In vitro antioxidant activities of four medicinal plants on the basis of DPPH free radical scavenging

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Abstract: The present research investigation was aimed at the evaluation of antioxidant activities of methanolic (70%) extracts of whole plant of *Pentanema vestitum* and fruits of *Pistacia integerrima*, *Withiana somniferra* and *Withiana coagulans* on scavenging of 2, 2-diphenyl-2-picrylhydrazyl (DPPH) free radical. The rank of order of free antioxidant activity of the selected plants was; *P. integerrima* > *P. vestitum* > *W. somniferra* > *W. coagulans* as compared to standard Ascorbic acid. *P. integerrima* showed significantly higher activity at all concentrations as compared to Ascorbic acid at P<0.05. The percent inhibition caused by *P. integerrima* at lowest concentration (40 ppm) was 68.16±0.5 and that of Ascorbic acid was 62.00±0.5. The IC_{50} value of *P. integerrima* was 5.75ppm as compared to ascorbic acid having 15.09 ppm. The percent inhibition at all concentrations caused by *P. vestitum* was not significantly different from Ascorbic acid at P<0.05. The IC_{50} value of *P. vestitum* was 13.00ppm and that of Ascorbic acid was 15.09 ppm. The percent inhibitions caused by *W. somniferra* (IC_{50}=46.85 ppm) and *W. coagulans* (IC_{50}=84.40 ppm) were most significantly lower than Ascorbic acid at P<0.05. It is inferred from the current study that the methanolic (70%) extracts of the *P. integerrima and P. vestitum* could be used in preparation of potent antioxidant drugs.

Keywords: Antioxidant activity, *Pistacia integerrima*, *Pentanema vestitum*, $IC_{50.}$

INTRODUCTION

Free radicals damage other molecules by extracting electrons from them in order to attain stability. These free radicals are ions, atoms or molecules (Halliwell, 1995). Free radicals cause oxidation of reduced molecules found in cellular structures. These free radicals are causative agents of more than hundred disorders in man. These include arthritis, ischemia, atherosclerosis, reperfusion injury of many tissues, injury of central nervous system, gastritis and cancer (Kumpulainen *et al.,* 1999; Cook *et al.*, 1996). In oxidative stress, reactive oxygen species (ROS) such as super oxide (O_2^-), hydroxyl (OH^-) and peroxyl radicals are generated (Confort *et al.,* 2008). Reactive oxygen species play important role in defense mechanisms against infections (Emamia *et al.,* 2011). ROS also have adverse pathological effects including DNA damage, carcinogenesis and other degenerative disorders such as aging, cardiovascular diseases and neuro-degenerative diseases (Gyamfi *et al.,* 2002; Osawa, 1994; Noda *et al.,* 1997).

These free radicals can be effectively eliminated with the help of synthetic and natural antioxidants by their decomposition (Rice-Evans *et al.,* 1995). Synthetic antioxidants have many side effects such as carcinogenic effects in living organisms. There is a need for investigation of natural antioxidants with less or no side effects. Use of nutraceuticals has significant impact on the human health and diseases prevention (Haque *et al.,* 2004). *Pentanema vestitum* (Linn.) is a medicinal plant of family Asteraceae, Sub family Inuleae (Qaiser and Abid, 2003). *Pistacia integerrima* (Stew.) is a medicinal plant (tree) belongs to family Anacardiaceae, it possess anti microbial, antioxidant and hepatoprotective activities (Rahman *et al.,* 2011; Ahmad *et al.,* 2008; Khan *et al.,* 2008). *Withiana coagulans* and *Withiana somniffera* are shrubby plants belong to Solanaceae are also medicinal and have been reported for their anti-hyperglycemic and antioxidant activities (Hoda *et al.,* 2010; Deepika *et al* 2011; Nadia *et al.,* 2011).

The aim of the present study was to evaluate and compare the *in vitro* antioxidant activities of *W. somniferra*, *W. coagulans*, *P. vestitum* and *P. integerrima* on the basis of scavenging of 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical.

MATERIALS AND METHODS

Plant Material

The whole plant of *P. vestitum* L. and the fruits of *P. integerrima* S., *W. somniferra* D. and *W. coagulans* D. were collected from wild in various parts of Malakand Division, Khyber Pukhukhwa Pakistan. The specimens were identified for their authenticity by Mr. Mahboob-ur-Rahman at Department of Botany, Government Post Graduate Jehanzeb College, Swat, Khyber Pakhtunkhwa. The parts of interest were harvested properly, rinsed with tap water to remove the impurities and dried in shade for seven days. All the plant materials were separately chopped by electric grinder (Chaudary *et al.,* 2012).

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Preparation of extracts

For extracts preparation, 25gm of the chopped plant material of each plant was soaked in 250 ml solvent of 70% methanol in air tight conical flasks. The flasks were kept at 25 C and 80rpm in shaker incubator for a week. Each extract in solution form was firstly separated from the mixture by using muslin cloth and then filtered with Whatman No.1 filter paper. The solvent was evaporated from each filtrate through rotary evaporator at 40 C at reduced pressure. The concentrated extract solutions were transferred to a shady and well ventilated room for evaporation of the remaining solvent. Fully dried extract of each plant was stored at 4 C. The yield of dry mass of *P. vestitum*, *W. coagulans*, *W. somniferra* and *P. integerrima* was 3.5gm (14% w/w), 8.5gm (34% w/w), 7.25gm (29% w/w) and 8.6gm (34.5% w/w) respectively.

Antioxidant activity

A 50ml stock solution with concentration of 500ppm was prepared by dissolving Ascorbic acid in methanol which was used as standard antioxidant for comparison in the activity. Same method was used for the preparation of 50ml stock solutions of each plant extract. From each stock solution of plant extract, a triplicate of 5ml solution each of 40, 60, 80 and 100ppm concentration was prepared in separate test tubes by using the formula of serial dilution $C_1V_1 = C_2V_2$. To each test tube, 1ml of stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) was added. All the test tubes were incubated for 30 minutes at room temperature in dark and then the absorbance was measured through spectrophotometer at 517nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The difference in the absorbance between the test solution and the control (DPPH in methanol) was calculated and expressed as percent scavenging or percent inhibition of DPPH radical. The percent inhibition of DPPH radical was calculated by using the equation; Percent inhibition of DPPH = Ac-As/Ac x 100.

Whereas, As; is the absorbance of the test solution and Ac; is the absorbance of the control.

RESULTS

In present study all the plant extracts showed antioxidant activity (table 1). Maximum inhibition was caused by *P. integerrima* at lowest 40 ppm concentration (P<0.001). The percent scavenging caused by *P. integerrima* at lowest concentration (40 ppm) was 68.16±0.5 and that of standard Ascorbic acid was 62.00±0.5. At highest concentration (100 ppm) the percent inhibition caused by *P. integerrima* was 75.50±0.5 and that of ascorbic acid was 73.00±1.3. The percent inhibition caused by *P. vestitum* at all concentrations (40, 60, 80,100 ppm) was similar to that of Ascorbic acid at P<0.05; the percent inhibition caused by *P. vestitum* at lowest concentration (40 ppm) was 62.66±0.7 and that of Ascorbic acid was 62.00±0.5. At highest concentration (100 ppm) the percent inhibition of *P. vestitum* was 72.00±0.5 and that of ascorbic acid was 73.00±1.3. The free radical scavenging activities of *W. somniferra* and Ascorbic acid were compared at both lowest and highest concentrations, the free radical scavenging activity of the extract was most significantly lower at all the tested concentrations (P<0.001). At lowest concentration (40 ppm) the free radical scavenging activity of *W. somniferra* was 48.16±0.5. At highest concentration (100 ppm), *W. somniferra* extract showed 63.50±1.0 and Ascorbic acid 73.00±1.3 free radical scavenging activity. The free radical scavenging activities of *W. coagulans* and Ascorbic acid were compared at both lowest and highest doses, free radical scavenging activity of the extract was significantly lower (P<0.001). At lowest concentration, the inhibition of free radical caused by *W. coagulans* was 36.33±1.2 and Ascorbic acid 62.00±0.5. At highest concentration (100 ppm) the percent inhibition caused by *W. coagulans* was 53.50±1.0 and ascorbic acid 73.00±1.3. The of the free radical scavenging activity of all the extracts and standard ascorbic acid at lowest 40 ppm and highest 100 ppm concentrations follows the general order; *P. integerrima*> *P. vestitum*> Ascorbic acid> *W. somniferra*>*W. coagulans*. The IC_{50} values for antioxidant activity of all the plants are shown in (table 2). The extract of *P. integerrima* caused maximum inhibition or scavenging of stable 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical. Minimum IC_{50} value was demonstrated by *P. integerrima* (5.75ppm) followed by *P. vestitum* (13.00ppm), Ascorbic acid (15.09ppm), *W. somniferra* (46.85ppm) and *W. coagulans* (84.40ppm) respectively.

Table 1: Percent DPPH scavenging of extracts and ascorbic acid in various concentrations

Extract/ standard concentration in (ppm)	Percent scavenging of stable DPPH free radical (%)					F-value
	Ascorbic acid	*Pentanema vestitum*	*Pistacia integerrima*	*Withiana somniferra*	*Withiana coagulans*	
40	62.00±0.5	62.66±0.7	68.16±0.5***	48.16±0.5***	36.33±1.2***	944.704
60	65.33±1.0	66.00±1.0	70.66±0.7***	52.83±1.6***	43.66±1.0***	307.430
80	69.16±0.7	69.00±1.3	73.50±1.0**	58.66±1.5***	48.50±0.5***	270.926
100	73.00±1.3	72.00±0.5	75.50±0.5*	63.50±1.0***	53.50±1.0***	292.214

*P<0.05, **P<0.01, ***P<0.001

Table 2: The IC_{50} values of 70 % methanolic extracts of four plants and ascorbic acid

Sample	IC_{50} value in ppm
Ascorbic acid	15.09ppm
Pentanema vestitum	13.00ppm
Pistacia integerrima	5.75ppm
Withiana somniferra	46.85ppm
Withiana coagulans	84.4ppm

DISCUSSION

The present study was conducted to evaluate the antioxidant activities of the methanolic extracts of whole plant of *P. vestitum* and fruits of *P. integerrima*, *W. somniferra* and *W. coagulans*. In present study, all the plant extract demonstrated scavenging of stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical. Among these plants maximum antioxidant activity was shown by *P. integerrima*, followed by *P. vestitum*, *W. somniferra* and *W. coagulans.* There are no previous reports available for the antioxidant activity of *P. vestitum.* It is second best antioxidant agent having similar antioxidant activity to that of the standard ascorbic acid.

During the present study highest antioxidant activity was shown by *P. integerrima* supporting the work of Khalil *et al.,* (2011), who illustrated *In vitro* antioxidant activity of Ethyl acetate and n-butanol fractions of *P. integerrima*. *W. somniferra* was found at third place in the antioxidant activity among the examined four plant extracts which is in agrrement with the report of Lakshmi *et al.,* (2000), about the antioxidant activity of *W. somniferra*. *W. coagulans* also demonstrated considerable antioxidant activity. Our study supports the results of Deepika *et al.*, (2011), who evaluated methanolic and aqueous extracts of fruits of this plant showing significant antioxidant activities.

The antioxidant activity of plants is mainly due to the presence of phenolic compounds in it. During a study the extract of *P. integerrima* showed high amount of phenolic compounds and exhibited high antioxidant activity. This high scavenging property was attributed to hydroxyl groups existing in the chemical structure of phenolic compounds that can provide the necessary components as a radical scavenger (Nahak and Sahu, 2010). In another study the marked *In Vitro* antioxidant activity of *Mellilotus officinalis* was attributed to the high contents of phenolic compounds (Pourmorad *et al.,* 2006). The antioxidant activity and total phenolic content of *Tridax procumbens* there was direct correlation between the phenol contents and antioxidant activity (Habila *et al.*, 2010). This findings support earlier reports that plant metabolites like phenolic contents, tannins and flavonoids possesses antioxidant activity (Rice-Evans *et al.,* 1997). During the present study the 70% methanol extract of *P. integerrima* fruit and *P. vestitum* whole plant showed remarkable antioxidant activities. The higher antioxidant potential can be attributed to high contents of phenolic compounds in light of the earlier investigations made in this regard.

It is inferred from the present research study that among the four plants viz; *P. vestitum*, *P. integerrima*, *W. somniferra* and *W. coagulans*, the 70% methanolic extract of fruits of *P. integerrima* and the whole plant of *P. vestitum* possess high potential for scavenging 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical.

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