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RESEARCH ARTICLE

Phytochemical Constituents in *Putranjiva roxburghii* Seed Extracts with Potential Health Benefits

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ABSTRACT

Background: *Putranjiva roxburghii* Wall. is an underutilized but valuable plant. It plays significant role in the traditional Ayurvedic and Unani systems. Antioxidants are crucial in preventing the formation of reactive oxygen species (ROS), neutralizing existing ROS, and repairing damage caused by ROS.
Objectives: The objective of the study was to investigate phytochemical profiling of seed extracts of *P. roxburghii*.
Methodology: The seeds of plant were used for extraction with various solvents and the phytochemical screening and analysis including total phenolic contents (TPC), total flavonoid contents (TFC) and DDPH radical scavenging assay were carried out by standard methodologies. MS Excel was used to calculate the means, and standard deviation (SD) and to make graphs.
Results: The various extracts of *P. roxburghii* seeds showed the presence of phenols, flavonoids, saponins, alkaloids, carbohydrates sterols and terpenoids whereas aqueous extract did not show terpenoids. The glycosides and tannins were also absent in all these extracts. The mean value of TPC and TFC of methanolic, hydroethanolic and water extracts of seeds of *P. roxburghii* vary from 48.8 to 83.0 mg/g GAE and 565 to 915 mg/10 g CE, respectively. The mean ± SD of DPPH % inhibition of methanolic, hydroethanolic and water extracts of seeds of *P. roxburghii* was 69.2 ± 8.9, 32.8 ± 1.86 and 35.6 ± 9.60, respectively.
Conclusion: The phytochemical screening of *P. roxburghii* seeds showed the presence of all representative groups, except tannins and glycosides in all extracts and terpenoids in aqueous extract. The hydro ethanolic extract of *P. roxburghii* seeds contain high contents of both TPC and TFC while methanolic extract showed highest DPPH inhibition.

INTRODUCTION

Plants of medicinal values have been used to cure many diseases since ancient times. Several studies indicate that many medicinal plants exhibit antioxidant properties (Saeed *et al.* 2012). Antioxidants are crucial in preventing the formation of reactive oxygen species (ROS), neutralizing existing ROS, and repairing damage caused by ROS (Ighodaro and Akinloye 2018). *Putranjiva roxburghii* Wall is an underutilized but valuable plant (Emasushan and John Britto 2018). It has effective medicinal value and plays a significant role in the traditional Ayurvedic and Unani systems (Gupta 2016; Pandey and Flulara 2022). Various parts of *P. roxburghii* are used for the treatment of different diseases (Unnikrishnan *et al.* 2015; Mradu 2016). It has an anti-inflammatory, antipyretic, analgesic, and anti-rheumatic herb, that is also useful to treat gynaecological and fertility disorders (Naik *et al.* 2023; Pandey and Flulara 2022). It is used to treat many diseases such as treatment of mouth and stomach ulcers, hot swellings, smallpox, burning sensation, ophthalmopathy and liver diseases (Mishra *et al.* 2021; Pandey and Flulara 2022).

Among the attributes of *P. roxburghii*, the most important ones are anthelmintic, anticancer, anti-

inflammatory, antioxidant, aphrodisiac, diuretic and laxative. Leaves and seed paste are used to treat burning sensation, filarial, inflammatory and eye diseases (Samal and Dehury 2016). Seed paste had been used in the treatment of various diseases like elephantiasis, constipation, ophthalmic, semen disorders, infertility and diseases of the female genital. The bark and seeds are used as an antidote in the treatment of snake bites. The leaves are used in treating illness, phlegm, skin ailment, aridity, and are also helpful in curing rheumatism. *P. roxburghii* possess antioxidant, antipyretic, and anti-inflammatory activities (Pandey and Flulara 2022). Almost all parts of the plant such as bark, stem, leaves, root, fruits, and seeds contain numerous secondary metabolites such as flavonoids, phenolics, triterpenes, saponins, glycosides, alkaloids, saponins, and glucosinolates. The presence of these phytochemicals imparts efficient protection roles against various diseases. Various plant parts and their extracts can be used as a cure for different diseases, including cancer (Gupta 2016; Kumar *et al.* 2019; Naik *et al.* 2023).

Phytochemical information of plants is required to fully explore the different parts of plants and correlate its activity. *P. roxburghii* has remarkable ethnomedical significance. However, its phytochemical profile in different parts has not been fully explored. In this study, various extracts, including aqueous, methanol and hydro-ethanol were prepared from the seeds of *P. roxburghii* and subjected to phytochemical assessment. The qualitative analysis revealed the presence of all representative groups, except tannins and glycoside, in the samples. The primary objective of this study was to carry out an in-depth screening of the plant's phytochemical composition qualitatively and quantitatively.

MATERIALS AND METHODS

Collection of plant samples and identification

The seeds of plant were collected from University of Agriculture, Faisalabad (UAF) and authentication of plant was done by a taxonomist at the department of Botany at UAF. The photographic documentation of plant and a voucher sample was provided under reference number (255-1-2023) and identified as *P. roxburghii* (Fig. 1).

Preparation of seed extract

The seeds of *P. roxburghii* were washed and subjected to drying under shade. The dried seeds were subsequently ground using a high-speed blender and preserved in an airtight jar. *P. roxburghii* seed powder was used for preparing various extracts. For each extraction, 10 g of the powder was placed in 250 mL conical flasks, and 100 mL of methanol, hydro-ethanol (a mixture of water and ethanol in a 1:1 ratio) and distilled water were added separately. The extraction process was done by using an orbital shaker (IRMECO) set at 220 rpm for 24 h. Following the extraction period, the resultant mixture was filtered using Whatman No. 1 filter paper. The filtrates obtained were subjected to evaporation at 45°C using a vacuum drying oven (Memmert, GmbH, Dusseldorf, Germany) to obtain the dry extract. Subsequently, these dry extracts were transferred to Eppendorf's and preserved at 4°C for subsequent use. For analysis, evaporated extracts weighing 50 mg were dissolved in 5 mL of DMSO, stored at 4°C and subjected to phytochemical screening and analysis including total phenolic contents (TPC), total flavonoid contents (TFC), and DDPH radical scavenging assay.

Phytochemical screening

The various extracts of *P. roxburghii* seeds were used for qualitative analysis of various groups of compounds, such as flavonoids, carbohydrates, phenols, saponins, alkaloids, glycosides and tannins by employing standard methodologies as presented (Sharma1995; Treare and Evans 1985; Peach and Tracy 1956; Varma *et al.* 2010).

Test for phenols: Potassium dichromate test: two mL of extract was treated with 5% potassium dichromate solution. Positive result was confirmed by a formation of brown precipitate (for phenol). Ferric chloride test: 2 mL of extract was treated 2–3 drops of 5% ferric chloride solution. Formation of bluish-black color showed presence of phenols and black color showed tannins.

Test for flavonoids: Lead acetate test: one mL extract was treated with 1 mL 10% lead acetate ($Pb(OAc)_4$) solution. Formation of yellow color precipitate indicated the presence of flavonoids.

Test for tannins: Braymer's test: 2 mL of extract was treated with 2 mL H_2O and followed with 2–3 drops of $FeCl_3$ (5%). Green precipitate proved the presence of tannins.

Test for saponins: Foam test: two mL extract was diluted with 10 mL of distilled water and warmed gently. It was shaken for 5 min. Persistent froth indicated the presence of saponins. The same extract was added with a few drops of olive oil. Formation of a soluble emulsion confirmed the presence of saponins (Treare and Evans 1985).

Test for glycosides: For Keller Kiliani test of glycosides, 2 mL extract was treated with 1 mL glacial acetic acid, one drop of 5% $FeCl_3$ and 1 mL of conc. H_2SO_4. The brown ring of the interface indicated the presence of cardiac glycosides (Treare and Evans 1985).

Test for alkaloids: Wagner's test: Two mL of extract was treated with few drops Wager's reagent. Formation of reddish-brown precipitate indicated the presence of alkaloids (Treare and Evans 1985).

Test for sterols: Salkowski's test: two mL of extract was treated with 2 mL chloroform and 2 mL of conc. H_2SO_4. Chloroform layer appeared red, and the acid layer showed greenish yellow fluorescence, which indicated the presence of sterols.

Fig 1: Leaves (left) and fruits (right) of *P. roxburghii*

Test for terpenoids: Salkowski's test: Two mL of chloroform and 1 mL of conc.H_2SO_4 was added to 1 mL of extract and observed for reddish brown color that indicated the presence of terpenoids (Sharma 1995).

Test for coumarins: Two mL of extract was treated with 3 mL of 10% NaOH solution. Yellow coloration indicated the presence of coumarins.

Analysis of Total phenolic and flavonoids contents: A quantitative assessment was conducted to determine Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC) in the raw extracts was carried out by following standard protocols.

Total phenolic content: The total phenolic contents in the extracts were determined by using Folin-Ciocalteu procedure with Folin-Ciocalteu reagent (MERCK). Standard concentrations of 5, 10, 20, 30, 40, and 50 µg/mL of Gallic acid were prepared from a stock solution of Gallic acid (1 mg/mL) in methanol. For each standard, 100 µL was combined with Folin-Ciocalteu reagent, vortexed, and then 800 µL of sodium carbonate was added and vortexed. After 1 h incubation, the absorbance was recorded at 765 nm with a spectrophotometer using a blank to set the instrument at zero. The blank was composed of 100 µL of methanol instead of the standard. The determination of total phenolic contents (TPC) was conducted for crude extracts obtained from methanol, hydro-ethanol, and water were determined following the same procedure as the standards (Jagadish *et al.* 2009). The findings are presented in equivalence to Gallic acid (mg GAE/g). The standard curve of gallic acid with the equation are given in Fig. 2a.

Total flavonoid contents

The determination of Total flavonoid content was conducted using the aluminium chloride method. A range of standard concentrations (50, 100, 200, 300, 500, and 1000 µg/mL) of Catechin was prepared from a stock solution of Catechin (1 mg/mL) in methanol. To 1 mL of each standard, 1 mL of 2% $AlCl_3$ was added, vortexed, and absorbance was measured by using spectrophotometer at 417 nm after a 15 min incubation. The spectrophotometer was set at zero by using blank and it was prepared by substituting 100 µL of methanol in place of standard. The total flavonoid contents (TFC) of crude extracts from methanol, hydro-ethanol, and water were determined

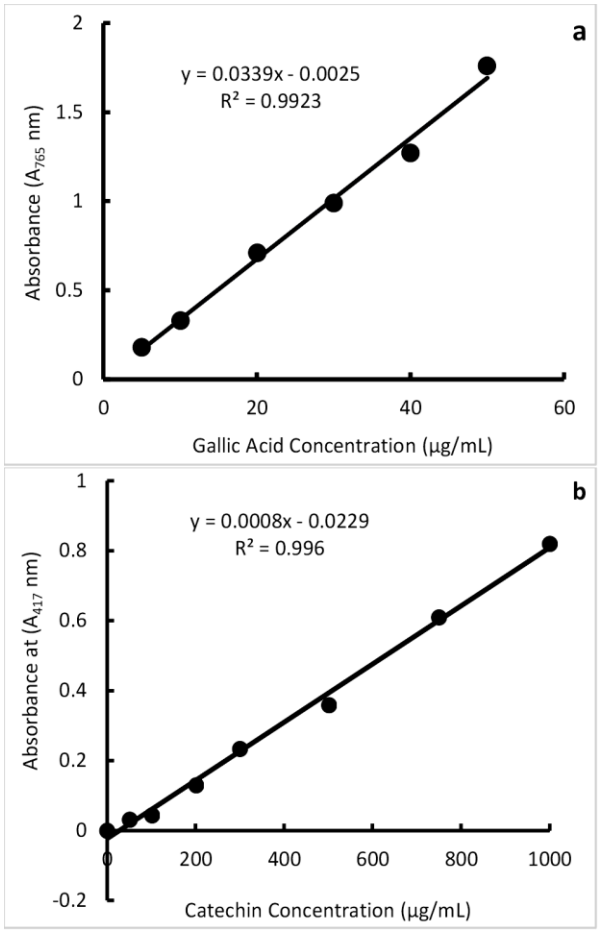

Fig. 2: Standard curve of gallic acid (a) and catechin (b)

following the same procedure as the standards (Riaz *et al.* 2019). The results are expressed in equivalence to catechin (mg Catechin E/10 g). The standard curve of catechin with the equation are presented in Fig. 2b.

DPPH radical scavenging assay

The antioxidant capacity of the extracts was determined by their capability to neutralize DPPH assay (Riaz *et al.* 2019). In a 250 µL plant extract, 1 mL of the DPPH solution was added, thoroughly mixed, and the mixture was left for 30 min for incubation in darkness. Subsequently, the spectrophotometer was used to measure the absorbance of both DPPH and extracts at 515 nm. The spectrophotometer was set at zero with blank (methanol) (Gulcin and Alwasel 2023). The percentage DPPH scavenging capability was calculated using the formula given below:

$$\% \text{ inhibition} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

Where A_{control} is absorbance by the DPPH and A_{sample} is absorbance by the test samples

Statistical analysis

Triplicate analysis was done for each parameter and the data have been presented as mean ± standard deviation of triplicate analysis. Correlation coefficient (*r*) of phytochemical activities was calculated by employing the correlation and regression function of Microsoft Excel program (Microsoft, Redmond, WA, USA).

RESULTS AND DISCUSSION

Importance of phytochemical constituents

Phytoconstituents are generated by the plants as a defense system against pathogens and predators. They are helpful for the treatment of diseases including antimicrobial, antioxidant, and stimulation as well as inhibition enzymes (Ahmed *et al.* 2017). Plant-based therapeutic agents possess fewer side effects as compared to synthetic agents and are also cost-effective (Mustafa *et al*. 2017). Plants contain a wide range of constituents such as alkaloids, polyphenolics, tannins terpenoids, etc. which are attributable to therapeutic potential (Verma and Singh 2008). Phytochemicals, either as crude extracts or isolated compounds, provide opportunities for drug discovery (Sasidharan *et al.* 2011). The various extracts of seeds were used for qualitative analysis of various groups of compounds, such as flavonoids, carbohydrates, phenols, saponins, alkaloids, glycosides and tannins by employing standard methodologies as presented in Table 1 (Auwal *et al*. 2014; Gul *et al*. 2017; Amine *et al*. 2019; Hussain *et al*. 2023). Furthermore, it aimed to determine the qualitative phytochemical composition and antioxidant potential as TPC, TFC and DPPH % qualitatively of seed extracts of *P. roxburghii.*

Qualitative phytochemical screening

A comprehensive estimation of phytochemicals within their respective categories was carried out in *P. roxburghii*. Positive and negative results were obtained for all tests conducted. According to the identification of noteworthy chemicals entitles the various extracts of *P. roxburghii* seeds, including methanol (M), Hydro ethanol (HE) and aqueous (A) extract. The results of qualitative screening of phytochemicals of *P. roxburghii* seeds showed the presence of Phenols, Flavonoids, Saponins, Alkaloids, Carbohydrates, Sterols and Terpenoids in all examined samples (Table 1). Our results showed absence of tannins and glycosides in all extracts which are in line with Siwach *et al.* (2024) but contrary to Sarath and Sudha (2019) that shows presence of tannins in methanolic extract. Siwach *et al.* (2024) reported absence of cardiac glycosides, tannins and saponins in methanolic extracts of seeds of *P. roxburghii*.

Different parts like leaves, fruits, seeds, root and stem bark of *P. roxburghii* showed the presence of many phenols, alkaloids, saponins, steroids, flavonoids and glycosides and triterpenes (Raghavendra *et al*. 2010; Kumar 2020; Balkrishna *et al*. 2021; Mishra *et al*. 2023). Qualitative phytochemical tests help to understand the role of chemical compounds and their usefulness as pharmaceuticals (Gupta 2016; Emasushan and Jhon Britto; 2018; Pandey and Fulara 2022).

Total phenolic content

The results of quantification of total contents of phenols and flavonoid in the various extracts of the *P. roxburghii* seeds are presented in Table 2. The determination of TPC is expressed as milligrams of Gallic acid equivalent per grams of the extract, determined using a reference standard curve (Fig. 2). The contents exhibited variation among fractions. Total phenolic content ranged from 48.8 ± 3.29 to 83.0 ± 2.88 mg GAE/g of extract. The hydro-ethanol extract exhibited the highest phenolic contents (83.0 ± 2.88 mg/g of GAE), followed by the methanol extract with phenolic quantities of 65.0 ± 9.5 mg/g of GAE. In comparison the aqueous extract displayed a lower phenolic concentration (48.8 ± 3.29 mg GAE/ g). These results align with findings reported in leaf by Keshav *et al.* (2021) reported TPC in hydro ethanol (30:70) leaf extract of *P. roxburghii as* 46.58 ± 2.52 mg GAE/g which is lower than from present study. Shahwar *et al.* 2012 reported total phenols as 176.0 ± 1.3 and 36.9 ± 3.0 mg GAE/g of stem extract of methanol and distilled water, respectively. These values are higher for methanol extract and lower for water extract. TPC notified in our research was somewhat higher than a recently published study which revealed that *P. roxburghii* leaf (hydroethanol, 30:70) extract contains 46.58 ± 2.52 mg/g GAE polyphenolic content (Keshav *et al.* 2021). Alterations in agro-climatic conditions accompanied with temperature and rainfall impart a significant impact on the amount of phytoconstituents within similar species of plants growing in different regions (Kumar *et al.* 2017) and different parts of the same plant also show variation in TPC and TFC contents (Shahwar *et al.* 2012; Sarath and Sudha 2019; Nazli *et al.* 2022). Hence, differences in solvent composition, plant collection sites and parts might be responsible for the variations of estimated TPC**.**

Total flavonoid content (TFC)

The results of total flavonoid contents as in the extracts are presented as milligrams of catechin equivalent per 10 g of the extract are presented in Table 2. The flavonoid quantities ranged from 565 ± 121 to 915 ± 185 mg CE/10 g. Hydro-ethanol extract exhibited the highest flavonoid content (915 ± 185 mg CE/100 g), followed by the aqueous extract (728 ± 130 mg CE/10 g) while the methanol extract displayed the least quantity (565 ± 121 mg CE/10 g). The observed flavonoid contents in *P. roxburghii* seeds extracts were higher than previous reported (Nazli *et al.* 2022; Keshav *et al.* 2021).

Table 1: Qualitative analysis of phytochemicals in extracts derived from *P. roxburghii* seeds

Extracts	Tests	Methanol	Hydro-ethanol	Aqueous
Phenols	Potassium dichromate test	+	+	+
Flavonoids	Lead acetate test	+	+	+
Tannins	Ferric chloride test	-	-	-
Saponins	Foam test	+	+	+
Glycoside	Keller Kiliani test	-	-	-
Alkaloids	Wagner's test	+	+	+
Carbohydrates	Molisch test	+	+	+
Sterols	Salkowski's test	+	+	+
Terpenoids	Salkowski's test	+	+	-
Coumarins	10 % NaOH test	+	+	+

Methanol (M), hydro-ethanol (HE) and aqueous (A). (+) indicates the presence of observed representative groups. (-) indicates the absence of observed representative groups.

Table 2: Total phenolic and flavonoid contents, and DPPH percent inhibition of seeds of *P. roxburghii* in different extracts

Extracts	TPC (mg/g) [i] (GAE)	TFC (mg/10g) [ii] (CE)	DPPH (% inhibition)
Methanol	65.0 ± 9.5	565 ± 121	69.2 ± 8.0
Hydro ethanol	83.0 ± 2.88	915 ± 185	32.8 ± 1.86
Aqueous	48.8 ± 3.29	728 ± 130	35.6 ± 9.60

Methanol (M), hydro-ethanol (HE) and aqueous (A) (i) Total Phenolic Contents (TPC) were quantified in milligrams of gallic acid equivalents (GAE) per grams of extract. (ii) Total Flavonoid Contents (TFC) were measured in mg of catechin equivalents (CE) per 10 g of extract. Values are presented as means ± standard deviation (SD). Ascorbic acid served as the positive control (n=3).

DPPH radical scavenging assay

Metabolic processes within the body and environmental factors produce free radicals mainly reactive oxygen species (ROS) which cause various ailments including ageing, carcinogenesis, mutagenesis, and cardiovascular abnormalities. Antioxidants are the agents which counteract the effects of free radicals and limit oxidative stress (Kedare and Singh 2011). DPPH assay is a standard method to investigate the free radical scavenging ability of test samples (Mishra *et al.* 2012). The ability of various solvent extracts to donate hydrogen atoms or electrons was assessed by reducing a purple DPPH into 1,1-diphenyl-2-picryl hydrazine (Raclariu-Manolică and Socaciu 2023). The ability of each extract to scavenge DPPH radicals was then measured and presented in Table 2. The methanol extract demonstrated DPPH radical scavenging activity 69.2 ± 8.0% whereas hydro ethanol and aqueous extract show almost similar activity as 32.8 ± 1.86 and 35.6 ± 9.60, respectively. The plants rich in phenols and flavonoids possess substantial antioxidant potential and are believed to be accountable for the observed antioxidant capacity in the DPPH experiment. Nazli *et al.* (2022) reported maximum percent free radical scavenging activity by methanol stem (MeOH-S) and distilled water leaf (DW-L)-L extracts as 86 ± 0.56% which is higher than our study.

CONCLUSIONS

Based on the results, it has been established that hydro ethanolic extract of *P. roxburghii* seeds contain high contents of both TPC and TFC while methanolic extract showed highest percentage of DPPH inhibition. *P. roxburghii* seeds can be used for free radical scavenging and attributed to its flavonoids and phenolic acids. However, further testing is necessary to demonstrate specific chemical composition.

AUTHOR CONTRIBUTIONS

MJ did analytical work; TI conceptualized and supervised the work; SZ and RR wrote and reviewed the first draft; MS performed formal data analysis, partially supervised lab work. All authors read and approved the final draft.

CONFLICTS OF INTEREST

The authors affirm that they possess no conflicts of interest.

DATA AVAILABILITY

The data will be made available on a fair request to the corresponding author

ETHICS APPROVAL

Not applicable

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This project is not funded by any agency.

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