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Evaluation for Total Phenolic, Total Flavonoid and Antioxidant activity of leaves and roots of *Pyrus pashia*

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Abstract: The antioxidant activity and total phenolic content of methanolic extracts of leaves and fruits of *Pyrus pashia* were evaluated by using a model system containing DPPH free radical and Folin-Ciocalteu method. The total phenolic content of the extracts was determined spectrophotometrically according to the Folin-Ciocalteu procedure and found to be 325 ± 10 mg/g and 98 ± 5 mg/g in leaves and fruits respectively on fresh weight basis. The total flavonoid content of extracts of leaves and fruits were determined by Aluminium chloride colorimetric assay and ranged from 150 ± 20 mg/g to 10.30 ± 10 mg/g respectively.

Key words: *Pyrus pashia*, Antioxidant, Phenolic, Flavonoid, DPPH.

Introduction

Medicinal plants are best remedies used as alternative tools for the prevention and treatment of many ailments. The recent studies have investigated that the antioxidant effect of plant products is mainly attributed to phenolic compounds such as flavonoids, phenolic acids, tannins etc^{1,2}.

Rosaceae (the rose family) is a medium-sized family of flowering plants, including about 2830 species in 95 genera³. Rosaceae includes herbs, shrubs and trees. Most species are deciduous, but some are evergreen⁴.

Pyrus pashia is commonly known as wild Himalayan pear, Punjabi pear, Indian pear belongs to family Rosaceae. It is a small to medium size deciduous tree of the small and oval shaped crown with ovate, finely toothed leaves, attractive white flowers with red anthers and small pear-like fruits. *Pyrus pashia* is a tolerant tree that grows on sandy loamy soil that is well drained. It is adapted to a precipitation zone that ranges from 750 to 1500mm/yr or more, and a temperature that ranges from -10 to 35 C⁵. Its fruit is edible and characterized as being pome⁶. It looks like the russet apple and has an astringent but sweet taste when ripe. The astringent juice is used to stop diarrhoea.

It is native to southern Asia. Locally, it is known by many names such as batangi⁴ (Urdu), tangi (Kashmiri), mahal mol (Hindi) and passi (Nepal)⁵. Leaves are used as fodder, leaf extract is tonic for hair fall and wood of the tree are used as a source of fuel in Himalayan region⁷. The leaves are consumed as tea beverages by the Monpa community of Tawang, Arunchal Pradesh.



Figure 1: Pirus pashia Plant

Material and method

Extraction

Samples were shade dried at ambient temperature ($\pm 24^{\circ}\text{C}$) and powdered. Powdered leaves and fruits were extracted with methanol. The methanolic extract was evaporated in a rotatory evaporator and dried by vacuum pump. The methanolic extract was suspended on water and extracted successively with hexane, ethyl acetate and butanol respectively. Extract was filtered with Whatman no.1 filter paper and concentrated under reduced paper to dryness below 40°C .

Determination of Total Phenolic Content

Total phenolic compound contents were determined by the Folin-Ciocalteu method^{8,9}. The extract samples (0.5 ml) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagent for 5 min and 2.0 ml of 75 g C 1 sodium carbonate were then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents.

Determination of Flavonoid Content

Total flavonoids were estimated using the method of Ordonez et al.^{8,9}. 0.5 mL solution of each plant extracts in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). Total flavonoid contents were calculated as quercetin from a calibration curve.

DPPH Radical Scavenging Activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the extracts^{9,10}. Different concentrations of each extracts were added, at an equal volume, to methanolic solution of DPPH (100 μM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Quercetin were used as standard controls.

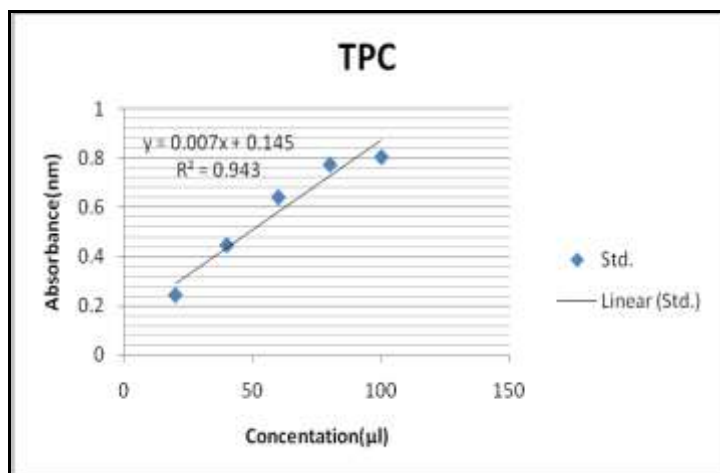
Statistical analysis

Experimental results are expressed as means \pm SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range test. The EC_{50} values were calculated from linear regression analysis.

Result and Discussion

Total Phenol Content

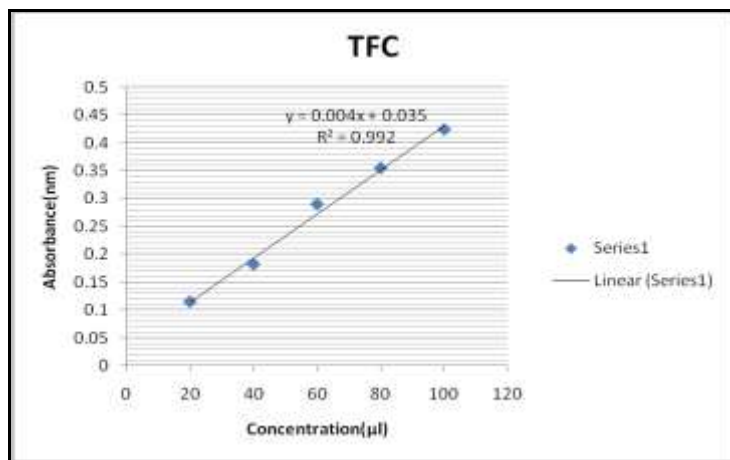
The Total phenolic content were reported as gallic acid equivalents by reference to standard curve . The total phenolic contents of leaves and fruits of *Pyrus pashia* were found to be 325 ± 10 and 98 ± 5 mg gallic acid equivalent/g of extract powder, respectively.



Graph 1. Standard curve of Gallic acid

Total Flavonoid Content

The Total flavonoid content of leaves and fruits of *Pyrus pashia* were found to be 150 ± 20 and 10.30 ± 10 mg quercetin equivalent g^{-1} of extract powder, respectively, by reference to standard curve .



Graph 2. Standard curve of quercetin

DPPH Radical Scavenging Activity

The radical-scavenging activity of leaves and fruits of *Pyrus pashia* for DPPH radical-scavenging activity was found to be 15.12 ± 10 and 8.5 ± 5 mg/ ml respectively.

Table 2 : Extractive yield , TPC, TFC and % Inhibition of extracts of *Pyrus pashia*

	Leaves	Fruits
Extraction yield (%)	20	22
Total phenolic content	325 ± 10 mg / g	98 ± 5 mg / g
Total flavonoid content	150 ± 20 mg / g	10.30 ± 10 mg / g
% Inhibition at 200 mg / ml	15.12 ± 10	8.5 ± 5

Conclusion

The present study evaluated the antioxidant activity, total phenolic and flavonoid contents in leaves and fruits of *Pyrus pashia*. It is important to measure the antioxidant activity using various radicals and oxidation systems in order to realize the health benefits from potential plant sources. *Pyrus pashia* possess significant antioxidant property.

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