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Screening and Evaluation of Bioactivity of Methanolic Extract of Leaf of *Putranjiva roxburghii* Wall. (*Putranjivaceae*)

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Abstract
The medicinal tree species, *Putranjiva roxburghii* Wall. is traditionally used in treatment of ailments and has a prominent role in Ayurvedic and Unani medications. The seeds (putrajeevak beej) and leaves are reported to have anti sterility and hypoglycemic potential. The present study is aimed at evaluation of bioactivity of the methanolic extract of leaves of *P. roxburghii* Wall. using various *in vitro* assays. The antioxidant capacity was screened by DPPH and ferric reducing power assays. Both assays indicated strong antioxidant potential of the extract in comparison to ascorbic acid and 91.33% scavenging of DPPH was evident. For antimicrobial screening, the extract was challenged for its efficacy against *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*, over a concentration range – 250 – 1000 mg/ml and the diameter of inhibition zone (IZ) in culture plates were measured. *A. niger* was monitored as the most sensitive strain showing 16 mm IZ. The cytotoxic effect of the extract was determined by MTT assay using L929 fibroblast cells for 6.25 – 100 µg/ml concentration and LC_{50} value was recorded as 211.776 µg/ml. Anti-inflammatory property of the extract was assayed using RAW 264.7 cell lines by analyzing the ability to inhibit cyclooxygenase (COX), 5-lipoxygenase (LOX), myeloperoxidase (MPO) and by estimating the reduction in levels of cellular nitrites and inducible Nitric Oxide Synthase (iNOS), over a concentration range 25 – 100 µg/ml. The screening tests suggested that the extract at 100 µg/ml has good anti-inflammatory activity in relation to the standard drug- Diclofenac sodium. The results of assays on bioactivity concluded that the leaf extract has antioxidant, antimicrobial, anti-inflammatory properties and supported validly its traditional use as medicine.

Keywords
Putranjiva roxburghii Wall., antioxidant, anti-inflammatory, cytotoxicity, MTT assay, COX, LOX, MPO, iNOS.

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INTRODUCTION
The traditional treatment systems are based on therapeutic potential of natural bioactive compounds present in medicinal plants. Plant extracts are rich source of a variety of secondary metabolites such as alkaloids, flavonoids, phenolic

compounds, glycosides, terpenes and terpenoids. These compounds have been investigated for their antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic properties. Flavonoids, phenolics and saponins present in plant extracts were attributed as agents with antioxidant and anti-inflammatory potential [1-5]. The plant *Putranjiva roxburghii* Wall is widely used as a traditional drug. Leaves and fruits are used as medicine for rheumatism [6]. The leaf extracts and bio-oil extracted from seeds are mostly utilized in Ayurveda, Herbal and Unani medications [7]. The paste of the leaf is applied to treat elephentiasis. Chemical investigation of the leaves of *P. roxburghii* Wall has resulted in the isolation of two new triterpenoids, putralone, a novel 10 alpha-hydroxy-25-nor D: A friedo-olean-9 (11) – en - 3 one and 3 beta-acetoxy-cycloart-24-en-23-one, along with a rare hopanoid, adian-5-en-3beta, 29-diol [8]. *P. roxburghii* Wall has been claimed to possess antidiabetic properties by many investigators. The ethanol extracts of leaves of *P. roxburghii* Wall has significant antihyperglycemic effect in an experimental model of diabetes mellitus [9]. In Thai folklore medicine, *P. roxburghii* Wall leaves and fruits have been traditionally used for the treatment of fever, muscle sprain, arthralgia, and rheumatism and the whole plant of *P. roxburghii* Wall has also been used for the treatment of fever and haemorrhoids [10]. The leaves are used traditionally for the treatments of phlegm, skin ailments and also in curing rheumatism [11].

The species - *P. roxburghii* Wall is distributed in Indo-Malasia, India, Bangladesh, Indo-china, Java, New Guinea, Myanmar, Sri Lanka and Thailand. It is a tree member of Putranjivaceae family, a moderate-sized, evergreen tree, growing up to 12–15 m in height. It has pendant branches and dark grey bark having horizontal lenticels. Leaves are simple, alternately arranged, dark green, elliptic-oblong, distantly serrated. Female flowers 1-3 in the axil of leaf, male flowers are with short stalks, in rounded axillary clusters, fruits ellipsoid or rounded drupes; seed normally one, stone pointed, rugose, very hard [12] . The traditional uses of leaves indicate that it could be a natural source of antioxidants and therefore an attempt is made to evaluate the antioxidant, anti-inflammatory, antimicrobial and anticancerous potential of the methanolic extracts using various *in vitro* assays.

MATERIALS AND METHODS

Preparation of Leaf extract: Dried powder of leaf (10 g) was suspended in 50ml of 80 % (v/v) methanol, placed in a water bath at a temperature of 40^{0}C for 10 hours and then in a gyratory shaker at 120 rpm for 48 hours. The extract was filtered and separated using Whatman No.1 filter paper, evaporated and dried to constant weight. The final residue was stored in a refrigerator at 4^{0}C for further use.

Analysis of Antioxidant Activity: The ability of methanolic extract to scavenge DPPH and the ferric reducing power were employed as assays to evaluate the antioxidant potential against the standard ascorbic acid.

DPPH Scavenging test: The extract at concentration ranges 10 – 100 µg/ml was screened by treating with 2 ml of 0.004% (w/v) DPPH in methanol [13]. After incubation of the reaction mixture under darkness for 10 min, the optical density was recorded at 517 nm against the blank. The control used was a mix of 2 ml of DPPH and 1ml of methanol. The assay was carried out in triplicates. The decrease in optical density of DPPH on the addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the 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.

Ferric Reducing Power Assay

Ferric reducing power of the leaf extract was analyzed for a concentration range of 10-100 µg/ml, using ascorbic acid as standard [14].

Test sample (1 ml) of extract in different concentrations (10 –100 µg) were mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The reaction mixtures were incubated at 50^{0}C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 min at 5000 rpm. To 1 ml supernatant was added 1 ml of deionised water and 200 µl of 0.1% $FeCl_3$. The blank was prepared with the same manner except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A700 after blank subtraction.

ANALYSIS OF ANTIMICROBIAL ACTIVITY

Microbial Strains: Both antibacterial and antifungal activity were evaluated using 2 bacterial (*Escherichia coli* and *Staphylococcus aureus*) and 2 fungal strains (*Aspergillus niger* and *Candida albicans*)

Culture Media 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- PDA (for Fungi) at 4^0C. For antimicrobial analysis 48 h old cultures of bacterial and fungal strains were raised in sterilized nutrient agar broth and potato dextrose broth respectively. For petriplate culture and assay, 20 ml media -nutrient agar and PDA – were used and inoculated with the respective strains. Determination of antimicrobial potential of the extracts at concentrations of 250, 500 and 1000 µg/ml was carried out using the agar well diffusion method [15]. For comparing the efficacy of extract, the antibiotic streptomycin and antimycotic clotrimazole (each 100 µg/ml) were used as positive controls. Negative control employed was methanol.

Extract samples at different concentrations were loaded in wells of 10 mm diameter and the plates were incubated for 24 h at 37°C. The antimicrobial activity was recorded by measuring the diameter of the inhibition zone formed around the well [16]. The experiment was carried out in the triplicates and the mean values were reported for the final consideration.

***IN-VITRO* CYTOTOXIC EFFECT DETERMINATION BY MTT ASSAY**

L929 - Fibroblast cells procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's Modified Eagles Medium-DMEM (Sigma Aldrich, USA) were employed. The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and an antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). The viability of cells was evaluated by direct observation of cells by inverted phase contrast microscope and followed by MTT assay method.

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO_2 incubator. After 24 hours growth, 100 µl samples in 5% DMEM with concentrations (µg /ml) in ranges - 6.25, 12.5, 25, 50 and 100 were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO_2 incubator. Non treated control cells were also maintained.

Cytotoxicity Assay by Direct Microscopic Observation

The entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation was recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method

Stock of MTT – 2, 5-diphenyl tetrazolium bromide (Sigma, M-5655) was prepared in phosphate buffer saline (5 mg/ml) and sterilized by filter sterilization. The cells were treated with different concentrations of extract and incubated for 24 h. 30 µl of MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO_2 incubator for 4 hours. MTT was removed by washing with PBS and 200 µL DMSO and kept at RT for 30 min. The absorbance values were measured by using microplate reader at a wavelength of 540 nm [17].

The percentage of growth inhibition was calculated using the formula:

$$\text{\% of viability} = \frac{\text{Mean OD Samples x 100}}{\text{Mean OD of Control group}}$$

ANTI- INFLAMMATORY ASSAYS

RAW 264.7 cells procured from NCCS, Pune, India and maintained in DMEM were used. The cell line was cultured under the same way as for L929 cells. The cells were grown to 60% confluency followed by activation with 1 µL lipopolysaccharide (LPS: 1µg/mL). LPS stimulated RAW cells were exposed with different concentration (25,50, 100 µg/mL) of the leaf extract and Diclofenac sodium, a standard anti-inflammatory drug in varying concentration corresponding to the sample was added and incubated for 24 h. After incubation, the anti-inflammatory assays were performed using the cell lysate.

Cyclooxygenase (COX) Activity

The COX activity was assayed by the method of Walker and Gierse, 2002 [18]. 100 µl cell lysate was incubated with Tris-HCl buffer (pH 8), glutathione 5 mM/L and haemoglobin 5 mM/L for 1 minute at 25°C. The reaction was initiated by the addition of arachidonic acid 200 mM/L and terminated after 20 minutes incubation at 37°C, by the addition 200µL of 10% trichloroacetic acid in 1 N hydrochloric acid. After the centrifugal separation and the addition of 200µL of 1% thiobarbiturate, the tubes were boiled for 20 minutes. After cooling, the tubes were centrifuged for three minutes. COX activity was determined by reading absorbance at 632 nm.

Percentage inhibition of the enzyme =
(Absorbance of control-Absorbance of the test)/Absorbance of control) × 100

Lipoxygenase (LOX) Activity

The determination of LOX activity was done as per Axelrod *et al* [19]. The reaction mixture (2 mL final volume) contained Tris-HCl buffer (pH 7.4), 50 µL of cell lysate, and sodium linoleate (200 µL). The LOX activity was monitored as an increase of absorbance at 234 nm, which reflects the formation of 5-hydroxyeicosatetraenoic acid.

Percentage of the enzyme inhibition =
(Absorbance of control-Absorbance of test)/Absorbance of control) × 100

Myeloperoxidase (MPO) Activity

Cell lysate was homogenized in a solution containing 50 mM potassium phosphate buffer and 0.57% hexadecyltrimethyl ammonium bromide (HTAB) the samples were centrifuged at 2000 g for 30 minutes at 4°C, and the supernatant was assayed for MPO activity [20]. MPO in the sample was activated by the addition of 50 mM phosphate buffer (pH 6) containing 1.67 mg/mL guaiacol and 0.0005% H_2O. The change in absorbance at 460 nm was measured. MPO activity was presented as units per mL of cell lysate. One unit of MPO activity was defined as that degrading 1 µM of peroxide per minute at 25°C.

Enzyme units for MPO were determined using the formula.
U= (OD x 4 x Vt x dilution factor)/ L x €460 x t x Vs

'OD' is optical density, 'Vt' is total volume in ml, 'L' is light path in cm, '€460' is extinction coefficient of tetraguaiacol, 't' is the time of measurement in minutes and 'Vs' is sample volume in ml.

Inducible Nitric Oxide Synthase (iNOS)

Nitric oxide synthase was determined by the method described by Salter *et.al* [21]. Cell lysate was homogenized in 2ml of HEPES buffer. The assay system contained substrate 0.1ml L-Arginine, 0.1ml manganese chloride, 0.1ml 30µg dithiothreitol (DTT), 0.1ml NADPH, 0.1ml tetrahydropterin, 0.1 ml oxygenated haemoglobin and 0.1ml enzyme (sample). Increase in absorbance was recorded at 401nm.

Estimation of Cellular Nitrite Levels

The level of nitrite level was estimated by the method of Lepoivre *et al*. (1998) [22]) To 0.5 mL of cell lysate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein-free supernatant was used for the estimation of nitrite levels. To 200 µL of the supernatant, 30 µL of 10% NaOH was added, followed by 300 µL of Tris-HCl buffer and mixed well. To this, 530 µL of Griess reagent was added and incubated in the dark for 10–15 minutes, and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

RESULTS AND DISCUSSION

Antioxidant Activity

The DPPH antioxidant activity of methanolic extract of the leaf was determined for the concentration range of 10 – 100 µg/ml, as more than 90% inhibition was attained (Table 1) the IC_{50} of the leaf is recorded as 49.96 µg/ml.

DPPH is a stable free radical and it can accept an electron or hydrogen radical to become a stable diamagnetic molecule [23]. 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 colourless product- 2,2-diphenyl-1-picryl hydrazine.

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.

Ferric Reducing Power Assay

The leaf extract of *P.roxburghii* Wall indicated concentration-dependent reducing power and the assessment 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.

Antimicrobial screening

The observation on ability of leaf extract to resist microbial growth measured in terms of diameter of inhibition zone (IZ) is depicted in Table 3. The antimicrobial potential of leaf extract exhibited a linear increase with increase in extract concentration. Among the strains compared, *Aspergillus niger* was monitored as most sensitive than other strains while *Escherichia coli* was detected as moderately resistant. The Gram positive *Staphylococcos aureus* exhibited more growth inhibition than the Gram negative *E. coli*. The sensitivity of *Candida albicans* in comparison to *A. niger* was found to be lesser with IZ ranging from 10 to 13 mm.

The antimicrobial activity of plant extracts could be explained as 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. The microbial strains respond differently to the extract as there are inter strain variations in cell metabolism and cell wall compositions and permeability. The ability of leaf extract at 1000 µg/ml to resist *A. niger* has been monitored as comparable to the standard antimycotic drug clotrimazole.

MTT Assay for evaluation of cytotoxicity

The effects of extract of leaf of *P. roxburghii* Wall on cell line were photographed (Fig 1) and the reduction in viability of the cell line by methanolic extract of the leaves of *P. roxburghii* and control are tabulated in Table 4.

A key factor about plant based bioactive compounds is that these compounds may exhibit toxicity to cells. In this study cytotoxic effect of methanol extracts of leaf at concentration ranging from 6.25 – 100 µg/mL was analyzed on L929 fibroblast cell lines by using MTT assay. As depicted in Fig 1, the morphology of cells was altered from its normal shape of fibroblast to oval shape, because of the toxic effect and suggested low toxic effect on cell line at lower concentrations while at high concentrations the extract are more toxic.

Methanolic leaf extract showed cytotoxic property against L929 Fibroblast cell line of mouse with LC_{50} value 211.776 µg/mL (Calculated using ED50 PLUS V1.0 Software). This result is comparable with an earlier report by Raghavendra *et al* [24] which suggest that the methanolic extract of seed of *Putranjiva roxburghii* Wall has cytotoxic effect in brine shrimp lethality test with LC_{50} 427.74 µg/ mL. In our study found that the LC_{50} of the leaf extract is higher (211.776µg/mL) than seed even though the methods are different.

Anti-inflammatory assays

The observations on anti-inflammatory activity of the leaf extract of *P. roxburghii* Wall and standard drug-Diclofenac sodium in comparison with control, estimated on basis of percentage inhibition of COX and 5-LOX enzymes, reduction in Myeloperoxidase (MPO) activity, Inducible Nitric Oxide Synthase and reduction in cellular nitrite level are tabulated in Tables 5 and 6 respectively. The leaf extract could cause moderate level of inhibition of COX (36.92%), 5-LOX (24.53%) and MPO at 100 µg/ml concentration and resulted in reduction in cellular nitrite concentration and could induce nitric oxide synthase in a dose dependent manner.

The observations of the present study indicated that the crude methanolic extract of leaves of *Putranjiva roxburghii* Wall. possess good antioxidant capacity and moderate level of anti-inflammatory property at a concentration level of 100 µg /ml. The extract could scavenge DPPH free radicals with a percentage scavenging of 91.33, exhibited better efficacy to reduce ferric ions in comparison to standard ascorbic acid and also had good anti-inflammatory activity by inhibiting COX and 5- LOX. These properties may be due to bioactive compounds present in leaf extract that could function as free radical scavengers and strong oxidants.

Biosynthesis of inflammatory lipid mediators such as prostaglandins (PG), thromboxanes (TX), leukotrienes (LT) and hydroxyeicosatetraenoic acids is regulated by cycloxygenases and lipoxygenases through metabolism of arachidonic acid. Diseases associated with oxidative stress and inflammation can be controlled through inhibition of these enzymes [25]. These enzymes are sensitive to antioxidants and the inhibition of COX and LOX by leaf extract appears to be due to appreciable level of antioxidant activity of the extract as evident from the results of DPPH and ferric reducing assays.

The present study also suggested that the leaf extract could bring down the level of myeloperoxidase (MPO), cellular nitrites and iNOS. Myeloperoxidase (MPO) is the neutrophil enzyme which promotes oxidative stress in numerous inflammatory conditions by catalyzing the production of hypohalous acids. Also, high amounts of nitric oxide induced by pathological conditions play an important role in inflammatory joint disease in animal models [26]. The ability of leaf extract to inhibit MPO and to reduce the levels of cellular nitrite and iNOS in comparison to the standard drug – Diclofenac suggested good anti-inflammatory capacity of the crude extract. The observations concluded a positive correlation between antioxidant activity and anti-inflammatory efficacy of the extract.

CONCLUSIONS:

The observations documented in various *in vitro* assays concluded that the methanolic extract of leaf of *Putranjiva roxburghii* Wall. possess antioxidant, antimicrobial and anti-inflammatory properties and provided sufficient scientific basis to validate its medicinal properties.

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Table 1. Results of DPPH Assay of Methanolic extract of Leaf of *P. roxburghii* Wall.

Sl. No.	Ascorbic acid		Leaf Extract	
	Conc. (µg/ml)	% scavenging	Conc. (µg/ml)	% scavenging
1	10	23.42	10	11.81
2	20	38.50	20	23.13
3	30	45.70	30	36.26
4	40	51.12	40	46.75
5	50	66.54	50	56.63
6	60	71.90	60	65.90
7	70	79.35	70	74.46
8	80	88.75	80	82.17
9	90	97.64	90	87.95
10	100	99.08	100	91.33

Table 2. Results of Ferric Reducing Power Assay of Methanolic extract of Leaf of *P. roxburghii* Wall.

Conc. (µg/ml)	Absorbance	
	Ascorbic acid	Leaf extract
10	0.009	0.429
20	0.017	0.500
30	0.025	0.609
40	0.032	0.756
50	0.038	0.871
60	0.045	0.963
70	0.050	0.996
80	0.057	1.063
90	0.063	1.366
100	0.072	1.412

Table 3. Antimicrobial activity of methanolic extract of Leaf of *P. roxburghii* wall against different strains of bacteria and fungi.

Sample	*Escherichia coli*		*Staphylococcus aureus*		*Aspergillus niger*		*Candida albicans*	
	Conc. (µg/ml)	IZ (mm)	Conc. (µg/ml)	IZ (mm)	Conc. (µg/ml)	IZ (mm)	Conc. (µg/ml)	IZ (mm)
Control	Streptomycin (100µg)	25	Streptomycin (100µg)	25	Clotrimazole (100 µg)	17	Clotrimazole (100 µg)	24
Leaf extract	250	Nil	250	10	250	12	250	10
	500	10	500	11	500	15	500	11
	1000	12	1000	15	1000	16	1000	13

(Extract Concentration range 250 -1000 µg/ml, positive control streptomycin and clotrimazole -100 µg/ml)

Table 4. A cytotoxic study in L929 Fibroblast cell lines by Leaf extract of *P. roxburghii* Wall.

Sample	Concentration (µg/mL)	Average OD	Percentage Viability	LC_{50}
Control		0.722	100.00	
Methanolic Extract Of *P. roxburghii* Wall	6.25	0.708	97.99	**211.776 µg/ml**
	12.5	0.661	91.51	
	25	0.637	88.26	
	50	0.628	87.03	
	100	0.536	74.30	

Table 5. Cyclooxygenase and 5-Lipoxygenase Inhibitory Assay of Methanolic extract of Leaf of *P. roxburghii* Wall.

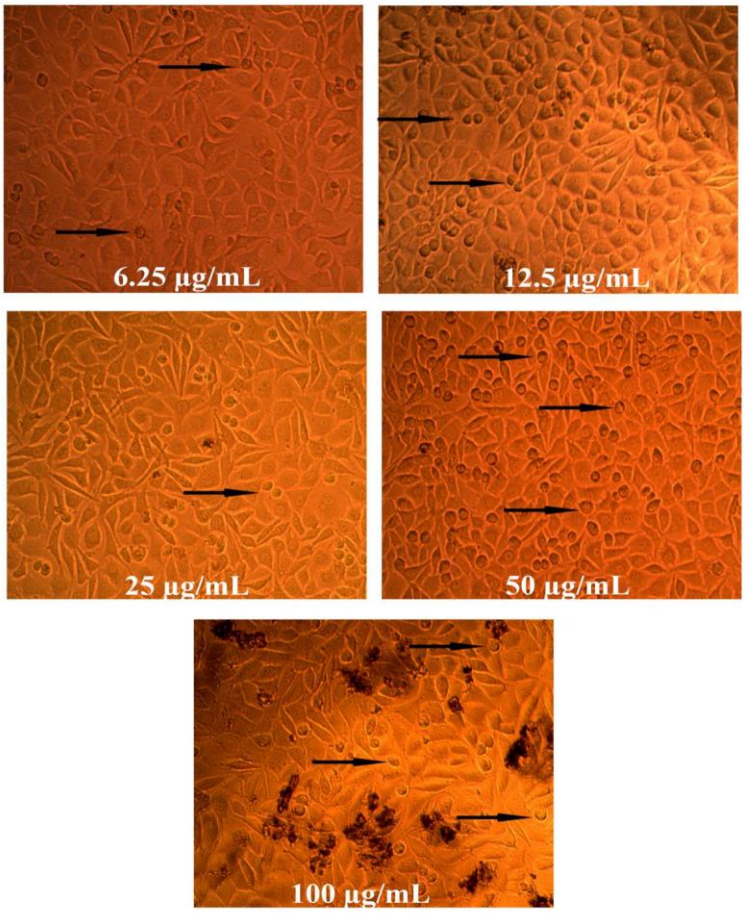
Sample	Concentration (µg /ml)	COX		LOX	
		OD at 632nm	Percentage inhibition	OD at 632nm	Percentage inhibition
Control		0.031	0	1.263	0
Standard Control (Diclofenac sodium)	25	0.089	72.022	0.071	71.937
	50	0.063	80.195	0.034	86.561
	100	0.048	84.911	0.005	98.024
Methanolic Extract of leaf (*P. roxburghii* Wall)	25	0.029	12.000	1.158	8.300
	50	0.026	20.620	1.075	14.910
	100	0.021	36.920	0.953	24.530

Table 6 - Myeloperoxidase, levels of cellular nitrite and inducible Nitric Oxide Synthase (iNOS) in RAW 264.7 cell lines by standard and Methanolic extract of Leaf of *P. roxburghii* Wall.

Sample	Conc (µg /ml)	MPO		Cellular nitrite level		iNOS level
		OD	Enzyme (u/ml)	OD	Nitrite conc	OD
Control		0.010	0.006	0.118	584.595	0.102
Standard Control (Diclofenac sodium)	25	0.004	0.001	0.147	729.596	0.029
	50	0.003	0.001	0.118	588.448	0.010
	100	0.002	0.001	0.068	339.948	0.006
Methanolic Extract of leaf (*P. roxburghii* Wall)	25	0.006	0.004	0.110	543.015	0.035
	50	0.005	0.003	0.101	501.435	0.029
	100	0.004	0.003	0.100	493.515	0.017

FIGURE 1

Cytotoxic effect of methanolic extract of leaf of *Putranjiva roxburghii* Wall. at different concentration (arrow mark indicate dead cells)



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