ppm)					
	10	20	40	60	80	100
Ethanol	18.55±1.77	28.51±1.44	35.33±1.11	60.11±1.23	70.99±1.03	78.54±1.22
Methanol	55.20±1.00	78.43±1.76	84.40±3.09	87.00±1.20	92.54±1.88	96.88±1.03
Ethyl acetate	15.44±1.33	20.22±1.34	30.21±2.76	40.62±1.03	52.66±1.87	57.10±1.70
Chloroform	30.22±1.13	58.31±1.03	60.01±1.03	72.42±1.89	80.21±1.03	91.22±1.76
n-hexane	4.00±1.23	8.11±1.88	16.90±1.03	20.10±1.22	30.91±1.55	40.11±1.63

Table 2. Antioxidant effect of isolated compounds.

Compound	Tested concentrations (ppm)						
	5	10	20	40	60	80	100
1	4.22±1.55	10.0±2.11	20.21±1.22	30.81±1.89	40.80±1.89	54.21±1.890	70.50±1.03
2	6.58±1.23	10.31±2.98	18.30±1.44	35.50±1.88	50.210±1.89	72.20±1.89	90.510±1.03
3	2.21±1.03	6.48±3.00	14.50±1.73	20.28±1.46	28.40±1.89	34.30±1.89	44.10±1.03

side effects. With the hope of finding natural, safe and effective antioxidants we scrutinized the crude ethanolic extract and the various solvent fractions of *P. evestita* and further the three compounds were isolated from the chloroform fraction of this valuable medicinal plant. Antioxidants protect the human body from many ailments. The production of various types of free radicals is believed to be the cause of a large number of disease conditions. Several medicinal uses of *P. evestita* has been reported such as antimicrobial, anti-inflammatory, anti-diarrheal, anti-diabetic, hepatoprotective, anticancer, antispasmodic and as remedy for ulcerative colitis (Tomczyk et al., 2009). The use of this plant in these diseases is directly attributed to its antioxidant potential.

The evaluations of various solvent fractions for various bioassays are due to the changes in the chemical composition of these solvent fractions (polar, non-polar or of intermediate polarity) (Muhammad et al., 2012; 2013). In the current study the maximum antioxidant effect was observed with the methanolic fraction followed by the chloroform extract. This deference in antioxidant effect is due to the different chemical composition. Therefore it is concluded that in the methanolic fraction, maximum antioxidant chemical moieties are accumulated as compared to the other solvent fractions.

For any compound showing antioxidant effect, the reactive site like hydroxyl groups or unsaturated bonds are very necessary. Compounds having maximum

number of unsaturated bonds or hydroxyl groups are probably exhibit more antioxidant activity. These unsaturated sites are more reactive than saturated sites and responsible for the scavenging effect of free radicals. In the present study, the maximum effect was demonstrated with compound **2**, followed by compounds **1** and **3**. Compounds **1** and **2** are structural analog and the only difference between them is the lack of the methoxy group in compound **1**. This methoxy group causes a shielding hindrance which makes the reaction weaker to complete. The presence of methoxy group might reduce the antioxidant potential of compound **1**. It is therefore recommended to medicinal chemists to synthesize various derivatives of these isolated natural compounds which may be proved to be good antioxidants.

Conclusion

It is concluded that *P. evestita* is a rich source of antioxidant molecules and its methanolic extract is a significant antioxidant. This research work provides strong scientific background to the folkloric uses of this plant in various ailments. The therapeutic potential of *P. evestita* is therefore due to its antioxidant effect which is due to the presence of these three isolated compounds.

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