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Evaluation of antioxidant and antibacterial activity of *Putranjiva roxburghii* Wall. fruit peel

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Abstract

Putranjiva roxburghii Wall. (Euphorbiaceae) has long been used in folk treatment. Considering this folkloric background, this study was designed to evaluate *in vitro* antioxidant and antibacterial activity of *P. roxburghii* fruit peel (PRFP) enthanolic (95%) extract. Antioxidant activity was checked based on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity. Antibacterial aptitude of PRFP was assessed using disc diffusion assay against two bacterial strains *Bacillus subtelis* and *Enterobacter xiangfangensis*. Disc diffusion assay was performed with three different PRFP doses (250, 500, and 1000 µg/disc). PRFP showed excellent antioxidant activity with 17.3 µg/mL IC_{50} (concentration that scavenged 50% DPPH radical) value. In antibacterial test, PRFP inhibited growth both of *B. subtelis* and *E. xiangfangensis* by revealing inhibition zone in dose-dependent fashion. Overall, PRFP is a rich source of pharmaceutical agents in terms of antioxidant and antibacterial activity.

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Introduction

Several factors such as environmental pollutants, radiation, chemicals, and toxins stimulate physiological and biochemical processes in cells of living organism and lead to generate free radicals. This generates an imbalance in the formation and neutralization of prooxidants that subsequently seek steadiness through electron pairing with biological macromolecules such as lipids, DNA, and proteins leading to oxidative stress in the physiological system (Al-Abd *et al*., 2015; Hasan *et al*., 2018; Joty *et al*., 2019). These adverse conditions cause lipid peroxidation as well as protein or DNA damage or both in human body cells. Subsequently, cellular damage provokes aging and several chronic diseases such as atherosclerosis, diabetes, and cancer as well as inflammatory, cardiovascular, and other degenerative diseases in humans (Al-Abd *et al*., 2015). However, antioxidant compounds from plant origin have the ability to halt these free radicals. The plant derived antioxidants have been reported to be composed of phenolic (such as phenolic acids, flavonoids, and tocopherols) and nitrogen compounds (amines, amino acids, chlorophyll derivatives, and alkaloids) as well as ascorbic acid and carotenoids (Velioglu *et al*., 1998). These natural antioxidants are being used in traditional medicine because of minimal side effects as well as carcinogenicity of the synthetic antioxidants (Al-Abd *et al*., 2015, Al-Rifai *et al*., 2017).

Plants are one of the most promising sources of valuable medicinal agents in traditional medicine practices from the very beginning of human civilization worldwide. Moreover, medicinal plants are rich source of antimicrobial agents. The rising resistance of bacteria to antibiotics poses a considerable challenge when fighting against infectious diseases caused by bacteria (Raza *et al*., 2012; Bandara *et al*., 2018). Therefore, utilization of plant extracts and their isolated compounds as efficient agents against microorganisms has been increased (Hassine *et al*., 2014). *Putranjiva roxburghii* Wall. (Euphorbiaceae) is widely grown in Bangladesh, India, Indochina, Myanmar, Nepal, Sri Lanka and Thailand (Hasan *et al*., 2019). It is locally known as "Putranjiv" in Bangladesh. The seed of this plant is a good source of a trypsin inhibitor (Chaudhary *et al*., 2008), and a thermostable glycosyl hydrolase family 1 enzyme with β-D-glucosidase and β-D-galactosidase activities (Patel *et al*., 2012). The leaves of this plant have been reported to be spread over the floor of maternity room for an easy delivery (Singh and Bisht, 1999). This plant has long been traditionally used for the treatment of arthralgia, fever, muscle pain, rheumatism, hemorrhoids, and inflammation (Boonyaprapat and Chokechaicharoenporn, 1999; Phuphathanaphong, 2006; Reanmongkol *et al*., 2009). Moreover, a gas chromatography-mass spectrometry (GC-MS) study of fruit peel of this plant demonstrated that a total of 25 compounds are present having a wide range of bioactivity including anti-cancer, anti-oxidant, anti-microbial, anti-inflammatory, anti-hyperlipidemic, anti-noceptive, anti-convulsant, anti-depressive, anti-trypanosomal, anti-fungal, anti-viral analgesic, anxiolytic, cytoprotective, neuroprotective, anthelmintic, wound healing, mosquito repellent, trypanocidal sedative, hypocholesterolemic, insecticide, insectifuge, chemo-preventive, pesticidal, and cytotoxic (Hasan *et al*., 2019).

Hence, considering the information mentioned above, this study was designed to evaluate antioxidant and antibacterial activity of *P. roxburghii* fruit peel extract (PRFP).

Materials and methods

Chemical and reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), and dimethyl sulfoxide (DMSO) were bought from Sigma-Aldrich (USA). Nutrient agar (NA) media, Mueller Hinton Agar (MHA) media, and Kanamycin, antibiotic discs were purchased from (HiMedia, India). All other chemicals were of analytical grade.

Plant sample collectionand extract preparation

P. roxburghii fruits were collected from the University of Rajshahi (Rajshahi, Bangladesh) campus. Then the fruit was identified and

authenticated as preserved in the herbarium of the Department of Botany, University of Rajshahi, Rajshahi, Bangladesh under an accession number 00312 for further referencing. The separated fruit peel, however, was dried by using a drying cabinet (LJ-120A(S), Guangdong LIK Industry Co., Ltd., China) at 37°C temperature and grinded to make fine powder. The fine powder (5 g) was then dissolved in 50 ml of 95% ethanol. The mixture was sonicated by using sonicator (Soniprep 150, China) at 20 kHz for 10 min. The content was filtered using Glass Fiber Filter paper (Macherey NAGEL, GmBH, German) with DURAN® Filtering Apparatus (German). The resulting solution was dried by using VirTis BenchTop Pro Freeze Dryer (German). Finally, the concentrated extract was stored at 4°C for further use.

Preliminary phytochemical screening

Preliminary phytochemical screening was performed for the PRFP extracts as described previously (Yadav and Agarwala, 2011). The screening was carried out for flavonoids, phenols, tannins, saponins, glycosides, steroid, triterpenoids, and alkaloids. The color change or the precipitate formation was used as analytical responses to these tests.

Antioxidant activity test

The antioxidant activity was measured according to the method described previously with little modification (Al-Rifai *et al.*, 2017). The antioxidant activity of PRFP extracts against DPPH radical was determined by UV spectrophotometry at 517 nm. PRFP extract was prepared at 1 mg/mL methanol (1 µg/µL). PRFP (5, 10, and 25 µL) was added in 1.5 mL microcentrifuge tube and then methanol was added wherever needed to make total volume 100 µL. Then, 900 µL of 1.0 mM DPPH in methanol was added, mixed well, and allowed to react at room temperature for 30 minutes in dark place. Only methanol (100 µL) and DPPH (900 µL) were mixed to prepare the control solution. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The radical scavenging activity was calculated using the following formula:

$\%\ \text{inhibition} = [(A_c - A_e) / A_c] \times 100$

where, A_c is absorption of the control sample and A_e is absorption of the extract treated sample.

The IC_{50} value OF the PRFP extract was calculated from the plot of inhibition percentage against extract concentration.

Antibacterial activity test

Microbial strains

The antibacterial activity of the PRFP was individually tested against two strains *Bacillus subtelis* and *Enterobacter xiangfangensis*. Bacterial isolates were cultured overnight at 37°C in NA media.

Disc diffusion assay

Antibacterial activity against two bacterial strains (*B. subtelis* and *E. xiangfangensis*) was determined by disc diffusion method as described previously (Parekh *et al.*, 2005). PRFP extract was dissolved in 100% DMSO, added at 250, 500 and 1000 µg/disc which were screened against *B. subtelis* and *E. xiangfangensis*on on MHA medium. One negative control disc was also placed to nullify the effect of solvent (DMSO) on bacterial growth. Each bacterial strain was also screened for standard antibiotic (Kanamycin, 30000 µg/disc) disc which acted as positive control. After incubation of 24h at 37°C, the plates were observed for the presence of zones of inhibition as evidence of antibacterial activity.

The degree of sensitivity was determined by measuring the diameter of visible zones of inhibition to the nearest millimeters with respect to each bacterial strain and extract dose.

Statistical analysis

All of the results are presented as mean ± SD (standard deviation). Means and SD were calculated using Microsoft Excel 2007.

Results

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids in PRFP (Table 1).

Table 1. Phytochemical constituents of PRFP extract.

Phytochemical	Result (+ means present, - means absent)
Flavonoids	+
Phenols	-
Tannins	-
Saponins	+
Glycosides	+
Steroid	+
Terpenoids	+
Alkaloids	+

Antioxidant activity test

The PRFP extract showed excellent antioxidant activity in a dose-dependent fashion (Fig. 1). The DPPH scavenging percentages were 16.0 ± 1.3, 40.7± 0.8, and 66.7 ± 2.8 at 5, 10, and 25 µg/mL PRFP respectively. The obtained IC_{50} value of PRFP is 17.3 µg/mL.

Table 2. Antibacterial activity of PRFP against *B. subtelis* and *E. xiangfangensis* strains.

Bacterial strains	Treatment	Dose (µg/disc)	Inhibition zones (mm)
B. subtelis	PRFP	250	7.3 ± 0.9
		500	9.5 ± 0.8
		1000	11.8 ± 1.0
	Kanamycin	30000	25.1 ± 1.7
E. xiangfangensis	PRFP	250	-
		500	-
		1000	8.9 ± 1.1
	Kanamycin	30000	13.2 ± 1.3

Antibacterial activity test

The result regarding antibacterial activity of PRFP has been presented in Table 2. PRFP showed growth inhibitory effect against both of the bacterial strains (Fig. 2). But, PRFP showed better antibacterial activity against *B. subtelis*. In case of *B. subtelis*, all of the doses (250, 500, and 1000 µg/disc) of PRFP revealed inhibition zone (7.3 ± 0.9, 9.5 ± 0.8, and 11.8 ± 1.0 mm) in dose-dependent manner. On the other hand, in case of *E. xiangfangensis*, we observed that only the highest dose (1000 µg/disc) of PRFP revealed inhibition zone.

The standard antibiotic Kanamycin (30000 µg/disc) gave 25.1 ± 1.7, and 13.2 ± 1.3 mm inhibition zone against *B. subtelis,* and *E. xiangfangensis* respectively which are larger compared to inhibition zone given by PRFP. But, the dose of standard (30000 µg/disc) was 30 fold of the highest dose (1000 µg/disc) of PRFP.

Discussion

Recently, phytochemicals are of greater interest in alternative medicine from the points of less toxicity and cost benefit. However, we confirmed presence of flavonoids, phenols, tannins, saponins, glycosides, steroid, triterpenoids, and alkaloids in our experimental extract. All of these phytchemicals are well known for their medicinal as well as physiological activities (Yadav and Agarwala, 2011).

Natural antioxidants are now getting much attention to fight against free radicals. Flavonoids are very strong and well known plant derived antioxidant compound (Kumari *et al.*, 2017). PRFP extract contain flavonoids, and showed remarkable

antioxidant activity. Moreover, a GC-MS study following similar extraction method confirmed presence of "citronellal", "caryophyllene oxide", "hexadecanoic acid, methyl ester", and "hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester" which were reported as antioxidant compound previously (Hasan *et al*., 2019). Hence, this antioxidant capacity of PRFP is consistent with previous reports (Kumari *et al*., 2017; Yadav and Agarwala, 2011; Hasan *et al*., 2019).

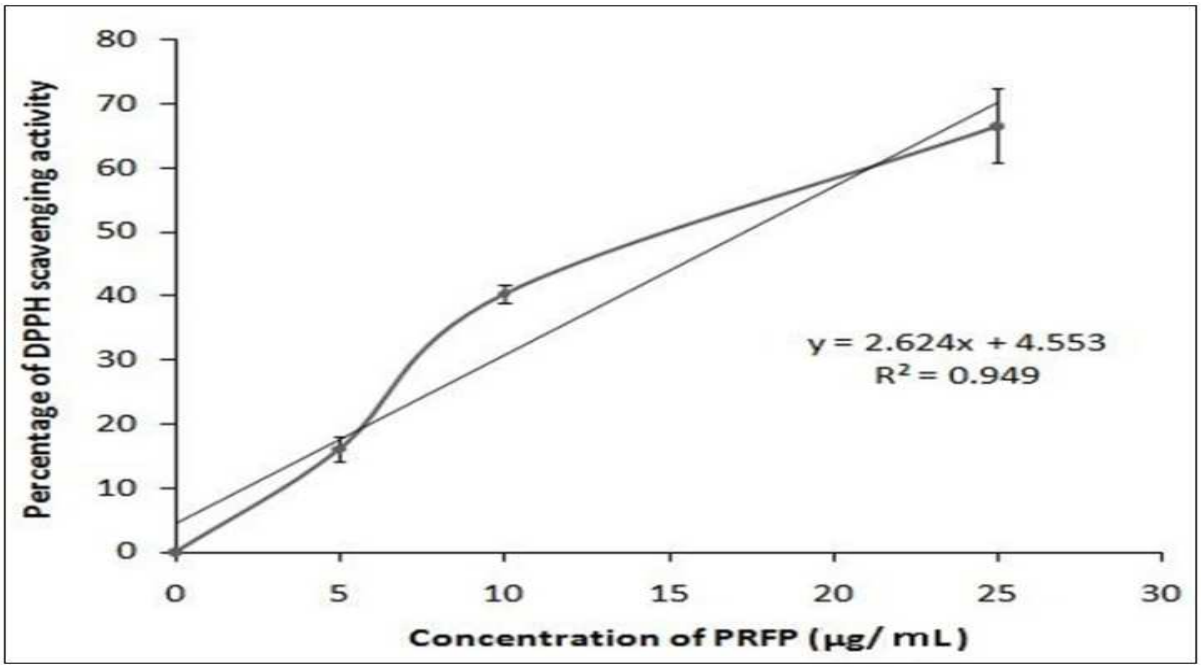

Fig. 1. Antioxidant activity of PRFP. PRFP scavenged DPPH radical in a dose-dependent manner. The IC_{50} value (17.3 µg/mL) was calculated using regression equation y = 2.624x + 4.553. Data are presented as mean ± SD (n=3).

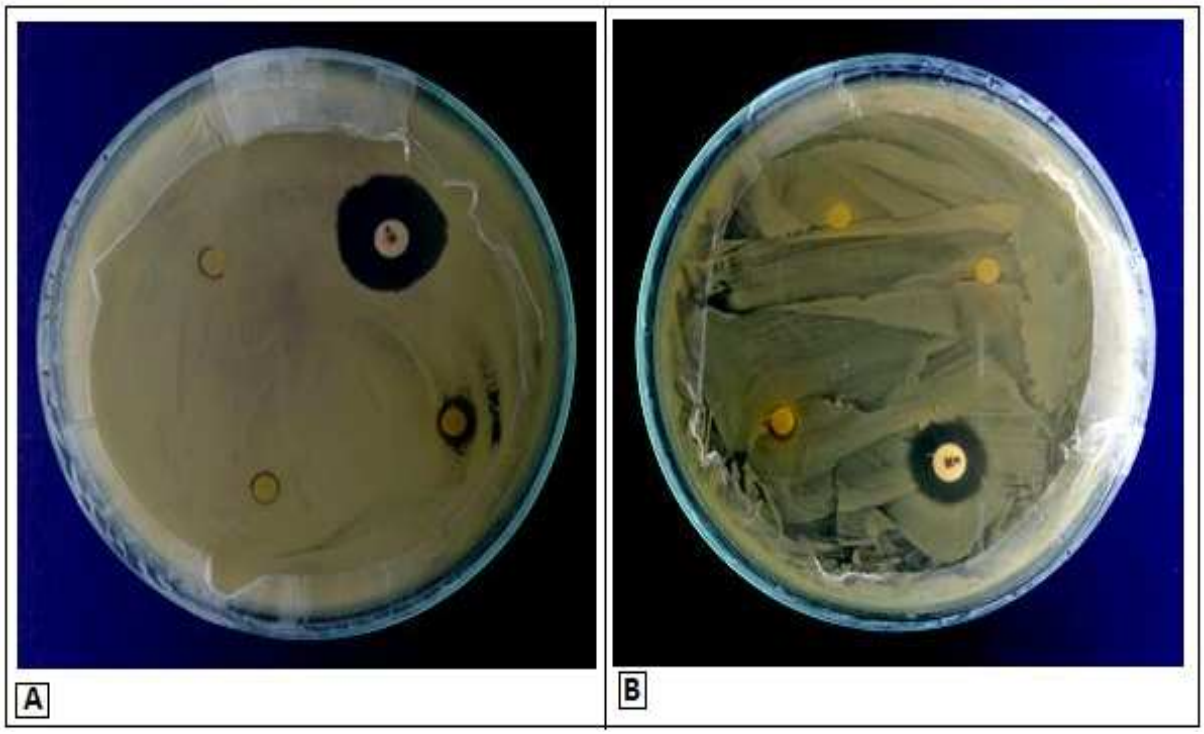

Fig. 2. Antibacterial activity of PRFP against *B. subtelis* (A) and *E. xiangfangensis* strains (B).

In case of antibacterial activity, we observed that PRFP extract inhibited growth of *B. subtelis* and *E. xiangfangensis*. Though the antibacterial activity of PRFP was lower in terms of inhibition zone compared to the standard antibiotic (Kanamycin), this was noteworthy in terms of dosing. Because, the highest dose (1000 µg/disc) of PRFP was 30 times diluted compared to the standard. However, it was reported that the presence of the secondary metabolites such as saponins, flavonoids, terpenoids, and steroids in the plant extract has a promising activity against pathogens and helps the antibacterial activities of plants (Raquel, 2007; Okwu and Okwu 2004; Khan *et al.*, 2011; Kumari *et al.*, 2017). Hasan *et al.* (2019) also reported the presence of "linalool", "citronellal", "cyclohexanol, 5-methyl-2-(1-methylethenyl)-", "2,6-Octadienal, 3, 7-dimethyl-, (Z)-", "geraniol", "2, 6-Octadienal, 3, 7-dimethyl-, (E)-", "6-Octen-1-ol,3,7-dimethyl-, formate", "(1R,2S,5R)-2-(2-Hydroxy-2-propanyl)-5-methylcyclohexanol", and "hexadecanoic acid, methyl ester" which are well known antimicrobial agents.

Conclusion

Overall, *P. roxburghii* fruit peel is an effective source of antioxidant and antibacterial agents. This study may attract a great deal of attention in using *P. roxburghii* fruit peel in traditional medicine as well as in conducting further study.

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Conflict of interest

The authors have no conflict of interests.

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