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Determination of physicochemical and pharmacological screening of leaves and flowers part of *Pyrus pashia*

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Abstract
Medicinal plants are best remedies used as alternative tools for the prevention and treatment of many ailments. Rosaceae (the rose family) is a medium-sized family of flowering plants, including about 2830 species in 95 genera. Rosaceae includes herbs, shrubs and trees. Most species are deciduous, but some are evergreen. In this study, the physicochemical evaluation; ash values, namely total ash, water-soluble ash, and acid-insoluble ash, and extractive values; namely ethanol-soluble extractive and water-soluble extractive values, moisture content by using loss of drying method, total phenolic and total flavonoid contents in *Pyrus pashia*leaves and flowers were used for illustrate the quality as well as purity and also investigated the pharmacological effect; namely antioxidant effect by using DPPH free radical assay. The total ash, water-soluble ash, and acid-insoluble ash in *Pyrus pashia* leaves was found to be 5%, 3.1%, and 1.9%, respectively. In other side in *Pyrus pashia* flowers the total ash, water-soluble ash, and acid-insoluble ash in *Pyrus pashia* leaves was found to be 5.12%, 3.05%, and 2.07%, respectively. The water-soluble extractive value was higher than alcohol-soluble extractive value in this study, and it was found to be 4.53% in leaves and 4.65% in flowers. The moisture content of the crude drug of leaves and flowers was found below 6.33% and 8.20%, respectively. The total phenolic content in the leaves and flowers extracts was found 180 ± 7 mg and 70 ± 9 mg of Gallic acid equivalent weight/g of extract, respectively, and the concentration of flavonoids in leaves and flowers extract of *Pyrus pashia* was found 371 ± 12 and 130± 10 mg of Quercetin equivalent weight/g of extract, respectively. The percentage inhibition of scavenging activities of the *Pyrus pashia* leaves and flowers showed 51.60% and 43.69% DPPH inhibition at 50 µg/mL concentrations. Whereas ascorbic acid showed 66.25% DDPH inhibition at 50 µg/mL.

Keywords: Ash value, flavonoid content, phenolic content, *Pyrus pashia.*

Introduction
Pyrus pashia Buch-Ham. Ex D. Don. (Rosaceae), is a medium size fruiting tree, known locally as Indian pear, Himalayan pear, Batangi, Tangai and Mehal [1]. It has 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 35C [2]. Its fruit is edible and characterized as being pome [3]. It is native to southern Asia. Locally, it is known by many names such as batangi [4] (Urdu), tangi (Kashmiri), mahalmol (Hindi) and passi (Nepal) [2]. 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 region [5]. The leaves are consumed as tea beverages by the Monpa community of Tawang and Arunchal Pradesh. Previously published data on *Pyrus pashia* revealed it to possess diverse pharmacological properties. Crushed leaves are used to improve cosmetic appearance by staining palms, feet and nails [6]. The fruits are useful in constipation [7]. Fruit juice is astringent and diuretic [8]. Leishmaniasis [9], eye problems [10], digestive disorder, sore throat, irritability, abdominal pain, anemia [11]. The barks are used to manage sore throat, fever, peptic ulcer, gastric ulcer [12]and typhoid fever [13]. Fruits possesses antimicrobial activity against *Klebsiella pneumonia, Shigella flexneri* and *Eschericia coli* [14]. Leaves are also used in inflammation [15] and depression [16]. Adulteration, impurities and substitution are the major problems among the natural drugs of plant origin. These problems can be overcome by using various modern analytical techniques to ensure quality control of medicinal plants and their products.

1. Materials and methods
1.1. Plant materials
The leaves and flowers were collected in the month of August- September from Bhimtal region, Dist. Nainital, Uttarakhand, India. The leaves and flowers were identified and

authenticated (NP-01) on dated 27 July 2017 by Dr. K. S. Negi, Principal Scientist, National Bureau of Plant Genetic Resources (N.B.P.G.R.) Niglat, Bhowali, Uttarakhand, India. The collected leaves and flowers were shade dried for 40 days and finally pulverized by electronic grinder machine into coarse powder. It was stored in a well-closed container free from environmental climatic changes or any other contamination till usage for the further studies.

1.2. Extraction of the plant material

For removal of fat materials, prior to extraction the powder of *Pyrus pashia* leaves and flowers soaked with the petroleum ether and then methanolic extraction was done by soxhlet method. By vacuum rotavapor (Perfit) the methanolic extracts of leaves and flowers were concentrated under reduced pressure and then dried in vacuum desiccators. Afterward dried extracts of both leaves and flowers were kept in refrigerator (8 ± 2°C) and these *Pyrus pashia* extracts were used for study. These extractions was done separately for both leaves and flowers.

1.3. Physicochemical screening of *Pyrus pashia* leaves and flowers

1.3.1. Determination of total ash: [17]

Place about 2-4g of the ground air-dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the heat to 450°C until it is white, indicating the absence of carbon. Cool in a desiccator and weigh. Ash value can be calculated by below using formula:

$$\text{Ash value} = \frac{\text{Initial Weight - Final Weight}}{\text{Initial Weight}} \times 100$$

1.3.2. Water soluble ash [17]

The total ash obtained above was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at low temperature. The weight of the insoluble matter was subtracted from the weight of total ash, represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug. The result was calculated with reference to the air-dried drug.

1.3.3. Acid insoluble ash: [17]

The total ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on tarred grouch crucible, washed with hot acidulated water, ignited, cooled and weighed. The percentage acid insoluble ash was calculated with reference to the air dried drug. The same procedure was repeated with other ash obtained.

1.3.4. Determination of extractive values [17]

Place about 15gm of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Add 300ml of water and weigh to obtain the total weight including the flask. Shake well and allow to stand for 1 hour. Attach a reflux condenser to the flask and boil gently for 6 hour; cool and weigh and filter rapidly through a dry filter. Dry the extracted powder in oven till the weight is constant. Calculate the content of extractable matter in mg per gram of air-dried material by below using formula:

$$\text{Extractive value} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

1.3.5. Moisture content: [17]

In this present paper, loss on drying method was taken in determining the moisture content. The powdered Leaves and Flowers of *Pyrus pashia* was dried in the oven at 100-105^{0}C.C to constant weight.

1.3.6. Total phenolic content (TPC) [18]

Total phenolic content in *Pyrus pashia* leaves and flowers extracts was determined using Foline Ciocalteu assay. Solutions of each extract (100 mL; 1 mg/mL) were taken individually in test tubes. To this solution, 2.5 ml of 10- fold diluted Folin Ciocalteu reagent was added, and the test tubes were thoroughly shaken. After 3 min, 2.0 mL of 7.5% Na_2CO_3 solution was added and the mixtures were incubated for 30 min. The absorbance of the reaction mixtures was measured at 760 nm by using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Gallic acid was used as a standard and TPC of *Pyrus pashia* leaves and flowers extracts was expressed in milligram Gallic acid equivalents (mg GAE/g extract)

The grouping of aggregate phenolic mixes in the concentrate was dictated by utilizing the equation:

T=CV/M

Where, T= Total phenolic content mg/gm. of plant extract in GAE,

C= Concentration of Gallic acid from the calibration curve,

V= volume of the extract in ml,

M= wt. of the pure plant methanol extract

1.3.7. Total flavonoid content (TFC) [19]

Total flavonoid content was determined by the aluminum chloride calorimetric method, with some modifications. Briefly, the test samples were individually dissolved in methanol. Then, the sample solution (2 mL) was mixed with 2 mL of 2% $AlCl_3$. After 10 min of incubation at ambient temperature, the absorbance of the solutions was measured at 435 nm by using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The flavonoid content was expressed as milligram Quercetin equivalent (mg QE/g extract).

The convergence of aggregate phenolic mixes in the concentrate was dictated by utilizing the equation:

T=CV/M

Where, T= Total flavonoid content mg/gm. of plant extract,

C= Concentration of Quercetin from the calibration curve,

V= volume of the extract in ml, M= wt. of the pure plant methanol extract.

2. Statistical analysis

Information was expressed as mean ± Standard Error Mean (SEM). Disparity were assayed as significant at ***P<0.001, or **P < 0.01 or *P<0.05 when compared test (different concentration of MEPPL) groups v/s control (distilled water) group. For numerical outcomes, one-way analysis of variance (ANOVA) with Tukey-Kramer Multiple Comparisons post tests were performed using GraphPadInStat Version 3 (GraphPad Software) and all graphs were made by utilizing Microsoft office 2013 software.

3. Results

Table 1: Ash Value of *Pyrus pashia* Leaves and Flowers

S. No	Types of ash	% yield of *Pyrus pashia* leaves	% yield of *Pyrus pashia* flowers
1.	Total Ash	5	5.12
2.	Water soluble Ash	3.1	3.05
3.	Acid insoluble Ash	1.9	2.07

Table 2: Extractive Value of *Pyrus pashia* Leaves and Flowers

S. No	Extractives	% yield of *Pyrus pashia* leaves	% yield of *Pyrus pashia* flowers
1.	Ethanol	2.7	2.92
2.	Water	4.53	4.65

Table 3: Percentage loss in weight on drying of Pyrus pashia Leave and flowers

Extract	Weight of the powder taken (gm)	% loss on drying
Pyrus pashia Leaves	1.5	6.33
Pyrus pashia Flowers	1.5	8.20

Table 4: Total Phenolic and Flavonoid Content of *Pyrus pashia* Leaves and Flowers

S. No	Content	% yield of *Pyrus pashia* leaves	% yield of *Pyrus pashia* flowers
1.	Total Phenolic content	180 ± 7 mg/g	70 ± 9mg/g
2.	Total Flavonoid content	371 ± 12mg/g	130 ± 10mg/g

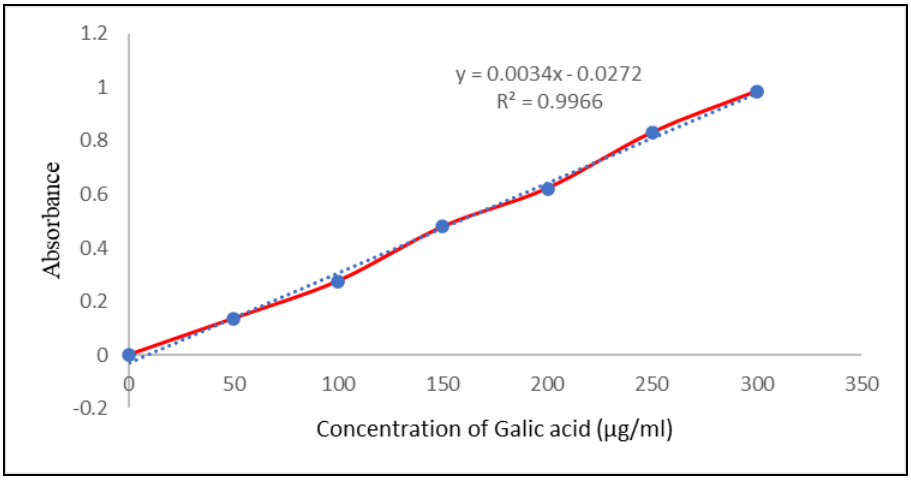


Fig 1: Total phenolic content for standard Gallic acid

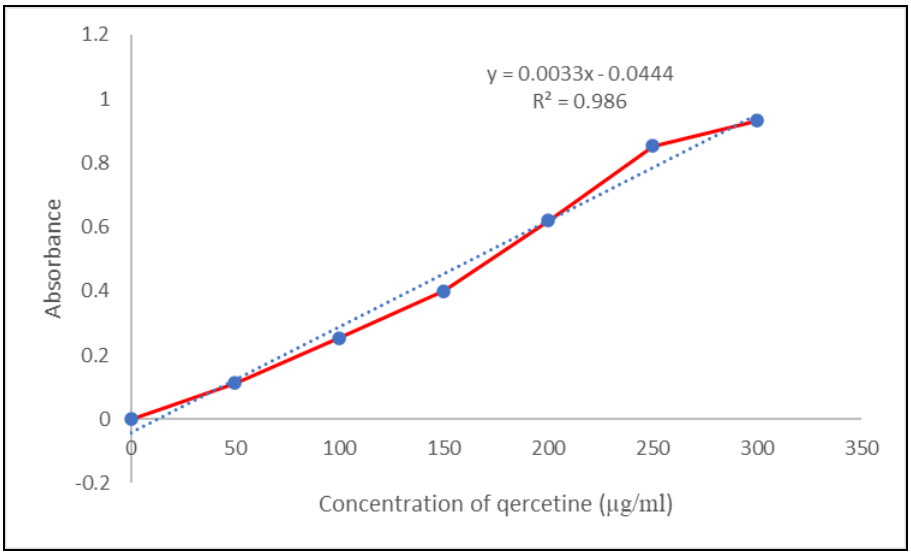
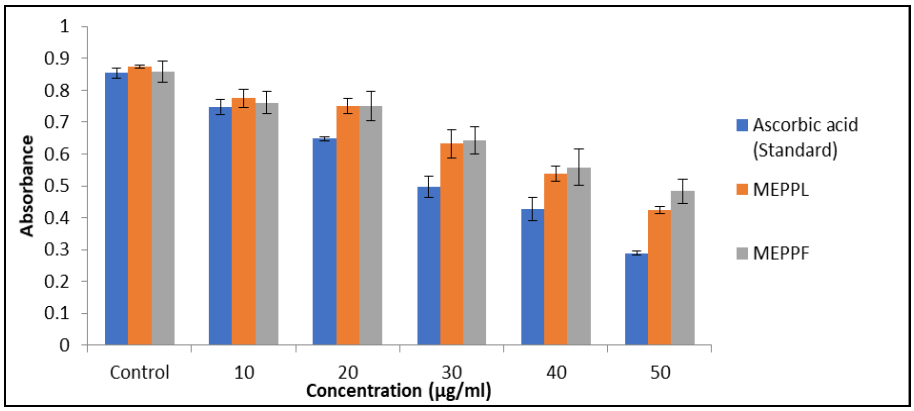


Fig 2: Total flavonoid content for standard Quercetin

Table 5: Absorbance of DPPH Free Radical of *Pyrus pashia* Leaves and Flowers Extract/ Ascorbic Acid at 517nm.

Concentration	Absorbance		
	Ascorbic acid	***Pyrus pashia* leaves**	***Pyrus pashia* flowers**
Control	0.853933333 ± 0.01461	0.8743 ±0.003604	0.858 ±0.03214
10	0.746433333 ± 0.02426	0.7759 ±0.02843	0.761067±0.03466
20	0.6477 ±0.005237	0.7509 ±0.02424	0.7512 ±0.04634
30	0.498033333 ±0.03335	0.633233333±0.04423	0.6422 ±0.04234
40	0.427766667 ±0.03753	0.537566667 ±0.0244	0.558367±0.05649
50	0.288133333±0.006435	0.423133333±0.01145	0.483133±0.03742

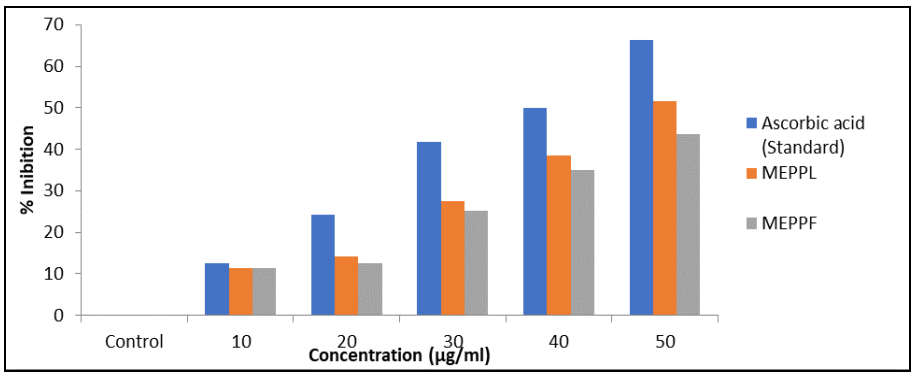
Values are mean ± SEM, n = 3.



Graph 1: Absorbance of DPPH Free Radical of *Pyrus pashia* Leaves and Flowers Extract/ Ascorbic Acid at 517nm.

Table 6: Percentage Inhibition of DPPH Free Radical of *Pyrus pashia* Leaves and Flowers Extract/ Ascorbic Acid at 517nm.

	% Inhibition		
	Ascorbic acid	***Pyrus pashia* Leaves**	***Pyrus pashia* Flowers**
Control	0	0	0
10	12.58880475	11.25471806	11.29759
20	24.15098759	14.11414846	12.44755
30	41.6777266	27.57253422	25.15152
40	49.90631587	38.51462122	34.9223
50	66.25809977	51.60318731	43.69075



Graph: Percentage inhibition of DPPH free radical of *Pyrus pashia* leaves and flowers extract/ ascorbic acid at 517nm

4. Discussion and result

The amount of impurities present in *Pyrus pashia* leaves and flowers can beanalyzed based on ash content, and ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards. The total ash, water-soluble ash, and acid-insoluble ash in *Pyrus pashia* leaves was found to be 5%, 3.1%, and 1.9%, respectively. In other side in *Pyrus pashia* flowers the total ash, water-soluble ash, and acid-insoluble ash in *Pyrus pashia* leaves was found to be 5.12%, 3.05%, and 2.07%, respectively. This percentage clearly indicates that the both leaves and flowers of Pyrus pashiaare best for drug action and effects. High extractive value indicates the presence of bioactive compounds in remarkable quantity and with fever impurity. The water-soluble extractive value plays an important role in the evaluation of crude drugs. Less extractive value indicates the addition of exhausted material, adulteration, or incorrect processing during drying or storage. The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value. The water-soluble extractive value was higher than alcohol-soluble extractive value in this study, and it was found to be 4.53% in leaves and 4.65% in flowers. This shows that the constituents of the drug in both leaves and flower are more extracted and soluble in water as compared to alcohol. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The moisture content of the crude

drug of leaves and flowers was found below 6.33% and 8.20% respectively. Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids, including flavones, flavanols, and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3 OH Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo*. The total phenolic content in the leaves and flowers extracts was found 180 ± 7 mg and 70 ± 9 mg of Gallic acid equivalent weight/g of extract, respectively, and the concentration of flavonoids in leaves and flowers extract of *Pyrus pashia* was found 371 ± 12 and 130± 10 mg of Quercetin equivalent weight/g of extract, respectively. The percentage inhibition of scavenging activities of the *Pyrus pashia* leaves and flowers showed 51.60% and 43.69% DPPH inhibition at 50 µg/mL concentrations. Whereas ascorbic acid showed 66.25% DDPH inhibition at 50 µg/mL.

Conclusion

The present study concluded that in both *Pyrus pashia* leaves and flowers found high contents of phenolic and flavonoid compounds, indicating that these compounds contribute to the antioxidant activity. Which can act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital role for good health.

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