INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

ANALYSIS OF *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF *Putranjiva roxburghii* Wall.

Sarath P and Sudha Bai R *

Post Graduate and Research Department of Botany, University College, Thiruvananthapuram, Kerala, India

*Corresponding Author Email: sudharvinayan@gmail.com

Article Received on: 01/01/19 Approved for publication: 19/02/19

DOI: 10.7897/2230-8407.1004133

ABSTRACT

Putranjiva roxburghii Wall. has been historically credited with a significant role in the traditional Unani and Ayurvedic systems of medicine. The present study was an attempt for evaluation of antioxidant and antimicrobial efficacy of the methanolic extracts from the bark and seeds using various *in vitro* assays. The antioxidant activity of the extracts was estimated using the DPPH radical scavenging assay and ferric reducing antioxidant power assay. Extract concentrations in the range of 50 – 500 µg/ml for bark and 200 – 2000 µg/ml for seed were assayed to determine the IC_{50} values in DPPH assay. For the evaluation of Ferric reducing capacity, concentrations in the range 50 – 1000 µg/ml were compared. The extracts were also analyzed for antibacterial and antifungal activity against *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*. The results suggested that bark has significant antioxidant and antimicrobial properties than the extract of seed. The IC_{50} for bark and seed extract were 129.47 µg/ml and 1978.7 µg/ml respectively. Both the DPPH and ferric reducing assays suggested a dose dependent increase in extract efficiency to scavenge and to reduce the free radicals. The antimicrobial activity suggested that bark extract could inhibit bacterial (IZ 10 – 12 mm) and fungal growth (IZ = 12 – 14 mm) at 1000 µg/ml concentrations.

Key Words: *Putranjiva roxburghii* Wall. Antioxidant assay, Antimicrobial activity, DPPH, Ferric reducing capacity.

INTRODUCTION

Putranjiva roxburghii Wall. is widely grown tree in Thailand, Nepal, Bangladesh, India, Indochina and Sri Lanka. In traditional medicine and Ayurveda, Its leaves and fruits are being used for the treatment of fever, muscle twisting, arthralgia, and rheumatism. Its usage in other clinical conditions such as azoospermia, catarrh, and constipation have also been documented [1]. Ayurvedic classics have highlighted the importance of Putranjivaka as helps in pregnancy. The powder of the seed is given in a dose of 1-3 g with milk to improve the sperm count in males and help in maintaining the foetus in pregnant women[2]. The seed oil of *P. roxburghii* Wall. has potential as a preservative for peanut seeds against spoilage by fungi and insects during storage. The oil was found to be fungicidal and thermostable at its minimum inhibitory concentration (MIC) of 400 ppm [3]. The Putranjivaceae is a mysterious family, it is the only known lineage outside the Capparales to possess the glucosinolate biochemical pathway. Glucosinolate pathway is the basis of an induced chemical defense system against herbivores - the "mustard oil bomb"[4]. The genus *Putranjiva* has only 4 species - *Putranjiva formosana* Kaneh. & Sasaki ex Shimada, *Putranjiva matsumurae* Koidz, *Putranjiva roxburghii Wall*. and *Putranjiva zeylanica* (Thwaites) Müll. Arg.

The objectives of the present study comprise an evaluation of antioxidant capacities of extracts from the bark and seed of *P. roxburghii* Wall. using *in vitro* assays such as DPPH scavenging and ferric reducing tests. A comparison of *in-vitro* antimicrobial activity of the extracts using selected strains of bacteria and fungi is also carried out to assess the ability of extracts to resist microbes.

MATERIALS AND METHODS

Collection of Plant Material. The plant was collected during November- December 2017 from Trivandrum, identified with the help of Flora of Presidency of Madras [5]. The specimen of the species was confirmed with the authenticated Herbarium of Department of Botany, University College, Trivandrum.

Preparation of Extracts: Bark and seeds of *Putranjiva roxburghii* were collected, washed in tap water, shade dried to constant weight, finely powdered and used for extraction. Ten gram of dried powder of bark seed was suspended in 50ml of 80 % (v/v) methanol, placed in a water bath at a temperature of 40^0C for 12 hours and then in a gyratory shaker at 120 rpm for 48 hours. After treatment in methanol, the extracts were separated by filtration using Whatman No.1 filter paper, evaporated and dried till the residue weights were recorded as consistent. The final residues were stored in a refrigerator at 4^0C for further use.

Evaluation of Antioxidant capacity: Stock solutions (2000 mg/ml) of bark and seed extract were reconstituted from the residue using methanol and used in varying concentration ranges for antioxidant assays.

DPPH (2,2-Diphenyl-L-Picrylhydrazyl) Radical Scavenging Assay

The antioxidant activity of the two extracts was estimated using the DPPH radical scavenging protocol [6]. A stock solution of DPPH solution – 0.004% (w/v) prepared in 95 % methanol. Methanolic extract and standard ascorbic acid were prepared in the concentration of mg/ml. From stock (methanolic extract and ascorbic acid) solution different aliquots were taken in test tubes.

With solvent made the final volume of each test tube up to 1 ml to get the required concentration ranges (50 – 500 µg/ml for bark and 200-2000µg/ml for seed). In each of the test tubes 2 ml of DPPH working solution (0.004% w/v) was added. After incubation of the reaction mixture under darkness for 10 min, the absorbance was recorded at 517 nm against the blank. The control used was a mix of 2 ml of DPPH solution in methanol and 1ml of methanol. The assay was carried out in triplicates. The antioxidant capacity of the plant extract was determined on basis of decrease in absorbance of DPPH in comparison to the control that contained no extract. The antioxidant activity was computed as percentage inhibition (%IP) of DPPH radical. The capability of extracts to scavenge DPPH radical was estimated using the following equation.

DPPH scavenged (%) = (A control – A Test) / A control x 100

Where "A control" is the absorbance of the control reaction and "A test" is the absorbance of the sample of the extracts. IC_{50} values denote the concentration of the sample, which is required to scavenge 50% of DPPH free radicals.

Ferric Reducing Power Assay

Methanolic extracts of bark and seed samples were compared for their Ferric reducing power using ascorbic acid as standard [7].

In ferric reducing antioxidant power assay, the extract in varying concentrations in a total I ml volume were mixed with equal volume (1 ml each) of 0.2 M sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The reaction mixtures were incubated in a water bath at 50^0C for 20 min, and added 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 min at room temperature to rule out the interference by traces of precipitate. The supernatant obtained (1 ml) was mixed with 1 ml of deionised water and 200 µl of 0.1% $FeCl_3$. The blank was prepared by excluding 1% potassium ferricyanide and substituting with distilled water. The absorbance of the reaction mixture was recorded at 700 nm. The reducing power was expressed as an increase in A700 after blank subtraction.

ANALYSIS OF ANTIMICROBIAL ACTIVITY

Microbial Strains

The microorganisms used for the evaluation of antibacterial activity were one Gram-negative strain – *Escherichia coli* and one Gram-positive strain – *Staphylococcus aureus*. The fungal strains selected were *Aspergillus niger* and *Candida albicans*.

Culture Medium And Inoculation

The stock cultures of microorganisms used in this study were maintained in Mueller Hinton agar slants (for bacteria) and in Potato-Dextrose agar (for Fungi) at 4^0C.

Mueller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Mueller Hinton Agar Medium (HI Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm petri plates (20ml/plate) while still molten.

Nutrient Broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (Himedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

Procedure

Petri plates containing 20ml Mueller Hinton Agar Medium were seeded with bacterial culture - *E.coli* and *S. aureus* (growth of culture adjusted according to McFards Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and sample of 250, 500, and1000 µg/ml concentrations were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well [8]. Streptomycin (mg/ml) was used as a positive control.

The inoculum was prepared by suspending a loopful of culture from stock slant into 25 ml of sterilized nutrient broth (prepared by dissolving 5 g Nutrient broth – readymade Himedia in 200 ml distilled water), maintained in a gyratory shaker at 120 rpm for 48 hours to get uniform growth of bacteria.

Medium For Culture Plate

11.2g Nutrient agar was dissolved in 400 ml of distilled water, as per the instruction provided by the suppliers (Himedia) and heated gently to dissolve the Nutrient agar completely. The medium was autoclaved at 121°C and 15 lb/in^2 pressure for 20 minutes. The sterilized Nutrient agar medium was cooled to 45 – 50^0C and 30 ml were poured into each culture plate. The plates were examined after 24 h for any contamination before carrying out the antibacterial analysis of the extracts.

Antibacterial Analysis

Determination of antibacterial potential of the extracts was carried out using the agar well diffusion method [9]. The solidified nutrient agar in the Petri plates was inoculated by dispensing 0.2ml of the inoculum using a sterilized pipetting device and spread evenly with sterile cotton swabs. Four wells were prepared on each plate with the help of a sterile cork-borer of 10mm diameter. The extract was then introduced into each well to get concentrations of desirable levels. Well introduced with 0.1 ml of streptomycin served as control. All the plates containing loaded wells were incubated for 24h at 37°C. After the incubation period, zone of inhibition in each plate, for each concentration of extract and antibiotic control were measured by calculating the difference between diameter of cork-borer and diameter of inhibition[8]. The same method of experiment was carried out in the triplicates and the mean values were reported for the final consideration.

Antifungal Activity

Agar well diffusion method was adopted to assess the the antifungal activity against *Aspergillus niger* and *Candida albicans*.

Preparation Of Inoculum

The fungal strains – *Aspergillus niger* and *Candida albicans* were grown at 27^0C on Potato Dextrose Agar (PDA) slants. The 48 h old cultures grown in potato dextrose broth were used for inoculation of fungal strains on PDA plates. An aliquot (0.02ml) of inoculum was introduced to the solidified PDA in petri dishes and swabbed. Appropriate wells (10 mm) were made on agar plate by using sterile cork borer and samples of different concentration (250; 500 and 1000 µg/ml) were added. The zone of inhibition was measured after overnight incubation and compared with that of standard antimycotic agent - Clotrimazole.

Table 1. Results of DPPH Assay of Methanolic extract of Bark and Seed of *P. roxburghii* Wall.

Sl No.	Ascorbic acid		Bark		Seed	
	Conc. (µg/ml)	% scavenging	Conc. (µg/ml)	% scavenging	Conc. (µg/ml)	% scavenging
1	50	82.65	50	26.18	200	11.57
2	100	94.81	100	37.67	400	12.17
3	150	97.84	150	58.59	600	19.52
4	200	99.97	200	68.28	800	22.29
5	250	100	250	72.58	1000	28.79
6	300	100	300	76.31	1200	29.88
7	350	100	350	85.87	1400	35.18
8	400	100	400	88.37	1600	40.96
9	450	100	450	89.47	1800	44.94
10	500	100	500	90.03	2000	50.60

Table 2. Results of FRAP Assay of Methanolic extract of Bark of *P. roxburghii* Wall.

Conc. (µg/ml)	Absorbance	
	Ascorbic acid	Bark
50	0.045	0.266
100	0.084	0.327
150	0.126	0.409
200	0.161	0.497
250	0.193	0.518
300	0.226	0.557
350	0.248	0.578
400	0.286	0.691
450	0.313	0.804
500	0.356	0.821

Table 3. Results of FRAP Assay of Methanolic extract of Seed of *P.roxburghii* Wall.

Conc. (µg/ml)	Absorbance	
	Ascorbic acid	Seed
100	0.083	0.257
200	0.157	0.311
300	0.233	0.348
400	0.331	0.364
500	0.398	0.379
600	0.442	0.428
700	0.496	0.516
800	0.573	0.525
900	0.615	0.587
1000	0.698	0.621

Table 4. Antibacterial activity of methanolic extract of Bark, Leaf and Seed of *P. roxburghii* wall. against different strains of bacteria.

	Escherichia coli		*Staphylococcus aureus*	
	Conc (µg/ml)	IZ (mm)	Conc. (µg/ml)	IZ (mm)
Control	Streptomycin (100µg/ml)	25	Streptomycin (100µg/ml)	25
Bark	250	Nil	250	Nil
	500	Nil	500	Nil
	1000	10	1000	12
Seed	250	Nil	250	Nil
	500	Nil	500	Nil
	100	10	1000	10

(Concentration range 250 µg-1000 µg, positive control streptomycin 100 µg)

Table 5. Antifungal activity of methanolic extract of Bark and Seed of *Putranjiva roxburghii* wall. against different strains of fungi.

	Aspergillus niger		*Candida albicans*	
	Conc. (µg/ml)	IZ (mm)	Conc. (µg/ml)	IZ (mm)
Control	Clotrimazole (100µg/ml)	17	Clotrimazole (100µg/ml)	24
Bark	250	11	250	Nil
	500	13	500	11
	1000	14	1000	12
Seed	250	10	250	Nil
	500	11	500	10
	1000	12	1000	10

(Concentration range 250 µg-1000 µg, positive control streptomycin 100 µg)

RESULTS

Antioxidant Activity

DPPH Scavenging Activity
The DPPH antioxidant activity of methanolic extract of the bark was determined for the concentration range of 50 – 500 µg/ml, and more than 90% inhibition was attained at 500 mg/ml (Table 1) and the IC_{50} of the extract was 129.47 µg/ml. Seed extract need higher concentration range of 200 - 2000 µg/ml to attain the IC_{50} (IC_{50} =1978.7µg/ml). The observations indicated a significantly higher efficacy of bark extract for free radical scavenging than seed extract.

Ferric Reducing Power Assay: The bark and seed extracts of *P.roxburghii* Wall. Indicated concentration-dependent reducing power and the data are depicted in Table 2. The higher absorbance value indicated a stronger reducing power of the extract. The extract, being a source of antioxidant, can donate an electron to free radicals. Reducing power was measured on basis of the reduction of $Fe^{3+}(CN^-)_6$ to $Fe^{2+}(CN^-)_6$ and the reaction product was monitored by means of the intense Prussian blue color of the complex that is measured at 700 nm.

The bark extract showed high reducing power than seed. At 500 µg/ml of bark extract the absorbance recorded was 0.821, seed extract has only poor reducing power even at 1000 µg/ml concentration (absorbance = 0.521), which suggested that seed has poor antioxidant power.

EVALUATION OF *In vitro* ANTIMICROBIAL ACTIVITY

Antibacterial activity: The methanolic extracts of bark, and the seed of *P. roxburghii* wall. were investigated for *in vitro* antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative *(Escherichia coli*) bacteria. The antibacterial sensitivity and potency were assessed quantitatively by measuring the diameter of the zone of inhibition in cultures in petri plates and the results are tabulated in Table 4

In antibacterial study by agar well diffusion method the antibacterial activity of each extract increased linearly with concentration. The zone of inhibition by bark extract is higher than that of seed. At 1000 µg/ml concentration of extract and the bark extract could restrict growth of 10mm IZ in *E.coli* culture and 12mm IZ in *S. aureus* culture, which is higher than that of seed. The Gram-negative bacteria (*E coli)* were more resistant to the *P. roxburghii* wall. extracts than Gram-positive bacteria (*S. aureus)*.

Antifungal Activity: The antifungal potency of each extract was assessed quantitatively by measuring the diameter of the zone of inhibition in potato dextrose agar culture in petri plate, and the result is tabulated in Table 5.

The bark extract of *P. roxburghii* Wall. has more antifungal activity than seed extract. *Aspergillus niger* is recorded as more susceptible than *C. albicans* against *P. roxburghii* wall. extract. Both bark and seed extracts have activity in 250 µg/ml concentration against *A.niger.*

DISCUSSION

Antioxidant activity: The results of assays on antioxidant efficiency of the extracts demonstrated that the bark and seeds possess antioxidant potential. In the present study, the result of DPPH scavenging activity suggested that there is an increase in the percentage of radical scavenging activity with an increase in the concentration of the extract for bark and seed. Among the plant parts compared, bark has the potential to effectively scavenge DPPH than the seed. The bark extract was monitored as more effective in reducing DPPH and ferric ions. The results of DPPH assay are found to be positively correlated with the ferric reducing power analysis and concluded that bark possess higher antioxidant property than seeds.

The molecule 1, 1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical due to delocalization of the spare electron over the molecule as a whole. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption peak at about 517 nm. When a solution of DPPH is mixed with a substrate that can donate a hydrogen atom, it gives rise to the reduced form with the loss of this violet color. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. DPPH is a stable free radical and it can accept an electron or hydrogen radical to become a stable diamagnetic molecule. A freshly prepared DPPH solution is of deep purple color with λmax 517 nm and in the presence of an antioxidant, the purple color disappears due to quenching of DPPH* free radicals and converting them into a colorless product- 2,2-diphenyl-1-picryl hydrazine [10].

Plants are a natural source of antioxidants by virtue of diverse types of secondary metabolites stored in them. The phytochemicals such as phenolics and flavonoids of most medicinal plants are important in defense mechanism and also have good antioxidant potential. It has been reported that the phenolics in plants possess strong antioxidant activity and levels of the antioxidant activity are proportional to the concentrations [11].

Antimicrobial Activity: Plant extracts are a potentially a rich source of antimicrobial compounds. Many plants have been known to synthesize active secondary metabolites such as phenolic compound with established potent insecticidal and antimicrobial activities [12].

In general the antimicrobial activity of plant extracts is due to the presence of secondary metabolites such as flavonoids, phenolic compounds, terpenoids, tannin and alkaloids that adversely affect the growth and metabolism of microbes. Studies on preliminary phytochemical screening of methanol extract of *Putranjiva roxburghii* Wall. seed reported the presence of phenols, alkaloids, saponins, steroids, flavonoids, and glycosides[13]. A comparative evaluation of bark and seed in our basic study also indicated much higher quantities of secondary metabolites in bark than seeds. The comparatively poor antimicrobial profile indicated by the seed extract which contains lesser levels of alkaloids and terpenoids, supports this correlation.

The microbial strains compared exhibited variable levels susceptibility to same dose of extracts from bark and seeds. The gram positive bacterium- *S. aureus* was detected as more sensitive among bacteria and *A. niger* was monitored as the more sensitive fungal strain. This difference could be attributed to the differences in cell wall constitution and permeability responses from strain to strain. In previous studies also the gram positive bacteria were reported as more sensitive to plant extracts[14,15].

CONCLUSION

The observations documented in the present investigation demonstrated that the bark of *Putranjiva roxburghii* has substantially high antioxidant and antimicrobial efficiency than the seeds. Though the bark of this medicinal species is not much

included in traditional systems of treatment, it could be explored for herbal treatments.

ACKNOWLEDGEMENT

The authors are thankful to the Principal, University College, Thiruvananthapuram for providing all laboratory facilities.

REFERENCES

1. Samal J, Dehury RK. *Putranjiva roxburghii* Wall: The controversies and the concurrences. International Journal of Green Pharmacy. 2016; 10(4):254–256.
2. Rahman AHM, Akter M. Taxonomy and Medicinal Uses of Euphorbiaceae (Spurge) Family of Rajshahi, Bangladesh, Research in Plant Sciences. 2013; 3: 517-523.
3. Tripathi NN, Kumar, N. *Putranjiva roxburghii* oil-A potential herbal preservative for peanuts during storage. Journal of Stored Products Research 2007; 43: 435–442.
4. Johnson SD, Griffiths ME, Peter CI, Lawes MJ. Pollinators, "mustard oil" volatiles, and fruit production in flowers of the dioecious tree *Drypetes natalensis* (Putranjivaceae). American Journal of Botany 2009; 96: 2080–2086.
5. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958; 181: 1199-1200.
6. Oyaizu M. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. The Japanese Journal of Nutrition and Dietetics. 1986.; 44: 307–315.
7. Hewitt W, Vincent S. Theory and application of microbiological assay. Academic Press. 1989; 5: 32-106.
8. Gamble JS. Flora of the Presidency of Madras, Vol. III. Botanical Survey of India. Calcutta, Pp. 1958;821-862.
9. Perez C, Pauli M, Bazerque P. An antibiotic assay by agar-well diffusion method. Acta biologeae et medicinae experimentalis. 1990; 15: 113–115.
10. Maharjan L, Bijaya, Baral, B. Antioxidant Capacity and Phenolic Content of Some Nepalese Medicinal Plants, American Journal of Plant Science. 2013; 21:, 23-36.
11. Kikuzaki H; Hisamoto M, Hirose K, Akiyama K. Taniguchi H. Antioxidant properties of ferulic acid and its related compounds. Journal of Agricultural and Food Chemistry 2002; 50: 2161–2168.
12. Nascimento G, Locatelli P, Freitas C, Silva G. Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic resistant Bacteria. Brazilian Journal of Microbiology. 2000; 31:247-256.
13. Raghavendra HL, Prashith Kekuda TR, Valleesha NC, Sudharshan SJ, Chinmaya A. Screening for cytotoxic activity of methanol extract of *Putranjiva roxburghii* Wall (Euphorbiaceae) seeds. Pharmacognosy Journal. 2010; 2: 335–337.
14. Ceylan E, Fung DYC. Antimicrobial activity of spices. Journal of Rapid Methods and Automation in Microbiology. 2004; 12: 1-55.
15. Mohamed Sham Shihabudeen H, Hansi Priscilla. D, Kavitha Thirumurugan; Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants ; International . Journal of Pharmaceutical Sciences and Research. 2010; 1(10):430-434

Cite this article as:

Sarath P and Sudha Bai R. Analysis of *In vitro* antioxidant and antimicrobial potential of *Putranjiva roxburghii* Wall. Int. Res. J. Pharm. 2019;10(4):106-110 http://dx.doi.org/10.7897/2230-8407.1004133

Source of support: Nil, Conflict of interest: None Declared