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## Phytochemical, Antioxidant and Antiinflammatory studies on the leaves of *Putranjiva roxburghii*

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### ABSTRACT

The *In-vitro* antioxidant and anti-inflammatory activity of the leaf extract of *Putranjiva roxburghii* were studied along with preliminary phytochemical investigation. The leaf of the plant contains mainly anthracene, cardiac and flavonoid glycosides along with phenolic compounds. Since many flavonoids have remarkable anti inflammatory activity the present work aims at evaluating the anti inflammatory activity of *Putranjiva roxburghii* by HRBC membrane stabilization. The ethanolic extract of the leaf was analysed for antioxidant activity by DPPH method at different concentrations. Throughout the studies leaf extract showed potent antioxidant activity. The antioxidant activity was found to be concentration dependent and may be attributed to the presence of high flavonoid content in the leaves of *Putranjiva roxburghii*

**Key Words:** Anti oxidant activity, Anti-inflammatory, *Putranjiva roxburghii*, HRBC Membrane stabilization, DPPH

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## INTRODUCTION

Plant based medicines have been in use against various diseases and disorders since time immemorial. *Putranjiva roxburghii* (Euphorbiaceae) is an important drug mentioned in the traditional medicinal texts. The plant is also known as Jivanputra in Sanskrit. Recent studies have shown that the plant possess significant febrifuge and sterility activity. The leaves of the plant were reported with important class of constituents like glycosides, steroids, alkaloids and carbohydrates<sup>9</sup>. Presence of these compounds in the leaves indicates that it can be used for treating various diseases because the therapeutic properties of many plant species are due to the presence of these class of compounds.



**Figure 1** *Putranjiva roxburghii*



**Figure 2** Leaves of *Putranjiva roxburghii*

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, cell migration, tissue breakdown and repair<sup>8</sup>. It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations<sup>3</sup>.

Antioxidants are a type of complex compounds found in our diet that act as a protective shield for our body against certain disastrous diseases such as arterial and cardiac diseases, arthritis, cataracts and also premature ageing along with several chronic diseases. The recent research on free radicals promises a revolutionary improvement in the health and life style of humans. The

human body has an elaborate antioxidant defense system. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken. After donating an electron an antioxidant becomes a free radical. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive<sup>11</sup>.

## MATERIALS AND METHOD

### Plant material

The Fresh leaves of *Putranjiva roxburghii* were collected from Hanumanthapura village of Tumkur District of Karnataka state in the month of January 2013. The plant material was identified and a voucher specimen (PUT-ROX.L) was deposited in the herbarium of the Department of Pharmacognosy of Academy of Pharmaceutical Sciences, Pariyaram, Kannur, Kerala.

### Preparation of extracts

The leaves were dried under shade and powdered. The powder was transferred to soxhlet extractor and subjected to extraction with ethanol. After extraction, the solvent was distilled off and the extract was concentrated on water bath to a dry residue and kept in a desiccator. For phytochemical screening, the leaf powder was extracted with various solvents at 50-70<sup>0</sup>C for two hours each. The different active ingredients in the leaves were screened by performing specific chemical tests using appropriate extracts<sup>2</sup>.

### Antioxidant activity

#### Reduction of 1, 1- Diphenyl- 2- Picryl Hydrazyl (DPPH) free radical

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1- diphenyl-2-picryl hydrazine. The ability to scavenge the free radical, DPPH was measured in the absorbance at 517 nm<sup>10</sup>.

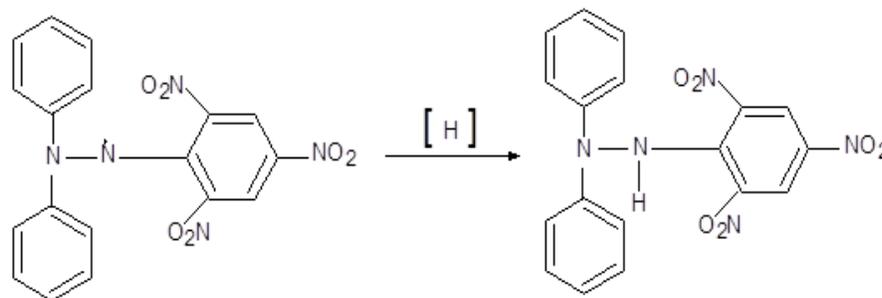


Figure 3 Reduction of DPPH free radical.

To the 1ml of various concentrations of methanolic extract in a test tube, 1ml of solution of DPPH 0.1 mM (0.39 mg in 10ml methanol) was added to the test tube. An equal amount of ethanol and DPPH were added to the control. Ascorbic acid was used as the standard for comparison. After 20 minutes incubation in the dark, absorbance was recorded at 517 nm. Experiment was performed in triplicate.

### Anti-inflammatory activity

The HRBC membrane stabilization has been used as a method to study the anti inflammatory activity<sup>1</sup>. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of sterilized Alsever solution The blood was centrifuged at 4000 rpm and packed cells were washed with isosaline and a 10 % v/v suspension was made with isosaline. The assay mixture contains the drug at various concentration ,1 ml phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Indomethacine was used as the reference drug. Instead of hyposaline 2 ml of distilled water was used in the control. All the assay mixtures were incubated at 37°C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100 %. The percentage of HRBC membrane stabilization or protection was calculated by using the formula,

$$\text{Percentage Protection} = 100 - (\text{OD sample} / \text{OD Control}) \times 100$$

\*OD=Optical Density

## RESULTS AND DISCUSSION

### Phytochemical Screening

**Table 1 Phytochemical screening of ethanolic extract of *Putranjiva roxburghii***

Sl No	Phytoconstituents	Ethanolic Extract of the Leaves
1	Cardiac glycosides	+
2	Flavonoid glycosides	+
3	Saponin glycosides	+
4	Steroids	+
5	Triterpenoids	+
6	Carbohydrates	+
7	Phenolic compounds	+
8	Alkaloids	+

+ indicates the presence of constituent in the ethanolic extract

### Antioxidant activity

The DPPH system is a stable radical generating procedure. DPPH is a potent scavenger for any other radicals, due to the easiness in following the procedure – violet colour of DPPH faints into the yellow colour of its reduced congener, with a high shift in the visible (from 520 nm to

330nm) spectra.

**Table 2 Effect of alcoholic extract of *Putranjiva roxburghii* on DPPH scavenging**

Sl.No.	Conc. µg/ml	Alcoholic extract		Ascorbic acid	
		Abs	% Sca.	Abs	% Sca.
1	5	0.857	3.57	0.879	8.40
2	10	0.867	7.41	0.678	12.49
3	15	0.765	15.45	0.610	38.58
4	25	0.543	39.44	0.457	54.87
5	50	0.517	47.45	0.019	89.72
6	100	0.457	66.89	0.003	94.30
7	250	0.339	70.45	0.002	98.55
8	500	0.245	72.56	0.002	98.69
9	1000	0.177	77.15	0.001	99.90
10	Control	0.762		0.799	

### Anti-inflammatory activity

The ethanolic extract of the leaves of *Putranjiva roxburghii* were subjected to erythrocyte (RBC) membrane stabilization induced haemolysis by hypotonic solution. The erythrocyte membrane resembles to lysosomal membran<sup>4</sup>. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane<sup>5</sup>. It was observed from the table 3 that the ethanolic extract shows significant anti inflammatory activity at the concentration of 500 mg/ml which is comparable to the standard drug Indomethacin. The anti inflammatory activity of the extract was concentration dependent, with the increasing concentration the activity was also increased. The phytochemical screening of the extract revealed the presence of flavonoids, phenolic compounds, steroids etc. The anti-inflammatory effect of the ethanolic extract may be due to the presence of flavonoids and steroids. Flavonoids and steroids show remarkable anti-inflammatory activity by inhibiting the cyclooxygenase and lipoxygenase systems<sup>6,7</sup>.

**Table 3 Anti inflammatory activity of the leaves of *Putranjiva roxburghii* at various concentrations**

Sl No	Concentration mg/ml	Anti-inflammatory activity		
		Ethanolic <i>Putranjiva roxburghii</i>	Leaf Extract of <i>Putranjiva roxburghii</i>	Indomethacin (Standard)
1	Control	-----		-----
2	100	65.2± 0.05		75.33± 0.01
3	200	69.4 ± 0.02		81.56 ± 0.03
4	300	74.6 ± 0.04		86.11 ± 0.08
5	400	78.1 ± 0.01		94.25 ± 0.05
6	500	84.2± 0.04		97.24± 0.06

(Values are expressed as SEM of 3 readings)

## CONCLUSION

The study reveals that, the ethanolic extract prepared from the leaves of *Putranjiva roxburghii* contains high amounts of phenolic compounds and flavonoid glycosides. The flavonoids are well documented to be having strong antioxidant activity and antiinflammatory activity. The ethanolic extract of the leaves exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane. From the above study it was concluded that the ethanolic extract of *Putranjiva roxburghii* has significant membrane stabilization property and it was comparable to the standard drug Indomethacin.

## REFERENCES

1. Gandhidasan R, Thamaraichelvan.A, Baburaj.S. Anti inflammatory action of *Lannea coromandelica* by HRBC membrane stabilization. *Fitoterapia*.1991; Voll LXII; No1:81-83.
2. Harborne J B. *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*1973; Cambridge University Press, Cambridge, UK.
3. Umopathy E, Ndebia EJ , Meeme A, Adam B, Menziura P, Nkeh-Chungag BN and Iputo JE. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *J Medicinal Plant Res* .2010;4 (5):789-795.
4. Omale J, Okafor PN .Comparative antioxidant capacity and cytotoxicity of the leaf and stem of *Cissus multistriata*. *African J Biotechnology*. 2008;7(17):3129-3133.
5. Rajendran Vadivu, Lakshmi KS. In vitro and in vivo anti inflammatory activity of leaves of *Symplocos cochinchinensis* (Lour) Moore ssp *Laurina*. *Bangladesh J Pharmacol*.2008; 3:121-124.
6. Robert J, Nijveldt, Elsvan Nood, Danny EC, Van Hoorn. Flavonoids: A Review of Probable Mechanisms of Action and Potential Applications. *Am. J. Clin. Nut*.2001; Vol. 74:418-425.
7. Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as Nutraceuticals”, A Review, *Tropical J Pharma Res* 2008;7(3):1089-1099.
8. Vane JR, Botting RM .New insights into the mode of action of anti-inflammatory drugs. *Inflammation Res* 1995; 44 (1):1-10.

9. Libani Rajan K et al .Pharmacognostic and Phytochemical evaluation of leaves of *Putranjiva roxburghii* , International journal of comprehensive pharmacy.2011;(11) 2:1-3.
10. Sreejayan N, Rao M. N. A. Free radical scavenging activity of curcuminoids. Drug Research. 1996; 46: 169.
11. Science Tech Entrepreneur. Antioxidants in Medicinal and Aromatic Plants. 2007; 1-10.

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