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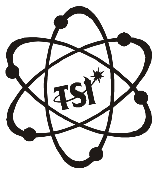

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In vitro antioxidant activity of *Putranjiva roxburghii* Wall seed by DPPH radical scavenging activity

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ABSTRACT

Antioxidants help to deal with oxidative stress, caused by free radical damage. The present study highlights the antioxidant activity of methanol extract of *P. roxburghii* by its DPPH free radical scavenging potential. Various phytoconstituents namely alkaloids, flavonoids, saponins, glycosides, coumarins, quinones, tannins and steroids were detected. A marked radical scavenging activity was observed and found to be dose dependent. The activity of extract was found to be slightly lower as compared to the scavenging activity of ascorbic acid. Though *in vitro* antioxidant assay has been carried out, *in vivo* tests remains to be done.

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KEYWORDS

Putranjiva roxburghii Wall;
Antioxidant activity;
Soxhlet extraction;
DPPH free radical scavenging assay;
Ascorbic acid.

INTRODUCTION

Antioxidants help to deal with oxidative stress, caused by free radical damage. Free radicals are chemical species containing one or more unpaired electrons that makes them highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability[1]. In recent years much attention has been devoted to natural antioxidant and their association with health benefits. *Putranjiva roxburghii* Wall., commonly called Putranjiva, is a deciduous, evergreen tree of about 18m tall having grey bark. Leaves elliptic-oblong to ovate-lanceolate, unequal sided at the base, dark green and shining inappearance. Flowers small; male in dense, rounded clusters, yellowish in colour;female flowers solitary or 2-3 together, green. Drupes ellipsoid or globose and white-tomentose, stone pointed, rugose, very hard and normally having a single seed. It is used in cold, fever and rheumatism[2,3] and seeds in inflammation[4]. The plant is cultivated in Hong Kong Zoological and Botanical Gardens. India, Sri Lanka, Myanmar, Cambodia, Laos, Indonesia, New Guinea[3]. There are several methods of assessing antioxidant activity of phytoconstituents[1]. The present study highlights the antioxidant activity of *P. roxburghii* by its DPPH free radical scavenging potential.

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MATERIALS AND METHODS

Collection and Extraction of plant material

The Seeds of *P. roxburghii* Wall were obtained from local shops of Udupi city, Karnataka, authenticated to identity by Dept. of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga and voucher specimen was deposited in the department for future reference. The dried Seed were powdered mechanically and subjected to soxhlet extraction using methanol for about 48 hours. The extract was filtered, concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the dessicator. The yield was recorded and the extract was kept in refrigerator until use[5]. The methanol extract was subjected to preliminary phytochemical analysis[6].

In vitro antioxidant activity by DPPH free radical scavenging assay

The antioxidant activity of methanol extract of *P. roxburghii* and the standard (Ascorbic acid) was tested on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH)-free radical activity[7,8]. Two concentrations of plant extract and standard namely 0.5mg/ml and 1.0mg/ml were prepared in methanol. 0.002% of DPPH was prepared in methanol. In clean and labeled test tubes, 2ml of DPPH solution was mixed with 2ml of different concentrations of plant extract and standard separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517nm using UV-Vis Spectrophotometer. The absorbance of extract solution without DPPH and the DPPH control (containing no sample) was also noted. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable DPPH* was calculated using the following equation.

$$\text{Scavenging activity (\%)} = 1 - \frac{A_1 - A_2}{A_0} \times 100\%$$

Where A_1 was the absorbance of DPPH* in the presence of extract, A_2 was the absorbance without DPPH* solution and A_0 was the absorbance of control (DPPH* without extract).

RESULTS AND DISCUSSION

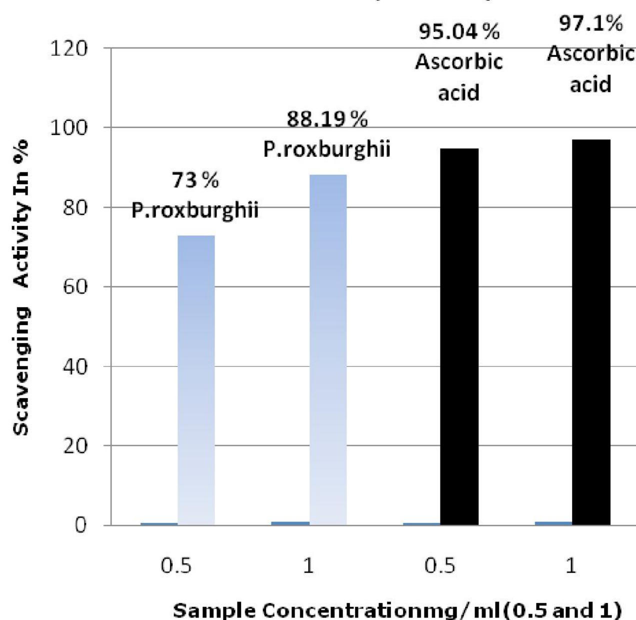
TABLE 1 : Phytochemical groups in methanol extract

Group	Methanol extract
Tannin	+
Alkaloid	+
Saponins	+
Steroids	+
Terpenoids	ND
Flavonoids	+
Glycosides	+
Coumarins	+
Quinones	+

'+' – Detected; 'ND' – Not detected

TABLE 2 : *In vitro* antioxidant activity of methanol extract and Ascorbic acid

Test compound	Concentration (mg/ml)	Scavenging activity in %
Methanol extract	0.5	73.00
	1.0	88.19
Ascorbic acid (standard)	0.5	95.04
	1.0	97.10



Graph 1 : *In vitro* antioxidant activity of methanol extract of *P. roxburghii* and Ascorbic acid

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Various phytoconstituents namely alkaloids, flavonoids, saponins, glycosides, coumarins, quinones, tannins and steroids were detected in the methanol extract of *P. roxburghii* whereas triterpenoid was not detected (TABLE 1). A marked radical scavenging activity of *P. roxburghii* extract was observed (TABLE 2; Graph 1). The activity was found to be dose dependent and a scavenging activity of 73% and 88.19% was observed in case of extract concentrations 0.5mg/ml and 1.0mg/ml respectively. The activity of extract was found to be lesser when compared to the scavenging activity of standard (95.04% by 0.5mg/ml and 97.10% by 1.0mg/ml). Literatures on antioxidant activity of *P. roxburghii* is lacking. A sensitive HPTLC method for estimation of amentoflavone, a bioactive principle from *Biophytum sensitivum* (Linn.) DC. and *P. roxburghii* Wall. was developed[9]. Ethnopharmacological and phytochemical screening of *P. roxburghii* Wall. was carried[10]. The oil of *P. roxburghii* showed potential as a preservative for peanut seeds against spoilage by fungi and insects during storage. It did not exhibit any adverse effect on seed germination, seedling growth and general health and morphology of plants[11]. Cytogenetic toxicity of leaf extract of *P. roxburghii* was tested by oral administration of leaf extract in young weaning Swiss albino mice. The results showed that the extract significantly induced mitosis-disruptive chromosomal changes in bone marrow cells. It is proposed that the extract might have interfered with the spindle and other proteins causing polyploidy, aneuploidy, c-mitosis, etc[12]. A highly stable and potent trypsin inhibitor of approximately 34 kDa was purified and characterized from *P. roxburghii* seeds by acid precipitation, cation-exchange and anion-exchange chromatography[13].

CONCLUSION

Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological activities. Though *in vitro* antioxidant assay has been carried out, *In vivo* tests remains to be done. The clinical efficacies of plant preparation used are not yet validated. Further experiments in animal models have to be carried to justify *in vivo* potential of the extract.

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