Pyrus pashia: A persuasive source of natural antioxidants

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Abstract: *Pyrus pashia* Buch. & Ham. was subjected to extraction with methanol. Methanolic extracts of fruit, bark and leaf were partitioned separately with four organic solvents in order of increasing polarity, as*n*-hexane, chloroform, ethyl acetate and *n*-butanol after dissolving in distilled water. Phytochemical screening revealed the presence of phenolics, flavonoides, alkaloids and cardiac glycosides in large amount in chloroform, ethyl acetate and *n*-butanol soluble fractions. The antioxidant activity of crude methanolic extracts, all the obtained fourorganic fractions and remaining aqueous fractions was evaluated by different methods such as: 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, ferric reducing antioxidant power (FRAP) assay and total antioxidant activity by phosphomolybdenum complex method as well as determination of total phenolics. The results of antioxidant activity exhibited that chloroform soluble fraction of fruit showed the highest value of percent inhibition of DPPH (48.16± 0.21µg/ml) at the concentration of 10µg/ml. Ethyl acetate soluble fraction displayed the lowest antioxidant activity havingIC_{50} value of bark as (8.64±0.32µg/ml) relative to butylated hydroxytoluene (BHT), having IC_{50} of 12.1± 0.92µg/ml. The ethyl acetate soluble fraction of bark revealed the highest FRAPs value (174.618±0.11TE µM/ml) among all the three parts. This fraction also showed the highest value of total antioxidant activity as (1.499±0.90), determined by phosphomolybdenum complex method. Moreover, this fraction also conferred the highest phenolic content (393.19± 0.72) as compared to other studied fractions of fruit and leaf.

Keywords: *Pyrus Pashia*, solvent extraction, phytochemical screening and antioxidant analysis.

INTRODUCTION

In living organisms, free radicals are produced as a result of the normal metabolic pathways, and free radical chain mechanism that normally occur as respiratory chain reaction in the mitochondria, through xanthene oxidase activity, liver mixed function oxidases, atmospheric pollutants and from the catalysis of transition metals, xenobiotics and drugs (Ara and Nur, 2009). Oxidative stress is responsible for several pathological conditions e.g. cancer, neurological disorders, cardiovascular disease, diabetes and ageing. Overproduction of RNS, nitrosylation reactions can interfere the normal structure of proteins and hinder their regular functions, DNA fragmentation and lipid oxidation in human body (Barreira *et al.*, 2008). Antioxidants based on their ability to scavenge deleterious free radicals e.g. "reactive oxygen species" (ROS) as well as "reactive nitrogen species" (RNS) which are by product of normal cellular metabolism. Free radicals are described as molecules which have an unpaired electron in the outer orbit. These molecules are usually unstable hence are, very reactive and can be transformed to other non-radical reactive species. These radicals act as a double bladed sword since they can be either harmful or beneficial to living systems (Zhang *et al.*, 2009). At low concentrations, ROS can cause mutagenic response in the cells. NO (Nitric oxide) serves as a significant signaling molecule and is involved in leukocytes adhesion, platelets aggregation, the relaxation and proliferation of vascular smooth muscle cells, angiogenesis, vascular tone, thrombosis, and hemodynamics (Uttara *et al.*, 2009). However, ROS and RNS can be severely harmful to cell structures composed of proteins, lipids, membranes, and nucleic acids at higher concentrations (Valko *et al.*, 2006). Therefore, if overproduction of free radicals cannot be balanced by enzymatic and non-enzymatic antioxidants, oxidative and nitrosative stresses will damage the biological systems and the redundant free radicals can alter cellular lipids, proteins, and DNA and suppress their normal function (Barreira *et al.*, 2008). In the past years, because of the concern of toxicity and carcinogenic claims of synthetic antioxidants, finding new natural antioxidants without undesirable side effects have become more popular. As a result, studies have centered on antioxidants, such as phenolics, from natural sources, for instance, naturally present in fruits, vegetables, nuts and herbs (Frankel and Finley, 2008). Moreover, these naturally occurring antioxidants might be developed as nutraceuticals helping to inhibit oxidative damage occurring in the body (Dudonne *et al.*, 2009).

Pyrus pashia Buch. & Ham. occurs in China, Bhutan, India, Kashmir, Laos, Myanmar, Nepal, Pakistan, Sikkim, Thailand, Vietnam, and Afghanistan. It is a medicinal plant (Bhatt *et al*., 2010) and is stated as a new documentation from North-Eastern Iran. It has punctuated fruits, deciduous and ovate-lanceolate leaves with more or less long sharp apex. The genus *Pyru s*belongs to sub-family *Maloideae*, family Rosaceae. The genus contains

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about 38 species (Zamani *et al*., 2009). It is commonly known as Molz (Pant, 2010). *Pyrus pashia* is also known as wild pear and is edible (Kala, 2007). It is most commonly used as laxative, febrifuge and sedative (Matin *et al*., 2001). *Pyrus pashia* Buch. & Ham. has soft and sweet fruits. The fruit of *Pyrus pashia* is suitable for dehydration. *Pyrus pashia* Buch. & Ham. is an appropriate rootstock for pear. Wild plants of *P. pashia* can be top worked with pear (Parmar and Koushal, 1982). Its fruits are used in digestive ailments. These are also used to treat infected eyes of cattle in pterygium disease (Terpo, 1992). Its cell sap is used as conjunctivitis (Promila and Dinesh, 2005). *Pyrus pashia* has a number of medicinal uses. It is used in gastrointestinal disorders, fever and headache, sweating of body (diaphoretic), hysteria and epilepsy. The fruits of this plant are sedative, febrifuge and laxative (Murad *et al*., 2008). According to our knowledge, as no detailed study on antioxidant activity and phytochemical screening of this plant have been carried out yet, therefore, the aim of the present investigation, to evaluate the comparative *in vitro* antioxidant capacity of aqueous as well as organic fractions of *Pyrus pashia* by three different antioxidant methods like total antioxidant activity by phosphomolybdenum complex (PC) method, 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity and FRAP assay along with determination of their total phenolic contents relative to conventionally used standards. Phytochemical screening was carried out to understand the synergic effect of the natural constituents present, which are responsible for the antioxidant potential of the various aqueous and organic fractions of the plant selected.

METERIALS AND METHOD

Plant material

The plant *Pyrus pashia* Buch. & Ham. was collected from Azad Jammu Kashmir in December 2010, and identified by Mr. Muhammad Ajaib (Taxonomist), Department of Botany, GC University, Lahore. Voucher specimen number (G.C. Herb. Bot. 821) has been placed in the herbarium of the above mentioned university.

Extraction, filtration and partitioning

The leaves (273g), fruits (220g) and bark (731g) were separated and shade dried and ground separately. The leaves were extracted with (3L×4), fruits with (2L×3) and bark with (4L×5) of methanol. The extraction was done carefully at room temperature. The three extracts were evaporation to get the residues; 110g for fruits, 140g for leaves and366g for bark. These residues were then separately partitioned with *n*-hexane (1L×4), chloroform (1L×4), ethyl acetate (1L×4) and *n*-butanol successively. These organic fractions and remaining aqueous fractions were concentrated separately on rotary evaporator and these residues were used to evaluate their *in vitro* antioxidant potential.

Chemicals and standards

Trolox, TPTZ (2,4,6-Tripyridyl-s-triazine), DPPH˙ (1,1-Diphenyl-2-picrylhydrazyl radical), BHT (Butylated hydroxytoluene), Gallic acid, and Folin-Ciocalteu reagent were obtained from the Sigma Chemical Company Ltd. USA. Organic solvents i.e. chloroform, *n*-hexane, ethyl acetate, and *n*-butanol as well as sulphuric acid, ammonia, aluminium chloride, acetic acid, bismuth nitrate, potassium iodide, ceric sulphate, sodium phosphate, ferrous chloride, ammonium molybdate, and ferric chloride, from Merck (Pvt.) Ltd. Germany.

Phytochemical screening

Phytochemical screening was performed using the standard methods (Ayoola *et al*., 2008; Trease and Evans, 1989; Sofowara, 1993) and are described below.

Test for reducing sugars (Fehling's test)

Fehling's solution (A and B) was boiledin a test tube with sample solutions (0.5 g of sample in 5 ml of water). Formation of red precipitates indicated the presence of sugars.

Test for terpenoids

Two methods were employed to check the presence of terpenoids. First (Salkowski test), 3ml of concentrated H_2SO_4 was carefully added in test tube containing 0.5 g of each of the extracts in chloroform (2ml) to form a reddish brown coloration at the interface which gave the positive test forterpenoids. Second, TLC card having spots of samples was sprayed with ceric sulfate solution and heated further on a TLC heater. Appearance of brown color indicated the presence of terpenoids.

Test for flavonoids

Four methods were employed for detection of flavonoids in plant extracts. First, to a portion of an aqueous filtrate of the extract, 5ml of dilute ammonia was added. 1ml of concentrated sulphuric acid was also added. A yellow coloration that disappeared on standing indicated the presence of flavonoids. Second, a few drops of 1% aluminium chloride solution was added to sample solution. Yellow coloration indicated the presence of flavonoids. Third, TLC card having spots of samples was sprayed with Benedict's reagent. Green fluorescence in UV light indicated flavanoids. Lastly, TLC card having spots of samples was sprayed with lead acetate, which also showed Green fluorescence in UV light and indicated the presence of flavanoids.

Test for saponins

To 0.5g of extract in a test tube 5 ml of distilled water was added. The solution was shaken vigorously till the formation of a stable persistent froth. 3 drops of olive oil were added in the frothing and was shaken vigorously till an emulsion was formed, which indicated the presence of saponins.

Test for tannins
Mixture of 5ml of *n*-butanol-HCl and 2ml of sample solutions were warmed for 1hr in a water bath. Appearance of red color indicated the presence of tannins.

Test for alkaloids
TLC card having spots of samples was sprayed with Draggendroff' sreagent. Appearance of orange color indicated alkaloids.

Test for cardiac glycosides (Keller-Killiyani test)
0.5g of each extract was diluted with 5ml of water. Glacial acetic acid (2ml) containing one drop of $FeCl_2$ solution was added drop wise in the diluted extracts. This was underlayed with concentrated sulphuric acid (1ml). A brown ring appeared at the interface of two layers, which showed the presence of deoxysugar, a characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Antioxidant assays
Following antioxidant assays were performed on all studied fractions of *Pyrus pashia*.

DPPH radical scavenging activity
The DPPH radical scavenging activities of various fractions of plant were examined by comparison with that of known antioxidant, butylated hydroxytoluene (BHT) adopting the method of Lee and Shibamoto (2001). Briefly, various amounts of the samples (200µg/ml, 150µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 10µg/ml,) were mixed with 3 ml of 0.1mM solution of DPPH made in methanol. The mixture was allowed to stand at room temperature for one an hour after shaking vigorously. Then absorbance was measured at 517 nm against methanol as a blank in the spectrophotometer. Lower absorbance of spectrophotometer indicated the higher free radical scavenging potential.

The percent of DPPH discoloration of the samples was calculated according to the formula:

$$\text{Antiradical activity} = \frac{\text{Acontrol - Asample}}{\text{Acontrol}} \times 100$$

Each sample was assayed in triplicate and mean values were calculated".

Total antioxidant activity by phosphomolybdenum complex method
"The total antioxidant activities of various fractions of plant were evaluated by phosphomolybdenum complex formation method (Prieto *et al.*, 1999). Briefly, 4 ml of reagent solution was mixed with 500 µg/ml of each sample (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in sample vials.4ml of reagent solution was taken as blank. The vials were capped and incubated at 95°C for 90 minutes in water bath. The absorbance of mixture was measured at 695 nm against blank when the contents of the test tubes were cooled to room temperature. The antioxidant potential was expressed relative to that of butylated hydroxytoluene (BHT). All determinations were assayed in triplicate and mean values were calculated".

Ferric reducing antioxidant power (FRAP) assay
"The FRAP assay was done according to Benzie and Strain (1996) with some modifications. The stock solutions contained 10mM TPTZ (2, 4, 6-Tripyridyl-s-triazine) solution in 40mM HCl, 300mM acetate buffer (3.1g $CH_3COONa.3H_2O$ and 16 ml CH_3COOH), pH 3.6, and 20mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 2.5ml TPTZ solution, 2.5 ml $FeCl_3.6H_2O$ and 25 ml acetate buffer solution. The solution was then warmed at 37°C before using.

Table 1: Phytochemical screening of various fractions of *Pyrus pashia*

Parts	Samples	Alkaloids	Terpenoids	Tannins	Phenols	Sugar	Saponins	Flavonoids
Fruit	Crude methanolic extract	+++	++++	++++	++++	++++	++++	+++++
	n-Hexane soluble fraction	++	+++	+++	+++	+++	+++	+++
	Chloroform soluble fraction	++	+++	+++	+++	+++	+++	+++
	Ethylacetate soluble fraction	+	++	++	++	++	++	++
	n-Butanol soluble fraction	-	+	+	+	+	+	+
	Remaining aqueous fraction	-	-	-	-	-	-	-
Bark	Crude methanolic extract	+++	++++	++++	++++	++++	++++	+++++
	n-Hexane soluble fraction	++	+++	+++	+++	+++	+++	++++
	Chloroform soluble fraction	++	+++	+++	+++	+++	+++	+++
	Ethylacetate soluble fraction	+	++	++	++	++	++	+++
	n-Butanol soluble fraction	-	+	+	+	+	+	++
	Remaining aqueous fraction	-	-	-	-	-	-	+
Leaf	Crude methanolic extract	+++	++++	++++	++++	++++	++++	+++++
	n-Hexane soluble fraction	++	+++	+++	+++	+++	+++	+++
	Chloroform soluble fraction	++	+++	+++	+++	+++	+++	+++
	Ethylacetate soluble fraction	+	++	++	++	+++	++	++++
	n-Butanol soluble fraction	-	+	+	+	+	+	++
	Remaining aqueous fraction	-	-	-	-	-	-	-

The solutions of plant samples and that of trolox were formed in methanol (250µg/ml). 10µl of each of sample solution and BHT solution were taken in separate test tubes and 2990µl of FRAP solution was added in each to make total volume up to 3ml. The FRAP solution was allowed to react with plant samples in the dark for 30 minutes. Absorption readings of the colored product [ferrous tripyridyltriazine complex] were taken at 593 nm by spectrophotometer. The FRAP values were determined as micromoles of trolox equivalents per mL of sample by computing with standard calibration curve constructed for different concentrations of trolox. Results were expressed in TE µM/Ml".

Total phenolic contents

"Total phenolics of various fractions of plant were determined by the method of Makkar *et al*. (1993). The 0.1ml (0.5mg/mL) of sample was combined with 2.8 ml of 10% Na_2CO_3 and 0.1ml of 2N Folin-Ciocalteu reagent. After 40 minutes absorbance at 725 nm was measured by UV-visible spectrophotometer. Total phenolics were determined as milligrams of gallic acid equivalents per gram of sample by computing with standard calibration curve constructed for different concentrations of gallic acid. The standard curve was linear between 50µg/ml to 500µg/ml of gallic acid. Results were expressed in GAE µg/ml".

STATISTICAL ANALYSIS

All the measurements were done in triplicate and statistical analysis was performed by Microsoft excel 2007. Results are presented as average ± S.E.M.

RESULTS

Results of phytochemical constituents are shown in table 1 while for antioxidant assays have been shown in table 2 and table 3. *Pyrus pashia* contains alkaloids, flavonoids, terpenoids, and tannins in an appreciable amount. DPPH Radical Scavenging Activity, Total Phenolic Contents, Total Antioxidant Activity by Phosphomolybdenum Complex Method and Ferric Reducing Antioxidant Power (FRAP) Assay were adopted to evaluate the antioxidant potential of different organic fractions and the remaining aqueous fractions of leaves, fruit and bark separately. In general, the ethyl acetate soluble fractions of all parts showed the highest antioxidant activity against all antioxidant assays applied.

DISCUSSION

Phytochemicals are defined as non-nutritive bioactive plant chemicals in fruits, vegetables, grains, and other plant foods having protective or disease preventive properties and are considered to reduce the risk of chronic diseases. Many individual phytochemicals have been identified and classified in fruits, vegetables, and grains. However, still large amount of phytochemicals need to be characterized to comprehend their health benefits. Some well-known phytochemicals include carotenoids, phenolics or polyphenols, alkaloids, nitrogen-including compounds and organosulfur compounds (Dong and Liu, 2007). On all the fractions of *Pyrus pashia*, the phytochemical screening was performed. The results are presented in (table 1). These results showed that flavanoids, phenolics and alkaloids were present in chloroform, ethyl acetate and *n*-butanol soluble fractions, however, *n*-hexane soluble fraction did not contain these constituents. Various fractions of *Pyrus pashia* also contained cardiac glycosides i.e. present in ethyl acetate and *n*-butanol soluble fraction in higher concentration but totally absent in *n*-hexane and chloroform extracts. All fractions contained tannins and sugars except chloroform and *n*-hexane while *n*-butanol contained sugars in significant amount. Most of the terpenes were present in *n*-hexane soluble fraction. Conversely, all other fractions have terpenes in lesser amount. Saponins were present in all the fractions except *n*-hexane and chloroform. The phenolics, flavonoids, cardiac glycosides, tannins, saponins and alkaloids showed their activity in residual water layer. These components are very important and are biologically active.

DPPH Radical Scavenging Activity

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) is a relatively stable Nitrogen containing free radical that is reduced easily by accepting an electron or hydrogen, of compounds that act as primary antioxidants. After reacting with suitable reducing agents, it loses color due to pairing up of electrons. Loss of color depends on the number of electrons taken up (Habila *et al.,* 2010). It is the odd electron of DPPH radical which is responsible for the absorbance at 515-517 nm and its visible purple color. The DPPH radical is decolorized, when it accepts an electron donated by an antioxidant compound. This can be quantitatively measured from the changes in absorbance (Abbasi *et al.,* 2011). During aerobic metabolism in our body free radicals such as superoxides, hydroxyl radicals and other reactive species (ROS) such as H_2O_2 and hypochloric acids are generated. Production of ROS in excess or inadequate antioxidant defense can be a cause of oxidative damage to a variety of biomolecules. Free radicals can neutralize the reactive oxygen species (Halliwel and Gutteridge, 1998). 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical is utilized to examine the scavenging activities of the various extracts of natural products. A diamagnetic molecule is produced when DPPH gains an electron or hydrogen radical. Antioxidants in plant fractions scavenge DPPH radical and *in vitro* converts it into reduced DPPH. The color changes from purple to yellow after reduction. As a result, the absorbance is decreased which is studied at wavelength 517nm. Free radical scavenging activity is enhanced when the free radical inhibition percentage is

enhanced (Haung *et al.,* 2005). In the current investigation, various extracts of fruit, bark and leaves of *Pyrus pashia* were assessed for their free radical scavenging potentialby using the DPPH radical assay. The DPPH radicals were reduced by *Pyrus pashia*. Results of %scavenging activity of DPPH radicals for fruit, bark and leaves are shown in (table 2). Choloroform soluble fraction of fruit exhibited the highest activity (48.16±0.21µg/ml) while ethyl acetate and methanolic fractions showed (45.22±0.08µg/ml) and (39.26±0.09µg/ml) respectively. Ethyl acetate soluble fraction of bark and leaves gave the highest activity as (43.50±0.1µg/ml) and (45.59±0.09µg/ml) respectively. Activities of choloroform soluble fractions of both bark and leaves were slightly less than that of ethyl acetate fractions at the concentrations of 10µg/ml relative to BHT which showed (23.57±0.31µg/ml at 08µg/ml concentration). Since IC_{50} measures the inhibitory concentration, its lower value shows greater antioxidant activity of the sample Ethyl acetate fraction displayed the lowest antioxidant activity with IC_{50} value of fruit as (11.42±0.23µg/ml), leaf as (10.92±0.36µg/ml) and bark as (8.64±0.32µg/ml), followed by chloroform fraction with IC_{50} value of fruit as (12.10±0.51µg/ml), leaf as (13.54±0.53µg/ml) and bark as (9.40±0.51µg/ml), crude methanolic extract with IC_{50} value of fruit as (37.16± 0.10µg/ml), leaf as (29.08±0.28 µg/ml) and bark as (23.32 ±0.25µg/ml), *n*-butanol fraction with IC_{50}value of fruit as (13.74±0.19µg/ml), leaf as (27.07±0.68µg/ml) and bark as (16.28±0.55µg/ml), *n*-hexane fraction with IC_{50}value of fruit as (57.13±0.94µg/ml), leaf as (65.66±0.44 µg/ml) and bark as (36.48±0.46µg/ml) and remaining aqueous fraction with IC_{50}value of fruit as (39.41±0.36µg/ml), leaf as (39.31±0.15µg/ml) and bark as (35.50±0.11µg/ml), relative to butylated hydroxytoluene (BHT), having IC_{50} of 12.1±0.92µg/ml.

Table 2: % age inhibition of plant extracts of *Pyrus pashia*

Part	Samples	Concentration	% inhibition
Fruit	Crude methanolic extract	200	81.15 ± 0.10
		150	77.59 ± 0.34
		100	72.60 ± 0.23
		50	58.28 ± 0.11
		25	42.32 ± 0.11
		10	39.26 ±0.09
	n-Hexane soluble fraction	200	79.14 ± 0.08
		150	67.71 ± 0.41
		100	60.90 ± 0.37
		50	51.14 ± 0.26
		25	42.56 ±0.09
		10	38.34 ±0.18
	Chloroform soluble fraction	200	84.29± 0.10
		150	75.39 ± 0.55
		100	65.35 ± 1.15
		50	59.78 ± 0.57
		25	54.56 ± 0.29
		10	48.16 ±0.21
	Ethyl acetate soluble fraction	200	81.41 ± 0.13
		150	78.88 ± 0.07
		100	67.71 ± 0.04
		50	61.11± 0.31
		25	55.59 ±0.19
		10	45.22 ±0.08
	n-Butanol soluble fraction	200	85.25 ± 0.16
		150	81.50 ± 0.71
		100	76.70 ± 0.37
		50	62.30± 0.36
		25	54.16 ±0.26
		10	39.78 ±0.19
	Remaining aqueous fraction	200	85.86± 0.25
		150	81.93 ± 0.46
		100	76.03 ± 1.07

Counted…

Table 2: *Continue…*

Part	Samples	Concentration	% inhibition
Fruit	Remaining aqueous fraction	50 25 10	64.50 ± 0.43 46.56 ±0.26 26.93 ±0.33
Bark	BHT[b)]	60 30 15 08	91.25 ± 0.13 75.56 ± 0.07 42.67 ± 0.04 23.57 ± 0.31
	Crude methanolic extract	200 150 100 50 25 10	86.73 ± 0.10 74.08 ± 0.34 69.37 ± 0.23 62.23 ± 0.11 51.59 ± 0.11 39.99 ± 0.09
	n-Hexane soluble fraction	200 150 100 50 25 10	96.32 ± 0.08 90.90 ± 0.41 85.65 ± 0.37 70.38 ± 0.26 40.94 ±0.20 25.56 ±0.11
	n-Butanol soluble fraction	200 150 100 50 25 10	97.90± 0.10 92.11± 0.55 84.55 ± 1.15 72.55 ± 0.57 55.38 ± 0.29 31.01 ±0.33
	Ethyl acetate soluble fraction	200 150 100 50 25 10	84.29 ± 0.13 71.64 ± 0.07 67.53 ± 0.04 63.08± 0.31 58.27 ±0.25 43.59 ±0.17
	Chloroform soluble fraction	200 150 100 50 25 10	89.59± 0.16 86.21 ± 0.71 78.62 ± 0.37 65.88± 0.36 58.35 ±0.09 40.55 ±0.18
	Remaining aqueous fraction	200 150 100 50 25 10	97.81 ± 0.25 88.36 ± 0.46 74.38 ± 1.07 52.33 ± 0.43 45.94 ±0.23 41.55 ±0.15
	BHT[b)]	60 30 15 08	91.25 ± 0.13 75.56 ± 0.07 42.67 ± 0.04 23.57 ± 0.31
Leaf	Crude Methanolic extract	200 150 100 50 25 10	92.47 ± 0.10 85.77 ± 0.34 76.93 ± 0.23 62.57 ± 0.11 54.74 ± 0.11 29.99 ±0.09

Counted…

Table 2: *Continue…*

Part	Samples	Concentration	% inhibition
Leaf	Chloroform soluble fraction	200	94.24 ± 0.16
		150	86.73 ± 0.71
		100	75.38 ± 0.37
		50	63.89± 0.36
		25	54.55 ±0.31
		10	42.01 ±0.28
	Remaining aqueous fraction	200	82.72± 0.25
		150	81.55 ± 0.46
		100	80.97 ± 1.07
		50	55.84 ± 0.43
		25	40.92 ±0.19
		10	36.21 ±0.28
	BHT[b)]	60	91.25 ± 0.13
		30	75.56 ± 0.07
		15	42.67 ± 0.04
		08	23.57 ± 0.31

Ferric Reducing antioxidant Power (FRAP) Assay

To estimate the antioxidant capacities of various extracts of selected parts of medicinal plant, two assays i.e., ferric reducing antioxidant power (FRAP) and that of Trolox equivalent antioxidant capacity (TEAC) was used. The mechanism of FRAP assay is based on the capability of antioxidants to reduce Ferric (III) ions (electron donating antioxidants produced colorless oxidized Fe^{3+}) to Ferrous (II) ions as deep blue Fe^{2+}tripyridyltriazinecomplex (Hodzic *et al.*, 2009). Reducing ability of antioxidants is measured by the FRAP assay against oxidative stress of reactive oxygen species. Antioxidants are called as reductants because they donate electrons and this inactivation of oxidants by reductants can be described as redox reactions. Formation of ferrous ions from FRAP reagent containing TPTZ and $fecl_3.\ 6H_2O$, causes an increase in absorbance which is a measure of antioxidant potential. These measurements are taken by spectrophotometer at 595nm (Abbasi *et al*., 2010). The present study revealed that the extract and fractions of *Pyrus pashia* showed effective antioxidant power with FRAP values expressed in µM/ml and are shown in (table 3). The ethyl acetate fraction gave the highest FRAP value of fruit (78.618±0.32TE µM/ml), bark (174.618±0.11 TE µM/ml) and leaf (105.818±0.14 TE µM/ml), chloroform soluble fraction of fruit (73.618± 0.59 TE µM/ml), bark (158.018±0.51 TE µM/ml) and leaf (89.618±0.54 TE µM/ml), followed by *n*-butanol soluble fraction of fruit (65.618±0.66 TE µM/m), bark (129.218 ±0.60 TE µM/m) and leaf (82.818±0.65 TE µM/ml), crude methanolic extract of fruit (51.218±0.19 TE µM/ml), bark (97.818±0.13 TE µM/ml) and leaf (71.418±0.29 TE µM/ml), aqueous fraction of fruit (50.018±0.29 TE µM/ml), bark (91.818±0.12 TE µM/ml) and leaf (55.218±0.19 TE µM/m)while the lowest value was shown by *n*-hexane fraction of fruit (49.618 ± 0.44 TE µM/ml), bark (97.818±0.13 TE µM/ml) and leaf (52.618±0.45 TE µM/ml).

Total Antioxidant activity by Phosphomolybdenum complex method

Phosphomolybdenum method is used to determine the total antioxidant power of different extracts of *P. pashia*. This method basically involves the reduction of Mo (VI) to Mo (V) by the antioxidants. A green phosphate molybdenum complex is produced in acidic conditions with maximal absorption at 695nm.Antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids are usually detected by phosphomolybdenum method (Riaz *et al*., 2011). The antioxidant activities of various extracts of *Pyrus pashia* were compared with the reference standard antioxidant BHT. The highest total antioxidant capacity shown in table 3 and are discussed in decreasing order, as ethyl acetate soluble fraction of fruit (1.420±0.34), bark (1.499±0.90) and leaf (1.513±0.47), chloroform soluble fraction of fruit (1.305±0.45), bark (1.452±0.79) and leaf (1.481±0.11), crude methanolic extract of fruit (1.084±0.12), bark (1.271±0.36) and leaf (1.196±0.92), water fraction of fruit (0.920±0.25), bark (1.258±0.56) and leaf (1.095±0.99), *n*-butanol soluble fraction of fruit (1.248±0.75), bark (1.356±0.81) and leaf (1.447±0.33) and lowest by *n*-hexane fraction of fruit (0.751±0.31), bark (0.188±0.83) and leaf (0.743±0.16). The results were compared with BHT, a reference standard, having total antioxidant potential 12.15±0.22.

Total phenolic contents

Phenolic compounds constituting one of the most widespread groups of substances are the products of secondary metabolism in plants. More than 8000 phenolic structures have been characterized, which involve essential activities in the reproduction and growth of the plants, play important role as defense agents against pathogens, parasites, and predators, as well as promotion to the color of plants (Dong and Liu, 2007). Phenolic acids are related to sensory qualities, color, antioxidant and nutritional aspects of foods. Organoleptic properties

Table 3: IC_{50}, Total Antioxidant Activity, FRAP value and total phenolics of different fractions of *Pyru spashia*

Part	Samples	IC_{50} of DPPH assay (mg/ml) ±S.E.M[a)]	Total anti-oxidant activity ±S.E.M[a)]	FRAP value TE (mM/ml) ±S.E.M[a)]	Total phenolics (GAE) mg/g of sample) ±S.E.M[a)]
Fruit	Crude Methanolic extract	37.16±0.10	1.084±0.12	51.218±0.19	141.02±0.34
	n-Hexane soluble fraction	57.13±0.94	0.751±0.31	49.618±0.44	14.06±0.22
	Chloroform soluble fraction	12.10±0.51	1.305±0.45	73.618±0.59	180.58±0.31
	Ethyl acetate soluble fraction	11.42±0.23	1.420±0.34	78.618±0.32	237.32±0.89
	n-Butanol soluble fraction	13.74±0.19	1.248±0.75	65.618±0.66	159.06±0.13
	Remaining aqueous fraction	39.41±0.36	0.920±0.25	50.018±0.29	17.54±0.99
Bark	Crude Methanolic extract	23.32±0.25	1.271±0.36	97.818±0.13	320.80±0.41
	n-Hexane soluble fraction	36.48±0.46	0.188±0.85	60.818±0.43	158.41±0.53
	Chloroform soluble fraction	09.40±0.51	1.452±0.79	158.018±0.51	332.97±0.44
	Ethyl acetate soluble fraction	08.64±0.32	1.499±0.90	174.618±0.11	393.19±0.72
	n-Butanol soluble fraction	16.28±0.55	1.356±0.81	129.218±0.60	331.45±0.21
	Remaining aqueous fraction	35.50±0.11	1.258±0.56	91.818±0.12	317.54±0.55
Leaf	Crude Methanolic extract	29.08±0.28	1.196±0.92	71.418±0.29	161.67±0.39
	n-Hexane soluble fraction	65.66±0.44	0.743±0.16	52.618±0.45	87.54±0.50
	Chloroform soluble fraction	13.54±0.53	1.481±0.11	89.618±0.54	305.58±0.41
	Ethyl acetatesoluble fraction	10.92±0.36	1.513±0.47	105.818±0.14	321.23±0.74
	n-Butanol soluble fraction	27.07±0.68	1.447±0.33	82.818±0.65	256.02±0.21
	Remaining aqueous fraction	39.31±0.15	1.095±0.99	55.218±0.19	128.63±0.53
	BHT[b)]	12.1±0.09	1.215±0.22		

[a)] Standard mean error of three assays [b)] Standard antioxidant

(flavor, astringency, and hardness) of foods are the basic reason behind the analytical investigations about phenolic compounds. It is known that there are many mechanisms. However, the dominant model of antioxidant potential is thought to be hydrogen atom donation in radical scavenging process. In addition, singlet oxygen quenching and electron donation are defined as other antioxidant-radical quenching mechanisms. The stabilization of phenolic acids is influenced by substituents on its aromatic ring, which, on the other hand, affects its radical-quenching ability. (Robbins, 2003). Phenolic compounds contribute qualitatively and nutritionally in varying color, taste, smell and flavor and also supply health benefits. They play important part in plant's defense system and work against reactive oxygen species (Sengul *et al.,* 2009). The role of medicinal plants in control of various diseases has been credited to their constituents, usually wide range of polyphenolic compounds (Ivanova *et al.,* 2007). In addition to their antioxidant properties, phenolic compounds exhibit a wide range of medicinal properties, such as anti-inflammatory, anti-microbial, anti-allergic, cardio-protective anti-thrombotic and vasodilatory effects. Polyphenolic compounds also plays an important role in stabilizing lipid oxidation, the phenolic content of plant materials is associated with antioxidant activity (Demiray *et al.,* 2009). It is determined in different fractions, expressed as mg of Gallic acid equivalent (GAES) mg/g of fraction.

Gallic acid was used as a standard to determine total phenolic content. Different extracts of the plant components were measured at 725nm against Gallic acid spectrophotometrically. All the experiments were performed in three sets. The results showed (table 3) that ethyl acetate fraction gave the highest phenolic content of fruit (237.32±0.89), bark (393.19±0.72) and leaf (321.23 ±0.74), followed by chloroform fraction of fruit (180.58±0.31), bark (332.97±0.44) and leaf (305.58±0.41), *n*-butanol fraction of fruit (159.06±0.13), bark (331.45±0.21) and leaf (256.02±0.21), aqueous fraction of fruit (17.54±0.99), bark (317.54±0.55) and leaf (128.63±0.53), methanolic extract of fruit (141.02±0.34), bark (320.80±0.41) and leaf (161.67±0.39) and *n*-hexane soluble fraction of fruit (14.06±0.22), bark (158.41±0.53) and leaf (87.54±0.50) had the lowest phenolic contents.

CONCLUSION

All the antioxidant assays verified the appreciable amount of phytochemical constituents in *Pyrus pashia* particularly in ethyl acetate and chloroform soluble fractions. It also gave the excellent scavenging activity on DPPH free radical. Choloroform soluble fraction of fruit exhibited the highest activity (48.16±0.21µg/ml). Ethyl acetate soluble fraction of bark and leaves gave the highest activity as (43.50±0.1µg/ml) and (45.59±0.09µg/ml) respectively at the concentrations of 10µg/ml. Ethyl acetate fraction displayed the lowest antioxidant activity with IC_{50} value of bark as (8.64±0.32µg/ml) relative to

butylated hydroxytoluene (BHT), having IC_{50} of 12.1±0.92µg/ml. Ethyl acetate soluble fraction gave the highest phenolic content of fruit (237.32±0.89), bark (393.19±0.72) and leaf (321.23±0.74).The ethyl acetate soluble fraction gave the highest FRAP value of fruit (78.618±0.32TE µM/ml), bark (174.618±0.11TE µM/ml) and leaf (105.818±0.14TE µM/ml). With phosphomolybdenum complex method, the highest total antioxidant activity was shown by ethyl acetate soluble fraction of bark (1.499±0.90).The results were compared with BHT, a reference standard, having total antioxidant potential 1.215±0.22. This proves that the major contributors are flavonoids and polyphenols towards high antioxidant potential of ethyl acetate, chloroform while *n*-butanol soluble fraction and *n*-hexane has comparatively low polyphenolic content and consequently, showed lesser activity. The high antioxidant potential of various extracts of *Pyrus pashia* can broaden their applications towards the prevention of degenerative diseases, but the research needs to be continued in order to analyze the role of different classes of vegetal compounds, other than polyph*enols, in the antioxidant protection.*

REFERENCES

Abbasi MA, Nazir K, Shahzadi T, Riaz T, Aziz-ur-Rehman, Siddiqui SZ and Ajaib M (2011)**.** *Acrachne racemosa*: A Potent Source for Natural Antioxidants. *Asian J. Pharm. Biol. Res*., **1**(3): 375-386.

Abbasi MA, Zafar A, Riaz T, Aziz-ur-Rehman, Arshad S, Shahwar D, Jahangir M, Siddiqui SZ, Shahzadi T and Ajaib M (2010). Evaluation of comparative anti-oxidant potential of aqueous and organic fractions of *Ipomoea carnea. J. Med. Plants Res*., **4**(18): 1883-1887.

Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bellu AA, Obaweya K, Ezennia EC and Atangbayila TO (2008). Phytochemical screening and anti-oxidant activities of some selected medicinal plants used for malaria therapy in southern Nigeria. *Trop. J. Pharm. Res*., **7**(3): 1019-1024.

Barreira JCM, Ferreira ICFRM, Oliveira MBPP and Pereira JA (2008). Anti-oxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food Chem.*, **107**(3): 1106-1113.

Benzie IFF and Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as ameasure of Anti-oxidant Power: The FRAP Assay. *Anal. Biochem.*, **239**: 70-79.

Bhatt V, Purohit VK and Negi V (2010). Multipurpose tree species of Western Himalayawith an agroforestry prospective for rural needs*. J. Amer. Sci*., **6**(1): 73-80.

Demiray S, Pintado ME and Castro PML (2009). Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: *Tilia argentea, Crataegi folium* leaves and *Polygonum bistorta* roots.*World Acad. Sci. Engg. Tech.*, **54**: 312-317.

Dong M, He X and Liu RH (2007). Phytochemicals of black bean seed coats: Isolation, structure elucidation, and their anti-proliferative and anti-oxidative activities. *J. Agri. Food Chem.*, **55**: 6044-6051.

Dudonne S, Vitrac X, Coutiere P, Woillez M and Merillon JM (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J. Agri. Food Chem.*, **57**: 1768-1774.

Frankel EN and Finley JW (2008). How to standardize the multiplicity of methods to evaluate natural anti-oxidants. *J. Agri. Food Chem.*, **56**(13): 4901-4908.

Habila JD, Bello IA, Dzikwi AA, Musa H and Abubakar N (2010). Total phenolics and antioxidant activity of *Tridax procumbens Linn. Afri. J. Pharm. Pharmacol.*, **4**(3): 123-126.

Halliwel B and Gutteridge JMC. *Free radicals in biology and medicine, London: Oxford University Press.*1998**.**

Haung DJ, Chen HJ, Lin CD and Lin YH (2005). Antioxidant and antiproliferative activities of water spinach (*Ipomea aquatica* Forsk) constituents. *Bot. Bull. Acad. Sin*., **46**: 99-106.

Hodzic HZ, Pasalic A, Memisevic M, Srabovic M, Saletovic and Poljakovic M (2009). The influence of total phenols content on antioxidant capacity in the whole grain extracts. *Eur. J. Sci. Res.*, **28**: 471.

Ivanova D, Gerova D, Chervenkov T and Yankova T (2007). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *J. Ethnopharm.*, **97**(1-2): 145-150.

Kala CP (2007). Prioritization of cultivated and wild edibles by local people in the Uttaranchal hills of Indian Himalaya. *Indian J. Tradit. Know*., **6**(1): 239-244.

Lee K and Shibamoto T (2001). Anti-oxidant property of aroma extract isolated from clove bud [*Syzygiunaromaticum* (L.) Merr. et Perry]. *Food Chem.*, **74**: 443-448.

Makkar HPS, Bluemmel M, Borowy NK and Becker K (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agr*., **61**: 161-165.

Matin A, Khan MA, Ashraf M and Qureshi RA (2001). Traditional use of herbs, shrubs and trees of Shogran valley, Mansehra Pakistan. *Pak. J. Bio. Sci.*, **4**(9): 1101-1107.

Murad W, Ahmed A, Gilani SA and Khan MA (2011). Indigenous knowledge and folk use of medicinal plants by the tribal communities of Hazar Nao Forest, Malak and District, North Pakistan*. J. Med. Plants Res*., **5**(7): 1072-1086.

Pala NA, Negi AK and Todaria NP (2010). Traditional uses of medicinal plants of Pauri Garhwal, Uttrakhand. *Nature Sci*., **8**(6): 57-61.

Pant HM (2010). Study on traditional knowledge of Himalayan medicinal plants of rath region of Uttarakhand*. Res. J. Agri. Sci*., **1**(3): 277-279.

Parmar C and Kaushal MK (1982). *Wild Fruits*. Kalyani Publishers, New Delhi, India. Pp.78-80.

Prieto P, Pineda M and Aguilar M (1999). Spectrophotometric Quantitation of anti-oxidant capacity through the formation of a phosphomolybdenum complex, specific application to the determination of vitamin E. *Anal. Biochem*., **269**: 337-341.

Promila K and Dinesh Y (2005). Indigenous animal healthcare practices of Kangra District, Himachal Pradesh. *Indian J. Tradit. Know.*, **4**(2): 164-168.

Riaz T, Abbasi MA, Aziz-ur-Rehman, Shahzadi T, Siddiqui SZ, Khalid H and Ajaib M (2011). *In vitro* assessment of fortification from oxidativestress by various fractions of *Rhynchosia pseudo-cajan. J. Med. Plants Res*., **5**(30): 6597-6603.

Robbins RJ (2003). Phenolic acids in foods: An overview of analytical methodology. *J. Agri. Food Chem.*, **51**: 2866-2887.

Sengul MM, Yildiz HH, Gungor N, Cetin B, Eser Z, Ercisli Z (2009). *Pak. J. Pharm. Sci*., **22**(1): 102-106.

Sofowara A (1993). Medicinal plants and traditional medicine in africa. Spectrum Books, Ibadan, p.150.

Trease GE and Evans WC (1989). Pharmacognosy. 13th edition, Bailliere Tindall, pp.176-180.

Uttara B, Singh AV, Zamboni P and Mahajan RT (2009). Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. *Cur. Neuropharmacol.*, **7**: 65-74.

Valko M, Rhodes CJ, Moncol J, Izakovic M and Mazur M (2006). Free radicals, metals and anti-oxidants in oxidative stress-induced cancer. *Chemico-Biolog. Interact*, **160**: 1-40.

Zamani A, Attar F and Joharchi MR (2009). *Pyruspashia* (rosaceae), a new record for the flora of Iran. *Iran. J. Bot.*, **15**(1): 72-75.

Zhang Z, Liao L, Moore J, Wu T and Wang Z (2009). Antioxidant phenolic compound from walnut kernels (*Juglansregia L.). Food Chem.*, **113**: 160-165.