

Research Article

Multi-model confirmatory evaluation of anti-inflammatory, analgesic and antioxidant activities of *Putranjiva roxburghii* wall

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Abstract

Putranjiva roxburghii Wall is traditionally used by tribals for treating various health problems. In this study, Analgesic, anti-inflammatory and anti-oxidant activities of ethanolic extract of leaves and stem of *Putranjiva roxburghii* were evaluated in rats using different evaluatory models for each activity. Carrageenan-induced paw edema, croton oil-induced ear edema and anus edema models were used for evaluating anti-inflammatory activity. Analgesic activity was evaluated by acetic acid induced writhing model, Eddy's hot plate model and tail flick models. The plant extract was also evaluated for its *In-vitro* anti oxidant activity in an attempt to find its potency in providing protection against free radical induced diseases. The results indicates significant dose dependant anti inflammatory activity of ethanolic extract (250 and 500 mg/kg, p.o.) of *Putranjiva roxburghii* wall with respect to standard drug Indomethacin. The extract showed dose dependent analgesic activity in acetic acid induced writhing model but dose independent activity in case of hot plate and tail flick models as compared to standard drug Indomethacin. The *In-vitro* antioxidant activity of the ethanolic extract was also significant and comparable with ascorbic acid in both DPPH and H₂O₂ models.

Keywords: *Putranjiva roxburghii* wall, anti-inflammatory, analgesic, anti-oxidant activity, DPPH, carrageenan

1. Introduction

In the modern era, synthetic drugs have gradually replaced the natural drugs due to their cost-effectiveness, abundant availability and prompt therapeutic response. But off late, natural drugs are again gaining importance due to low incidence of side effects and untoward effects¹. Traditionally natural products are screened by testing their crude extracts obtained by different extraction processes followed by the crucial work of back tracking the active constituents from the successful extracts.²

The level of interest in the research of natural products has increased over the last few years, with particular emphasis being placed on the investigation of bioactive compounds of plant origin³. This has led to the discovery of potentially useful molecules considered worthy for clinical application⁴.

India is having a very rich resource of forests inhabited by different tribal groups. Many of these forests are not reachable for extending the modern system of medicine to the tribals and they depend mainly on these forests wealth for the treatment of many diseases⁵. If the forest wealth is properly explored by the scientific community mankind will be richly

benefited in treating many dreadful diseases⁶. *Putranjiva roxburghii* Wall commonly called in Telugu by names Kuduru, Putrajivika and in Hindi as Putijia belongs to the family Euphorbiaceae grows abundantly in forests of khammam district of Andhra Pradesh. It is widely grown in Thailand, Nepal, Bangladesh, India, Myanmar and Sri Lanka⁷.

It is used by tribals in the treatment of various health problem⁶. In Thai folklore medicine, *Putranjiva roxburghii* leaves and fruits have been traditionally used for the treatment of fever, muscle sprain, arthralgia, and rheumatism and the whole plant of *P.roxburghii* has also been used for the treatment of fever and hemorrhoids⁸.

Depending on the available traditional information regarding plant usage, the present investigation was undertaken with a view to prepare ethanolic extract of the *P.roxburghii* and to investigate and confirm the anti-inflammatory, anti-nociceptive and antioxidant effects of the ethanolic extract from the leaves and stem bark of *P.roxburghii*⁹ using different pharmacological activity models in rats.

2. Materials and Methods

2.1 Materials

2.1a Plant material

The leaves and stems of *Putranjiva roxburghii* Wall were collected from deciduous forest area of Khammam district, Andhra Pradesh, India and the sample was authenticated by Botanical Survey of India-Hyderabad, A.P, India (plant sample reference no: BSI/DRC/12-13/Tech./379.).

2.1b Drug substances and devices

Indomethacin was received as gift samples from Lupin Pharmaceuticals, Goa, India. Ascorbic acid, was received as a gift samples from Shantha Biotech, Hyderabad, India. Hydrogen peroxide and DPPH was purchased from SD Fine Chem, Mumbai, India. All other required chemicals were of AR grade.

Shimadzu 1800 U.V spectrophotometer was used for measurement of absorbance. Eddy's hot plate (Medicraft, India), Plethysmometer (Orchid scientific, India) were used.

2.1c Experimental Animals

Healthy wistar strain albino rats of either sex of approximately same average body weights were procured from National Institute of Nutrition, Hyderabad, A.P, India. Qualified personnel inspected each animal and judged them as healthy and suitable to be as subjects for experiment conducted as per Institutional Animal Ethical Committee guidelines. Each animal was assigned a distinct identification number and immediately placed in quarantine for at least 2 days for acclimatization. Each animal was observed for changes in general appearance and behaviour to ensure minimum inter subject variability. These animals were housed in clean polypropylene cages in animal house with $25 \pm 2^{\circ}\text{C}$, %RH 45-55% and supplied with water *ad libitum* and standard rodent feed obtained from Nutrilabs. A total of 186 rats were used for the complete study. The experimental animal groups are depicted in the table 1.

Table 1 : Experimental Animal Groups

Study	Grouping Of Rats						
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Toxicity study	6	6	6	6	6	6	6
Anti inflammatory activity							
Carrageenan induced paw edema	6	6	6	6	-	-	-
Croton oil induced ear edema	6	6	6	6	-	-	-
Croton oil induced anus edema	6	6	6	6	-	-	-
Analgesic activity							
Eddy's hot plate method	6	6	6	6	-	-	-
Tail flick by immersion	6	6	6	6	-	-	-
Acetic acid induced writhing	6	6	6	6	-	-	-

In toxicity study, groups I to VII indicate dose groups, in anti-inflammatory and analgesic studies there are only 4 groups where Group I indicates Control, Group II indicates Standard, Groups III and IV indicate ethanolic extract of lower and higher dose levels respectively.

2.2 Methods

The leaves and stems of *P.roxburghii* were collected and shade dried. They were powdered by using grinder to coarse powder, packed into Soxhlet column and extracted using 70% ethanol as solvent for 48 hrs¹⁰. The excess of solvent was removed using rotatory flash evaporator. The obtained crude extract was stored in air-tight container in refrigerator below 10⁰C for further studies.

2.2.1 Preliminary Phytochemical Screening¹¹

Ethanolic extract of the powder material was subjected for several chemical tests to find out the presence or absence of phyto-constituents like Carbohydrate and Glycosides (Molisch's Test), Alkaloids (Borntrager's test), Phytosterols (Liebermann Burchard Test), fixed oils (Spot Test), Gums and Mucilage, Saponins, Proteins and Free Amino Acids (Ninhydrin reagent), Phenolic Compounds and Tannins, Flavonoids (Shinoda's Test), terpenoids, Triterpenes, (Salkowski test) the results are depicted in table 2.

2.2.2 Acute oral Toxicity Study

Acute Toxicity studies were performed as per OECD-423 guidelines¹² to determine the safety doses. Acute Toxicity studies of the extract were carried out on female wistar strain albino rats. Rats were fasted over night and weight of each animal was recorded just before use. Animals were divided into seven groups. They were fed orally with the extract of wall in increasing dose levels of 500, 1000, 1500, 2000, 3000, 4000 and 5000 mg/kg body weight through oral feeding needle. The animals were observed continuously for changes in signs and symptoms and mortality. Table 3 depicts acute oral toxicity studies for ethanolic extract of *P.roxburghii* wall in rats.

2.2.3 Evaluation of Various Pharmacological Activities

2.2.3.1 Anti Inflammatory Activity

2.2.3.1a. Carrageenan Induced Paw Edema In Rats¹³

Among the different methods used for screening of anti-inflammatory activity of drugs, most commonly employed technique is based upon the ability of such drugs to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Carrageenan is most widely used phlogistic agent for these studies and was agent of choice in this study.

Procedure: The animals were fasted overnight. To ensure uniform hydration, the rats were given 5 ml of water by stomach tube, and were grouped randomly into control and treatment groups, drug and extract were given orally. Thirty minutes later, rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark.

The paw volume was measured plethysmographically immediately after injection, again after 1, 2, 3, 4, 5hrs. The increase of paw volume after 1, 2, 3, 4, 5 hrs was calculated as percentage increase and compared with the volume measured immediately after injection of the irritant for each animal. The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated.

2.2.3.1b Croton-Oil Induced Ear Edema In Rats¹⁴

This method has been developed primarily as a bioassay for the concomitant assessment of the antiphlogistic and thymolytic activities of topically applied steroids.

Procedure: The irritant is composed as follows (v/v): 1 part Croton oil, 10 parts ethanol, 20 parts pyridine, 69 parts ethyl ether. For tests in rats the following mixture was prepared (v/v): 4 parts Croton oil, 10 parts ethanol, 20 parts pyridine, 66 parts ethyl ether. The standard and the test compounds were dissolved in this solution. Six animals were used for control and each test group. The test compounds were dissolved in a concentration of 1 mg/ml, 10 times higher concentration for rats in the irritant solution. On both sides of the right ear, 0.02 ml of this solution was applied. Controls were given only the irritant solvent. The left ear remains untreated. Four hours after application the animals were sacrificed under anesthesia. Both ears are removed and discs of 8 mm diameter are punched. The discs were weighed immediately and the weight difference between the treated and untreated ear was recorded indicating the degree of inflammatory edema. The ears were removed by sharp, straight scissors 6 h after application and weighed as total.¹⁵

The anti-phlogistic effect can be determined by expressing the difference in weight of the treated ear as percentage of the weight of the contra lateral control ear. The difference of both ears and excised discs was calculated as the average values for treated and control groups.¹⁶

2.2.3.1c Croton Oil Induced Anus Edema In Rats¹⁷

Croton oil-induced anus edema was measured by modifying the method described by Nishiki *et al*¹⁸. A cotton swab soaked with the inducer, which consisted of 6% croton oil in diethyl ether (0.2 ml), was inserted into the anus of rats for 10 s. One hour after administration of croton oil, *P. roxburghii* 250, *P. roxburghii* 500, Indomethacin 15 mg/kg body weight were orally administered once daily for 3 days. On the fourth day, rats were anaesthetized, then the size of each rat's anus (mm) was measured by using a vernier callipers.

2.2.3.2 Analgesic Activity

Tests used in animals in the search of new analgesics are designed as models for the treatment of pathological pain in man, but they usually differ from the original in that the drug is given before the noxious stimulus (thermal, electrical, chemical and mechanical types of stimulus). Hence, these tests only measure the power of a drug to increase the resistance to stimulus required to elicit pain or nociceptive response¹⁹. The methodology to perform these tests using above stimuli is described below.

2.2.3.2a Hot Plate Method

In this method pain is induced with the source of heat. Rats were individually placed on a hot plate maintained at constant temperature (52⁰c) and the reaction of animals, such as paw licking or jumping response was taken as the end point. Analgesics increase this reaction time. This method is described by Eddy and Leimbach (1953)²⁰. Hot plate method has been widely used to evaluate opioid analgesics²¹. The time period (latency period) when animals were placed and until response occurs, was recorded by a stop watch. Test compounds were administered orally and latency period was recorded after 15, 30, 60, 90, 120 minutes. These values were compared with the values before administration of the test drug.²²

2.2.3.2b Tail-Flick Test Using Immersion of Tail²³

Procedure: Rats were placed into separate cages in such a way that their tail hangs out freely. The distal 5cm part of the tail was marked, immersed (15 sec cut off time) in a cup filled with warm water (temperature 55⁰C). A tail withdrawal reflex was seen within a few seconds. This reaction time was noted by stop watch. The test substances were given orally and reaction time was recorded after 15, 30, 60, 90, 120 minutes.

2.2.3.2c Acetic Acid Induced Writhing Test²⁴

Chemical test can be distinguished clearly from other models, in terms of their physical nature, duration and measurement of a behavioral score in units of time. Without doubt, these experimental models are the closest in nature to clinical pain.

Procedure: This is a model of visceral or peritoneal pain in animals, which involves the administration of algogenic agents. This test is used to detect peripheral analgesic activity of a compound. In this test, pain is induced by intra peritoneal administration of chemicals that irritate serous membranes and provoke a stereo typed behaviour known as writhing. Acetic acid (0.02%) was suspended in 1 % suspension of CMC. An aliquot of 0.2ml of this suspension was injected intraperitoneally in each animal. The animal reacted with a characteristic stretching behavior i.e., a series of constrictions that travel along the abdominal wall sometimes accompanied by turning movement of the body and extension of the hind limbs. This response was noted as writhing²⁵. In test groups, prior to acetic acid administration, test drugs were administered orally. The animals were placed individually into glass chambers and numbers of writhing were recorded for 10 mins in each animal. For scoring, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb²⁶. Formula for computing percent inhibition is as follows and the time period with greatest percent of inhibition was considered as the peak response.

$$\% \text{ inhibition} = \frac{\text{avg. writhings in the control group} - \text{writhings in the test group}}{\text{writhing in the control group}} \times 100$$

2.2.3.3 In Vitro Antioxidant Activity

2.2.3.3a Determination of 1,1- Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity²⁷

Procedure: 1,1- diphenyl-2-picrylhydrazyl (DPPH, 0.004%) solution was prepared by dissolving 4 mg of DPPH in 100 ml of ethanol and kept it overnight in dark place for the generation of DPPH[·] radical. An aliquot of 3 ml of 0.004% DPPH solution in ethanol and 0.1 ml of test sample at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of test sample.

The percentage inhibition of free radical by the test sample was calculated using the formula:

$$\text{Inhibitory ratio} = \frac{(A_o - A_1) \times 100}{A_o}$$

Where, A_o is the absorbance of control; A_1 is the absorbance with addition of test sample.

2.2.3.3b Determination of Hydrogen Peroxide Scavenging Activity²⁸

Hydrogen peroxide (0.002%v/v) solution was prepared by transferring 100 µl of hydrogen peroxide (30%) to a volumetric flask and the volume was made up to 15 ml with distilled water. From this, 1 ml of solution was transferred to a volumetric flask and was made to 100 ml with distilled water.

Phosphate buffer (100 mM, pH 7.4) solution was freshly prepared during the study. Solution A: 276 mg of NaH_2PO_4 was weighed, transferred to a volumetric flask and the volume was made up to 100 ml with distilled water. Solution B: 568 mg of Na_2HPO_4 was weighed, transferred to a volumetric flask and the volume was made up to 100 ml with distilled water. From the above solutions, 12 ml of solution A and 88 ml of solution B were mixed and pH was adjusted to 7.4. Phenol red (0.2 mg/ml) containing Peroxidase (0.1 mg/ml) solution was also freshly prepared during the free radical study. 2 mg of phenol red and 1 mg of horseradish peroxidase were dissolved in 10 ml of 100 mM phosphate buffer solution

The reaction mixture containing 100 µl of plant extract of different concentrations, 100 µl of 0.002% H_2O_2 and 0.8 ml of phosphate buffer were preincubated for 10 min at 37°C. To this reaction mixture, 1 ml of phenol red dye containing horseradish peroxidase solution was added. After 15 min 50 µl of 1 M NaOH was added and absorbance was measured at 610 nm immediately. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ ascorbic acid.²⁹ The percentage inhibition of radical by the test sample was calculated using the formula:

$$\text{Inhibitory ratio} = \frac{(A_o - A_1) \times 100}{A_o}$$

Where, A_o = absorbance of control; A_1 is the absorbance with addition of test sample.

3. Results and Discussion

3.1 Preliminary Phytochemical Screening

Presence of various phyto-constituents in the stem-leaves ethanolic extract of *P. roxburghii* was detected by respective chemical tests. The results are shown in table 2.

Table 2. Phytochemical Screening of *P. roxburghii* stem-leaves Ethanolic Extract

S. No	Phytochemical Constituent	Ethanolic Extract result	S.No	Phytochemical Constituent	Ethanolic Extract result
1	Carbohydrates	+	8	Proteins & amino acids	--
2	Alkaloids	+	9	Phenolic compounds	+
3	Phytosterols	+	10	Tannins	+
4	Fixed oils & fats	+	11	Flavonoids	+
5	Gums & mucilages	--	12	Triterpenes	+
6	Saponins	+	13	Volatile oils	--
7	Glycosides	+	14	Steroids & terpenes	+

Phytochemical analysis showed the presence of important classes of phytoconstituents like cardiac glycosides, saponin glycoside, Flavonoids, phenols, alkaloid, sterols, triterpenoids and carbohydrates. This indicates that the plant can be useful for treating different diseases because the therapeutic activity of a plant may be due to the presence of particular class of compounds.³⁰ Development of such a monograph would pave the way for isolation of phytoconstituents, therapeutic investigations and standardization of formulations of *Putranjiva roxburghii* Wall.

3.2 Results for Acute Oral Toxicity Studies

Female rats were chosen for acute toxicity testing of the plant's ethanolic extract because of their better responsive nature for toxic effects than male counter parts. The results are enumerated in table 3 below.

Table 3 : Acute Oral Toxicity Study for Plants Ethanolic Extract In Rats

Group	Dose (mg/kg)	Route	Death / total	Death%
I	500	Oral	00 /06	0
II	1000	Oral	00 /06	0
III	1500	Oral	00 /06	0
IV	2000	Oral	00 / 06	0
V	3000	Oral	00 /06	0
VI	4000	Oral	00 /06	0
VII	5000	Oral	03 /06	50

The LD₅₀ was 5g/kg body weight therefore the ED₅₀ was found to be 500mg/kg body weight. Basing on these results, the doses for the study have been selected and the experiments were carried out.

3.3 Multi-Model Anti-Inflammatory Activity

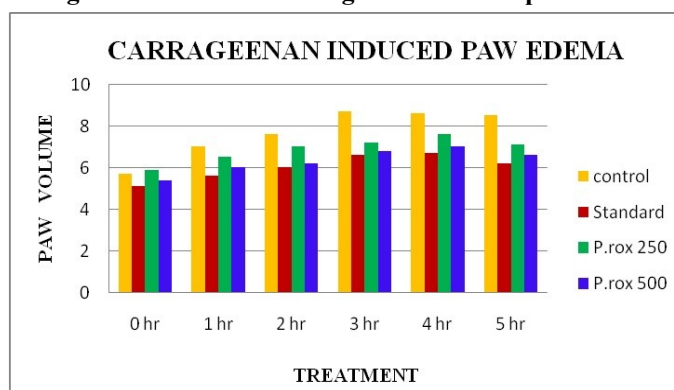
The anti inflammatory potency of *P.roxburghii* stem-leaves ethanolic extract was compared with the used standard drug i.e., Indomethacin to find the possible alternative treatment for inflammatory disease and also to validate the traditional use of the plant in thai medicine for treating inflammation and the results are enumerated in table 4 below.

Table 4 Anti Inflammatory Activity of *P. roxburghii* Ethanolic Extract

Carrageenan induced paw edema in rats								Croton oil induced edema			
Drug	Dose (mg/kg, p.o.)	PAW VOLUME (ml)						Size of anus (mm)	Ear		
		0hr	1hr	2hr	3hr	4hr	5hr		Dose mg/ ear topical	Ear swelling (ml)	% reduction in swelling
Control	-	5.7±0.3	7.0± 0.3	7.6± 0.2	8.7± 0.4	8.6 ±0.1	8.5± 0.2	45.4±1.0	-	14.2±0.44	-
Indomethacin	15	5.1± 0.2	5.6±0.3	6.0±0.2*	6.6±0.2*	6.7±0.5*	6.2±0.2*	38.2±0.7***	15	5.8±0.38**	59.1%
<i>P. roxburghii</i>	250	5.9 ±0.4	6.5± 0.1	7.0± 0.2	7.2 ±0.3	7.6± 0.4	7.1± 0.3	43.5±2.3	2.5	10.3±0.94	27.4%
<i>P. roxburghii</i>	500	5.4±0.4	6.0± 0.1	6.2± 0.1	6.8±0.2*	7.0±0.2*	6.6±0.1*	41.7±1.2***	5.0	7.2±0.54**	49.2%

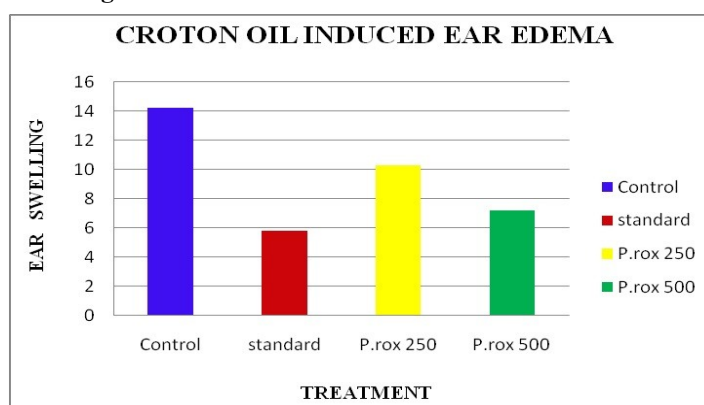
Values are expressed in mean±SEM. *P, **P, ***P <0.01 treatment groups Vs control group

Carrageenan-induced paw edema is an acute inflammatory model. It involves several mediators released in sequence for causing inflammation. An initial phase during the first 1.5 hr is caused by the release of histamine and serotonin, the second phase is mediated by bradykinin from 1.5 to 2.5 h and the third phase, in which the mediator is suspected to be prostaglandin, occurs from 2.5 to 6 h after carrageenan injection³¹. In the present study, *P. roxburghii* at the dose of 500 mg/kg significantly suppressed the paw edema induced by carrageenan in rats at the third phase, suggesting the possible primary mechanism of action of *P. roxburghii* extract may involve inhibition of prostaglandin biosynthesis. This anti inflammatory activity was dose dependant and comparable with the standard drug. Figure 1 shows comparative efficacy of different test substances in reducing paw edema in rats.

Figure 1: Results of Carrageenan induced paw edema

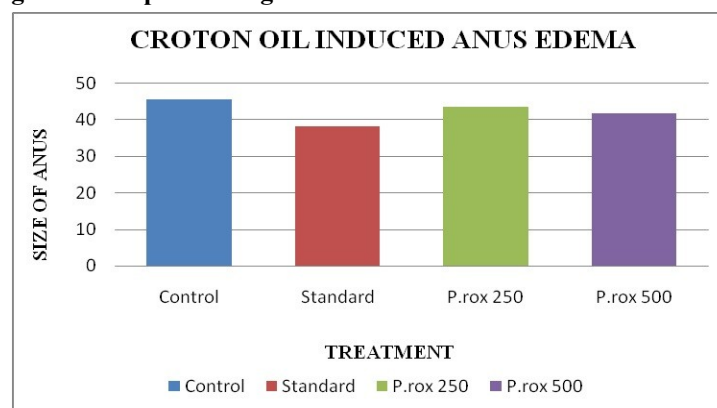
Croton oil-induced ear edema is a useful model for testing topical anti-inflammatory activity³². Topically applied *P.roxburghii* (5.0 mg/ear), as well as a reference drug Indomethacin, significantly reduced the inflammatory response caused by croton oil, since 12-O-tetra- decanoylphorbol-13-acetate (TPA), a kind of phorbol ester present in croton oil, has been reported to stimulate phospholipid-dependent protein kinase C³³ and this enzyme appears to be involved to the activation of cellular functions via protein phosphorylation³⁴. Of particular note the ethanolic extract showed effective anti-inflammatory activity when applied on the skin, which could be rationalized by cutaneous absorption of the extract. Figure 2 shows comparative efficacy of anti-inflammatory potency of different test substances in rats.

Figure 2: Results of Croton Oil Induced Ear Edema



Croton oil-induced anus edema model involves infiltration of inflammatory cells, vasodilation, and destruction of the mucosal epithelium³⁵. *P.roxburghii* 500mg/kg decreased anus swelling but needed a higher dose (>500 mg/kg) than those of other systemic models, as this model may produce very large amounts of inflammatory tissue. The ethanolic extract of *P. roxburghii* may be beneficial for the treatment of hemorrhoids which are partly involved in anus inflammation. Figure 3 shows comparative efficacy of different test substances in rats.

Figure 3: Graph showing result of Croton Oil Induced Anus Edema



Since Flavonoids or bioflavonoids are known to possess anti-inflammatory properties³⁶ and flavonoid compounds have been isolated from the *P. roxburghii* leaves^{37,38}, it can be stated that this plant has potent anti-inflammatory activity.

3.4 Multi-Model Analgesic Activity of *Proxburghii* stem-leaves Ethanolic Extract

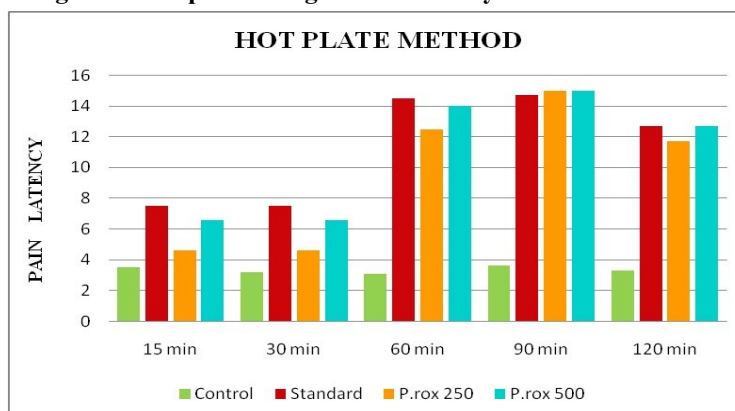
The anti nociceptive potential of *P.roxburghii* stem-leaves ethanolic extract was compared with the used standard drug in different nociceptive response stimuli testing models with the aspiration of finding the better replacement to the presently available medication in treating pain without any side effects and also to validate the use of this plant in thai folklore medicine for treating arthralgia. The results of these study models are enumerated in table 5 below.

Table 5: Multi Model Analgesic Activity of *P. roxburghii*

Animal model	Drug	Dose (mg/kg, p.o.)	Pain latency (seconds)				
			15 min.	30 min.	60 min.	90 min.	120 min.
Eddy's hot plate Method in Rats	Control	-	3.5±0.1	3.2±0.2	3.1±0.2	3.6±0.3	3.3±0.2
	Standard	15	7.5±0.2	12.5±0.1	14.5±0.1	14.7±*0.1	12.7±0.1
	<i>P.roxburghii</i>	250	4.6±0.1	7.3±0.2	12.5±0.1	15±*0.1	11.7±0.1
	<i>P.roxburghii</i>	500	6.6±0.1	11.6±0.3	14±0.3	15±*0.2	12.7±0.2
Tail flick test using Immersion of tail in rats	Control	-	3.3±0.3	3.2±0.1	2.9±0.2	3.2±0.1	2.85±0.1
	Standard	15	6.1±0.2	7.5±0.2	13±0.2	15±**0.3	14.2±0.1
	<i>P.roxburghii</i>	250	4.3±0.1	6.5±0.2	10.2±0.2	14.7±0.2**	13.2±0.2
	<i>P.roxburghii</i>	500	5.5±0.1	7.4±0.1	10.7±0.3	14.8±0.3	12.7±0.2
Acetic acid induced Writhing test in rats	Drug	Dose (mg/kg p.o)	Number of writhes		% Inhibition		
	Control	-	39.7 ± 1.3		-		
	Standard	15	7.5 ± 1.4***		81.1		
	<i>P.roxburghii</i>	250	25.7 ± 1.4***		35.2		
	<i>P.roxburghii</i>	500	16.7 ± 2.4***		57.93		

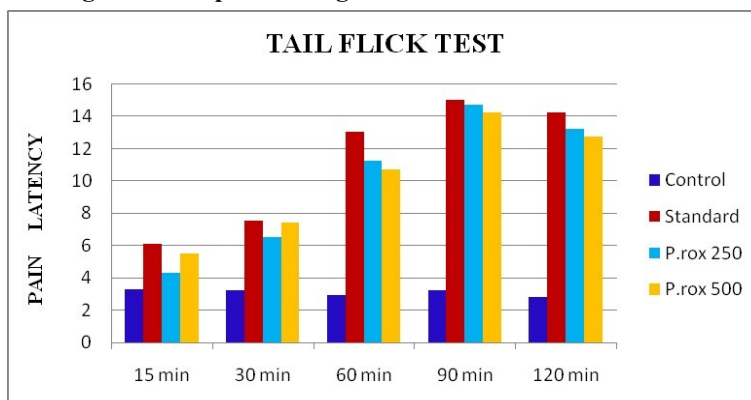
Values are expressed in mean±SEM (n=4) *P, **P, ***P <0.01 treatment groups Vs control group

In hot plate method pain is induced by thermal stimuli. This stimulus is usually known to be selective to centrally acting analgesic drugs. *P.roxburghii* has shown significant analgesic action when used in doses of 500mg/kg body weight orally at 90 mins but the effect decreases after 2 hrs. it is observed that the analgesic potency was dose dependant in acetic acid induced writhing model but not dose dependant in case of Eddy's hot plate, tail flick models shown in table 5 and figure 4. Hence it can be hypothesized that *P.roxburghii* may act by central pathway for analgesic activity.

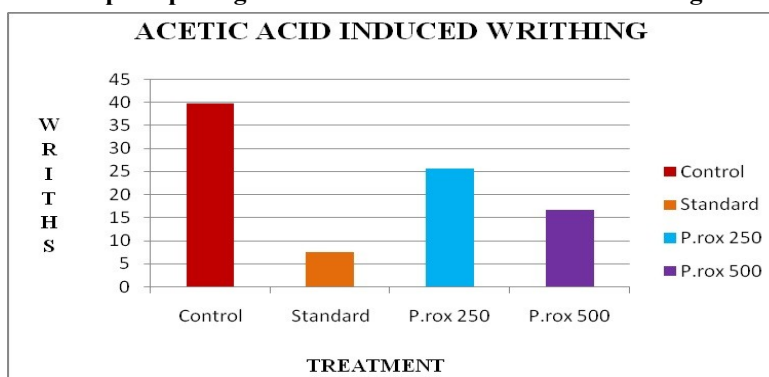
Figure 4: Graph showing result for Eddy's Hot Plate Method

Tail flick Test is the method of pain induced by thermal stimuli to screen the analgesic activity of the drug under investigation where the tail was immersed in hot water at constant temperature (52⁰c) *P.roxburghii* has shown considerable effect at dose of 500mg/kg at 90 min and the effect was gradually reducing after 2hrs (120 min) compared to the standard drug Indomethacin which has also shown significant effect.

The results indicate that the extract may have both central and peripheral mechanisms to show analgesic activity based on the results obtained from hot plate model and tail flick model respectively. Figure 5 shows comparative efficacy of different test substances in rats.

Figure 5: Graph Showing results of Tail Flick Test in Rats

In Acetic acid induced writhing model, *Proxburghii* showed significant inhibition on acetic acid-induced writhing response in rats and its potency was comparable with the reference drug Indomethacin. In comparison, the ether extract was slightly less potent than Indomethacin on nociceptive response in rats³⁹ as described by Wantana Reanmongkol *et al* 2009. These results indicated that the *Proxburghii* ethanolic extract may possess significant antinociceptive activity. Figure 6 depicts the results of antinociceptive efficacy of *Proxburghii* in comparison to the standard Indomethacin.

Figure 6: Graph depicting results of Acetic Acid Induced Writhing Test in Rats

3.5 In Vitro Antioxidant Activity of *Proxburghii* stem-leaves Ethanolic Extract

Studies reveal that the pathogenesis of many diseases like diabetes, ulcer and cancer may be due to involvement of free radicals⁴⁰. Free radical scavenging activity can be an indication of possession of potential for treating these diseases. The results of this activity *Proxburghii* ethanolic extract are tabulated in Table 6.

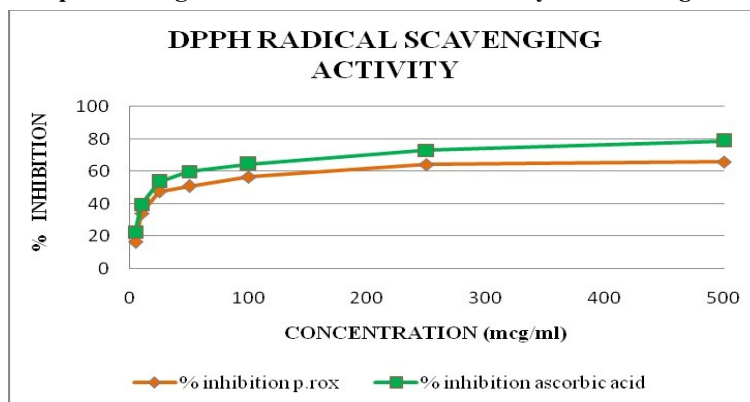
Table 6: Comparative Anti Oxidant Activity of *Proxburghii* with Ascorbic Acid

Animal Model Concentration Mcg/MI	1, 1- Diphenyl-2-Picrylhydrazyl (Dpph) Free Radical Scavenging Activity		Hydrogen Peroxide Free Radical Scavenging Activity	
	% Inhibition by Standard Ascorbic Acid	% Inhibition by <i>Proxburghii</i>	% Inhibition by Standard Ascorbic Acid	% Inhibition by <i>Proxburghii</i>
5	22.8	16.5	31.4	27.4
10	39.8	34.2	43.6	37.8
25	53.6	47.7	54.2	49.2
50	59.9	51.0	58.8	52.7
100	64.6	56.7	67.3	58.6
250	73.1	64.4	74.5	65.6
500	78.9	66.0	82.4	73.2

Putranjiva roxburghii Wall ethanolic extract was found to have significant anti oxidant activity. Moreover the antioxidant activity was found to be dose dependant.

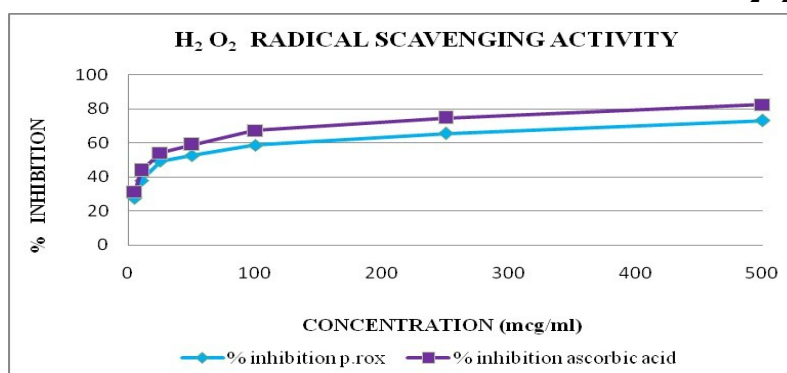
Free radical scavenging activity using DPPH (1, 1- diphenyl-2-picryl hydrazyl) is depicted in figure 7. The activity is slightly less in comparison to used standard ascorbic acid.

Figure 7: Graph showing results of Anti Oxidant Activity in *P.roxburghii* using DPPH



To consolidate the findings of antioxidant activity, the potency of *P.roxburghii* ethanolic extract was compared with antioxidant potential of Hydrogen peroxide, the results are enumerated in table 6. These results are graphically represented in Figure 8, which show that the antioxidant activity of the *P.roxburghii* ethanolic extract has significant hydrogen peroxide free radical scavenging same as its potential of scavenging DPPH free radical. But this potency was slightly less in comparison to the used standard Ascorbic acid.

Figure 8: Result of Anti Oxidant Activity in *P.roxburghii* using H₂O₂



Hence it can be inferred from the *In vitro* antioxidant study, that ethanolic extract of *P.roxburghii* has protective action against many diseases like diabetes, peptic ulcer, cancer, neuronal damage that may occur due to involvement of free radicals⁴⁰.

4. Conclusion

In the present study, the qualitative phytochemical analysis showed the presence of flavonoids and other active constituents in *P.roxburghii* wall, which may therefore be responsible for the activities of its leaves-stem ethanolic extract. Based on these results, we conclude that *P.roxburghii* wall leaves, stem ethanolic extract possesses anti-inflammatory, analgesic and anti oxidant activities in oral as well as topical models which validate the traditional use of *P. roxburghii* for the treatment of muscle sprain, inflammation, arthralgia in thai folklore medicine preparations.

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