Preliminary antioxidant profile of *Pistacia integerrima* Stewart

Abdur Rauf[1], Ghias Uddin[1], Haroon Khan[2] and Roohullah[3]
[1]Institute of Chemical Sciences, University of Peshawar, Peshawar, KPK, Pakistan
[2]Gandhara College of Pharmacy, Gandhara University, Peshawar, KPK, Pakistan
[3]Department of Pharmacy, Abasyn University of Peshawar, KPK, Pakistan

Abstract: To explore the free radical scavenging properties of crude ethanolic extract of galls, bark, leaves, roots of *Pistacia integerrima* and its subsequent solvent fractions viz., *n*-hexane, chloroform, ethyl acetate and methanol against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) stable. *In vitro* DPPH based free radical was employed using querceitin as standard antioxidant while methanol as negative control. Different parts of *P. integerrima* showed marked scavenging on DPPH in a concentration dependent manner. The ethanolic extract exhibited 60.51 88.51% scavenging effect on DPPH which differentiated upon fractionation. Of the part used, leaves of the plant were the least effective while *n*-hexane was the least dominant fraction. However, the rest of the parts and fractions demonstrated profound scavenging potential. This *in-vitro* study revealed an outstanding free radical scavenging potential of various solvent fractions of different parts of whole plant *P. integerrima*.

Keywords: *Pistacia integerrima*, galls, leaves, bark, roots, DPPH.

INTRODUCTION

Pistacia integerima (J. L. Stewart ex Brandis) belongs to family anacardiacea. It mostly grows at a height of 12000 to 8000 feet in Eastern Himalayan regions (Uddin *et al.*, 2012). It is a variable sized tree that can grow up to forty feet. *P. integrrima* has been used in the treatment of inflammation, diabetes, blood purification, gastrointestinal problems, and as an expectorant. Indian are using this plant as antiasthmatic, antipyretic, antiemetic and antidiarrheal (Pant and Samant, 2010; Uddin *et al.*, 2012b). In Pakistan, galls of *P. integrrima* are used for treatment of hepatitis and other liver disorders (Uddin *et al.*, 201; Ahmad *et al.*, 2010). Infections, diabetes, pain, inflammatory conditions, and fever (Ahmad *et al.*, 2008).

Phytochemically, monoterpenes triterpenoids sterols, dihydromalvalic acid and flavonoids have been isolated from the different parts of *Pistacia* species (Arfan *et al.*, 2011). In this research article, we present the results of various fractions of different parts of *P. integrrima* against DPPH based *in-vitro* assay.

MATERIAL AND METHODS

Collection of plant materials

P. integerrima were collected from Toormang, Razagram (District Dir), Khyber Pakhtunkhwa, Pakistan in the month of February, 2010. The identification of plant material was done by Dr Abdur Rashid plant taxonomist Department of Botany, University of Peshawar. A voucher specimen no (RF-895) was deposited in the herbarium of the same institution.

**Corresponding author:* e-mail: mashaljcs@yahoo.com

Extraction and fractionation

Shade dried and crushed galls, leaves, barks and roots of *Pistacia integerrima* (Stewart) was extraction with ethanol. The resulting ethanolic extract of each part was suspended in water and sequentially fractionacted with hexane, chloroform, ethyl acetate and methanol in order to get respective fraction.

DPPH radical scavenging assay

The antioxidant activity was performed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay according to standard protocol as earlier discusses (Uddin *et al.*, 2012c). The hydrogen atom or electron donation capabilities of the resultanting extracts/fractions and standards were measured from the bleaching of the purple-colored methanol solution of DPPH. Experiments were carried out in triplicate. Briefly, a 1 mM solution of the DPPH radical solution in methanol was prepared and 1 ml of this solution was mixed with 3 ml of sample (extracts/fractions) solutions in methanol (containing 10-100 µg/ml) and control (without sample). The solution was standing for 30 min in dark, followed by absorbance at 517 nm. Reduction in the absorbance of DPPH solution signify an increase in the scavenging activity of test article. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follows:

%DPPH=(OD control – OD sample) X 100 / OD control

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

RESULTS

Effect of galls extracts on DPPH

The galls of *P. integerrima* were tested at accumulative concentrations i.e. 10, 20, 40, 60, 80 and 100 ppm(table 1) against DPPH. The quenching effect was in a concentration dependent mode. The maximum antioxidant effect was observed with ethyl acetate fraction (98.10%) followed by methanolic (96.88%) at 100 µg/ml (fig. 1).

Effect of leaves extracts on DPPH

The antioxidant effect of crude ethanolic extract of leaves of the plant and its various solvent fractions of the title plant are presented in table 2. Among the tested samples, maximum anti-radical activity was demonstrated by the methanolic fraction (84.88%) followed by chloroform fraction with 81.14% scavenging at 100 µg/ml (fig. 2).

Effect of barks extracts on DPPH

The antioxidant profile of crude ethanolic extract and its various solvent fractions of barks of the plant are

Table 1: DPPH radical scavenging activities of various solvent extract of galls of *P. integerrima*

Conc (ppm)	% DPPH				
	Ethanol	*n*-hexane	Chloroform	Ethyl acetate	Methanol
10	33.55	8.22	41.21	44.44	39.2
20	47.55	18.12	66.33	77.8	61.43
40	58.33	28.90	77.02	88.21	71.40
60	66.22	35.1	80.4	90.6	79.0
80	70.22	52.9	82.29	92.6	84.54
100	80.55	60.11	94.55	98.10	96.88

Data are shown as mean of three different experiments.

Table 2: DPPH radical scavenging activities of various solvent extract of leaves of *P. integerrima*

Conc (ppm)	%DPPH				
	Ethanol	*n*-hexane	Chloroform	Ethyl acetate	Methanol
10	5.51	3.21	6.31	7.22	6.24
20	10.51	8.13	13.53	16.66	15.44
40	19.31	14.91	20.02	29.11	30.41
60	40.21	29.55	40.55	52.61	55.0
80	49.21	48.11	60.28	73.61	79.51
100	60.51	58.11	69.53	81.14	84.88

Data are shown as mean of three different experiments.

Table 3: DPPH radical scavenging activities of various solvent extract of barks of *P. integerrima*

Conc (ppm)	% DPPH				
	Ethanol	*n*-hexane	Chloroform	Ethyl acetate	Methanol
10	11.51	3.22	20.21	15.41	10.21
20	22.51	6.12	44.33	30.81	19.23
40	44.31	16.90	70.02	44.22	39.22
60	72.21	30.1	80.4	70.62	70.11
80	80.28	40.9	86.29	78.63	80.51
100	88.51	50.11	96.55	82.11	90.81

Data are shown as mean of three different experiments.

Table 4: DPPH radical scavenging activities of various solvent extract of roots of P. *integerrima*

Conc (ppm)	% DPPH				
	Ethanol	*n*-hexane	Chloroform	Ethyl acetate	Methanol
10	15.55	6.21	11.32	20.41	18.21
20	22.52	10.13	22.34	44.82	30.41
40	40.32	22.95	40.44	60.23	44.43
60	60.24	30.15	70.45	78.62	58.01
80	72.28	44.99	80.21	90.61	70.52
100	82.51	55.18	92.51	95.19	80.89

Data are shown as mean of three different experiments.

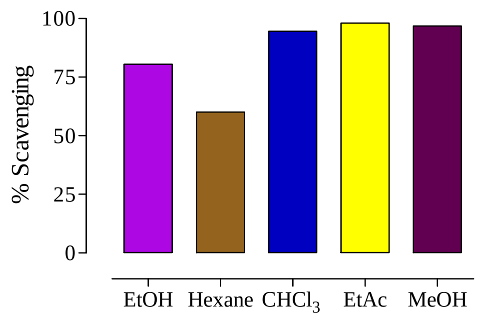

Fig 1: Percentage scavenging effect of extracts of galls of *Pistacia integerima* on DPPH at 100 µg/ml. Results are mean of three different experiments.

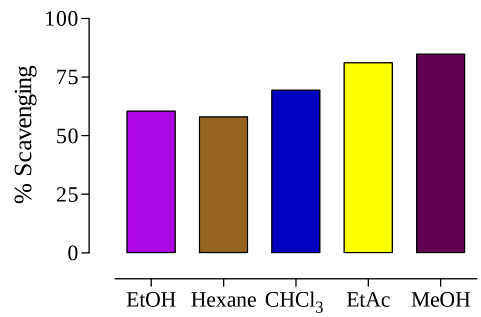

Fig 2: Percentage scavenging effect of extracts of leaves of *Pistacia integerima* on DPPH at 100 µg/ml. Results are mean of three different experiments.

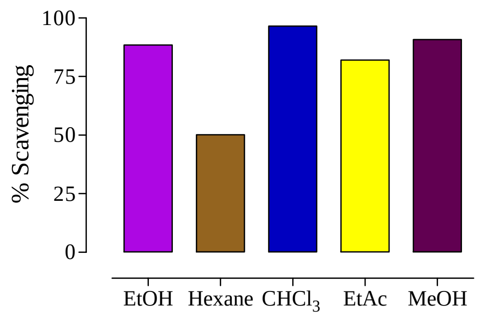

Fig 3: Percentage scavenging effect of extracts of barks of *Pistacia integerima* on DPPH at 100 µg/ml. Results are mean of three different experiments.

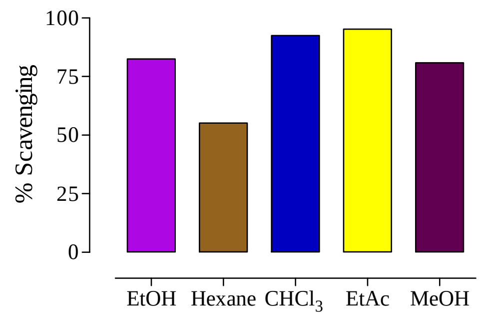

Fig 4: Percentage scavenging effect of extracts of roots of *Pistacia integerima* on DPPH at 100 µg/ml. Results are mean of three different experiments.

illustrated in table 3. In a concentration dependent manner, profound inhibition of DPPH was observed. The maximum antioxidant potential was 96.55% and 90.18% by the chloroform and methanolic fractions respectively (fig. 3).

Effect of roots extracts on DPPH

Table 4 presents the antioxidant profile of crude ethanolic extract and its various solvent fractions of roots of *P. integerrima.* Results reflected outstanding quenching effect of roots extracts in a concentration dependent manner with maximum scavenging activity of 95.19 and 92.51% by ethyl acetate and chloroform fractions respectively (fig. 4).

DISCUSSION

Free radicals are produced in several oxidative-reductive processes over expression of which may provoke oxidative damage in various human components (e.g., lipids, protein and nucleic acids). This may also contribute in different processes that causes mutations. Furthermore, free radical reactions may involved or augment several chronic diseases such as cancer, hypertension, heart disease, rheumatism, cataracts etc. that affect life style (Khan *et al*., 2012a). On the basis of their mode of action, antioxidants may be classified as free radical terminators, chelators of metal ions involved in catalyzing lipid oxidation or oxygen scavengers that react with oxygen closed system (McDowell *et al.,* 2011).

Antioxidants are free radical scavenger's 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 (Khan *et al.*, 2012a; Khan *et al.*, 2012b). These antioxidants play important roles in delaying the development of chronic disorders such as cardiovascular diseases, cancer, atherosclerosis and inflammatory diseases (Raziq *et al.*, 2011).

Antioxidants from natural sources play a paramount role in helping endogenous antioxidants to neutralize oxidative stress. Several epidemiological, clinical and experimental data suggest that plant based antioxidants have beneficial effects on prevention on chronic diseases (Khan *et al.*, 2012c; Lateef *et al.*, 2012). As a result, there has been a keen interest in evaluating the role bioactive constituents from medicinal plants in reducing the risk of the aforesaid diseases.

Numerous methods are employed for the estimation of free radical scavenging activity but DPPH has gained tremendous reputation in recent times due to its simplicity and easeness. Overall the years, It is also found rapid and sensitive technique when employed for the assament of plants extracts.

The results of our study revealed outstanding potential of various fractions of different parts of the plant in a concentration dependent manner. It could therefore be assumed the pharmacological active constituent(s) contained by fractions of the plants of different parts possessed interfered with the activity stable free radical, DPPH.

CONCLUSIONS

Thus it could be a significant natural source of anti-oxidant.

ACKNOWLEDGMENT

The author's greatly acknowledge to Institutor of Chemical Sciences, University of Peshawar and Higher Education Commission of Pakistan for financial support to conduct the research works.

REFERENCES

Ahmad NS, Farman M, Najmi MH, Mian KB and Hasan A (2008). *J. Ethanopharmacol.*, **117** (3): 478-482.

Ahmad NS, Waheed A, Farman M and Qayyum A (2010). Analgesic and anti-inflammatory effects of *Pistacia integerrima* extracts in mice. *J. Ethnopharmacol*., **129**: 250-253.

Arfan M, Rauf A, Tahir MN, Ali M, and Uddin G (2011). 2-Methyl-6-(4,4,10,13,14-pentamethyl-3-oxo2,3,4,5,6,7,10,11,12,13,14,15,16,-17-tetradecahy-dro-1H-cyclopenta[a] phenanthren-17-yl)hept-2-enoic acid. *ActaCryst,* E 67: o711.

Khan H, Khan MA and Abdullah (2012c). Antibacterial, antioxidant and cytotoxic studies of total saponin, alkaloid and sterols contents of decoction of Joshanda: components identification through TLC *Toxicol. Industrial Health*; DOI: 10.1177/0748233712468023.

Khan H, Khan MA, Muhammad N, Ashraf N, Gul F and Tariq SA (2012b). Antiinflammatory and antioxidant activity of Joshanda partially mediated through inhibition of lipoxygenase. *Phytopharmacol*; **3**: 19-28.

Khan H, Saeed M, Khan MA, Khan I, Ahmad M, Muhammad N and Khan A (2012a). Antimalarial and free radical scavenging activities of rhizomes of *Polygonatum verticillatum* supported by isolated metabolites. *Med. Chem. Res*., **21**: 1278-1282.

Lateef M, Iqbal L, Fatima N, Siddiqui K, Afza N, Zia-ul-Haq M and Ahmad M (2012). Evaluation of antioxidant and urease inhibition activities of roots of *Glycyrrhiza glabra*. *Pak. J. Pharm. Sci*., **25**: 99-102.

McDowell A, Thompson S, Stark M, Ou Z-Q and Gould KS (2011). AntioxidantActivity of Puha (*Sonchus oleraceus* L.) as Assessed by the Cellular Antioxidant Activity (CAA) Assay. *Phytoth. Res.*, **25**: 1876-1882.

Pant S and Samant SS (2010). Ethanobotanical observation in the Momaula Reserve Forest of Koumoun., West Himalaya. India Ethanobotanical Leaflets 1493.

Raziq N, Muhammad N, Chishti KA, Saeed M, Rahman S, Khan H (2011). Correlation of the antioxidant capacity with the phenolic contents of *Hypericum monogynum* and *Hypericum perforatum*. *Afr. J. Pharm. Pharmacol*., **5**:1872-1876.

Uddin G, Abdur Rauf, Abdulaziz M. Al-Othman, Simona Collina, Muhammad Arfan, Gowhar Ali, Inamullah Khan (2012b). Pistagremic acid, a glucosidase inhibitor from *Pistacia integerrim. Fitoterapia*, **83**(8):1648-1652.

Uddin G, Rauf A, Arfan M, Ali M, Qaisar M, Saadiq M and Atif M (2012c). Preliminary phytochemical Screening and antioxidant activity of *Bergenia caliata*. *Middle-East J. Sci. Res*., **11**: 1140-1142.

Uddin G, Rauf A, Rehman TU and Qaisar M (2011). *Middle-East J. Sci. Res*., **7**(5): 707-711.

Uddin G, Rauf A, Arfan M, Waliullah, Khan I, Ali M, Taimur M, Ur-Rehman I and Samiullah (2012a). Pistagremic acid a new leishmanicidal triterpene isolated from *Pistacia integerrima* Stewart. *J. Enz. Inhib. Med. Chem*. **27**(5): 646-8.