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ANTI-INFLAMMATORY ACTIVITY OF *PONGAMIA PINNATA* HYDRO-ALCOHOLIC LEAF EXTRACT IN WISTAR ALBINO RATS

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ABSTRACT

Keywords:

Pongamia pinnata,
Antioxidant, Hydro-alcoholic,
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The *Pongamia pinnata*, commonly known as 'Karanj', has been recognized in different system of traditional medicines for the treatment of different diseases and ailments of human beings. It contains several phytoconstituents belonging to category of flavonoids and fixed oils. In this study, Antioxidant activity of the hydro-alcoholic extract of *Pongamia pinnata* (HAPP) was determined by DPPH free radical, ABTS and total reduction methods. In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. In the present study, the antioxidant potential of the plant was used to screen the anti-inflammatory activity of its hydro alcoholic extract in acute model of inflammation which was assessed in rats. Oral administration of *Pongamia pinnata* leaves (HAPP) in doses of 200 mg/kg and 400 mg/kg, exhibited significant anti-inflammatory activity in acute (carrageenan) induced hind paw edema. HAPP did not show any sign of toxicity and mortality up to a dose level of 10gm/kg, p.o. in rats. These results clearly indicate that *Pongamia pinnata* can be effectively used against free radical mediated diseases and thus indicate that HAPP possesses significant anti-inflammatory activity suggesting its potential as an anti-inflammatory agent for use in the treatment of various inflammatory diseases mediated through free radicals.

INTRODUCTION

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Although rheumatism is one of the oldest known diseases of mankind affecting the majority of population, no substantial progress has been made in achieving a permanent cure. The greatest disadvantage in presently available potent synthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation. Therefore, the screening and development of drugs for their anti-inflammatory activity is still in progress and there is much hope for finding anti-inflammatory drugs from indigenous medicinal plants. *Pongamia pinnata* (Linn) Pierre (Leguminosae, *Pongamia glabra* Vent), popularly known as 'Karanj' in Hindi, is a medium sized glabrous tree, found throughout India and further distributed eastwards, mainly in the littoral regions of South Eastern Asia and Australia (Satyavati *et al.*, 1987). In the ayurvedic literature of India, different parts of this plant have been recommended as a remedy for various ailments. The seed and seed oil of this plant have been used for treating various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago, muscular and articular rheumatism (Nadkarni, 1954). The leaves are hot, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations (Kirtikar and Basu, 1933). A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scrofulous enlargement (Chopra *et al.*, 1933; Satyavati *et al.*, 1987). While different extracts of roots and seeds (ethanol, petroleum ether, benzene extracts and others) of *P. pinnata* have been reported to have anti-inflammatory activity (Singh and Pandey, 1996; Singh *et al.*, 1996), its leaves still remain unexplored. The present study is an attempt to address this issue. The 70% hydro-alcoholic extract of *P. pinnata* leaves (HAPP) was evaluated in acute, sub acute and chronic models of inflammation in rats. In addition, the effect of the extract on gastric mucosa of rats was also studied to find out whether it has any ulcerogenic activity.



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MATERIALS AND METHODS

Plant material

The mature green leaves of *P. pinnata* were collected locally in the month of February 2012 nearby Mohuda village, Berhampur, Ganjam district of Odisha. The plant was authenticated from Department of Botany, Khalikote College, Berhampur, Odisha.

Preparation of hydro alcoholic extract

The leaves of *Pongamia pinnata* were dried for 20 days under the shade to prevent the loss of volatile oils. The shade-dried and powdered leaves were subjected to extraction with hydro- alcoholic extract by soxhlation. The hydro-alcoholic mixture was prepared by ethanol 70% and water in the ratio of 7:3. The filtrate was collected and concentrated on heating mantle to obtain a syrupy mass.

Phytochemical screening

Phytochemical screenings were performed using standard procedures. (Gupta *Vet al.*, 2011)

a) Test for phenols

To 0.5 g each of the extract, 2 ml of ferric chloride was added. A reddish brown coloration at the interface indicates the presence of phenols.

b) Test for terpenoids (Salkowski test)

To 0.5 g each of the extract, 2 ml of chloroform was added. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of terpenoids.

c) Test for flavonoids

Three methods were used to test for flavonoids. First, diluted ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract followed by addition of concentrated sulphuric acid (1 ml). A yellow coloration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of diluted ammonia solution. A yellow coloration indicates the presence of flavonoids.

d) Test for saponins

To 0.5 g of extract 5 ml of distilled water was added in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

e) Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

f) Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate 2 ml of diluted ammonia was added followed by addition of 5 ml of chloroform and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one part of the extract and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

Experimental animals

Experiments were performed using Wistar albino rats (150– 180 g) of either sex. All the animals were maintained under controlled room temperature (22.2 °C) and relative humidity (50_5%). The animals were housed in colony cages (three animals per cage) with free access to feed and water. Guidelines of Institute Animal Ethics Committee were followed while using live animals. Institute Animal Ethics Committee permission was obtained before starting experiments on animals Regd. No: - 07/IAEC/2011. All the animals were acclimatized to the laboratory environment for 6 days before the experiment. The animals were divided into four groups (three rats in each) of which Group I served as control, Group II as standard and Group III and IV as test.

Group I- rats are treated with distill water

Group II- rats were treated with standard drug ascorbic acid

Group III- rats were treated with HAPP 200mg/kg.

Group IV- rats were treated with HAPP 400mg/kg.

Acute toxicity study (oral)

The HAPP was administered orally in graded doses of 3.0, 4.5, 6.75 and 10.125 g/kg to four different groups of rats, while the control group received vehicle (2 ml/kg, p.o.) alone. All treated animals were closely observed for any abnormal or toxic manifestations and for mortality up to the end of 24 h in each group to calculate LD₅₀ by the method described by Weil (1952). Based on the results obtained from preliminary toxicity study, the doses for further pharmacological studies were fixed to be 200 and 400 mg/kg, p.o.

Evaluation of hydro-alcoholic extract:***In- vitro* evaluation DPPH radical scavenging assay**

To the Methanol solution of DPPH [di (phenyl)-(2, 4, 6-trinitrophenyl) iminoazanium] (1 mM) an equal volume of the extract dissolved in alcohol was added at various concentrations from 250 to 2000 µg/ml in a final volume of 1.0 ml. An equal amount of alcohol was added to the control. After 20 min, absorbance was recorded at 517 nm. Experiment was performed in triplicate.

ABTS radical scavenging assay

To the reaction mixture containing 0.3 ml of ABTS [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] radical, 1.7 ml phosphate buffer and 0.5 ml extract was added at various concentrations from 250 to 2000 µg/ml. Blank was carried out without drug. Absorbance was recorded at 734 nm. Experiment was performed in triplicate.

Total reduction capability

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu *et al.*, with a slight modification. Different concentrations (250–2000 µg/mL) of extract (0.5 mL) were mixed with 0.5-mL Phosphate buffer (pH 6.6) and 0.5-mL 0.1% potassium hex-cyanoferrate, followed by incubation at 50°C in water bath for 20 min. After incubation, 0.5-mL 10% TCA was added to terminate the reaction. The upper portion of the solution (1 mL) was mixed with 1 mL of distilled water and 0.1 mL 0.01% FeCl_3 solution was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against appropriate blank solution. All tests were performed three times. A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as a positive control.

***In-vivo* evaluation:**

Carrageenin-induced hind paw edema in rats.

The acute hind paw edema was produced by injecting 0.1 ml of Carrageenin (prepared as 1% suspension in sterile normal saline) locally into the plantar aponeurosis of the right hind paw of rats (Winter *et al.*, 1962). HAPP (100, 300 and 1000 mg/kg, p.o.) .HAPP was administered 1 h prior to the injection of Carrageenin. The rat pedal volume up to the ankle joint was measured using plethysmometer (UgoBasile, Italy) at 0 (just before) and 3 h after the injection of Carrageenin. Increase in the paw edema volume was considered as the difference between 0 and 3 h. Percent inhibition of edema volume between treated and control groups was calculated as follows:

$$\text{Percent inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where V_c and V_t represent mean increase in paw volume in control and treated groups, respectively. The median inhibitory dose (ID₅₀) was calculated by the method of Reed and Muench *et al.*, 1938.

Statistical analysis

The experimental results were expressed as mean \pm SEM of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and two way analysis of variance (ANOVA). All these analysis was done by Graph Pad Prism Software program (version 5). P values < 0.05 were regarded as significant (Harvey J, Paige S Met *al.*, 1998)

RESULTS

Phytochemical Screening

Table 1:- Phytochemical screening of Hydro-alcoholic extract of *Pongamia pinnata* leaves

SL NO.	PHYTOCONSTITUENTS	HYDRO-ALCOHOLIC EXTRACT
1	Alkaloid	+ ++
2	Carbohydrate	+ - -
3	Glycoside (cardiac glycoside)	+++
4	Tanins and phenolics	+ + -
5	Protein & amino acid	- - -
6	Gum and mucilage	+++
7	Flavones & flavonoids	++
8	Saponins	+++
9	Steroids & sterols	+++
10	Triterpinoids	+++

+ = presence, – = absence.

Acute toxicity study (oral)

On gross examination, all animals given HAPP at the doses of 3, 4.5, 6.75 and 10.125 g/kg, p.o. were devoid of toxic symptoms and mortality.

Anti-oxidant assay

Table 2:- Study on DPPH scavenging activity in *Pongamia pinnata* leaves

Concentration ($\mu\text{g/ml}$)	Ascorbic acid (% scavenging activity)	<i>P. pinnata</i> (% scavenging activity)
0	0	0
250	90.2 \pm 0.004	43 \pm 0.005
500	91 \pm 0.009	68.7 \pm 0.004
1000	92.4 \pm 0.005	71 \pm 0.003
2000	93 \pm 0.007	76 \pm 0.007

Values are mean \pm SEM of three separate experiments; Statistical comparison has been done by student's t- test

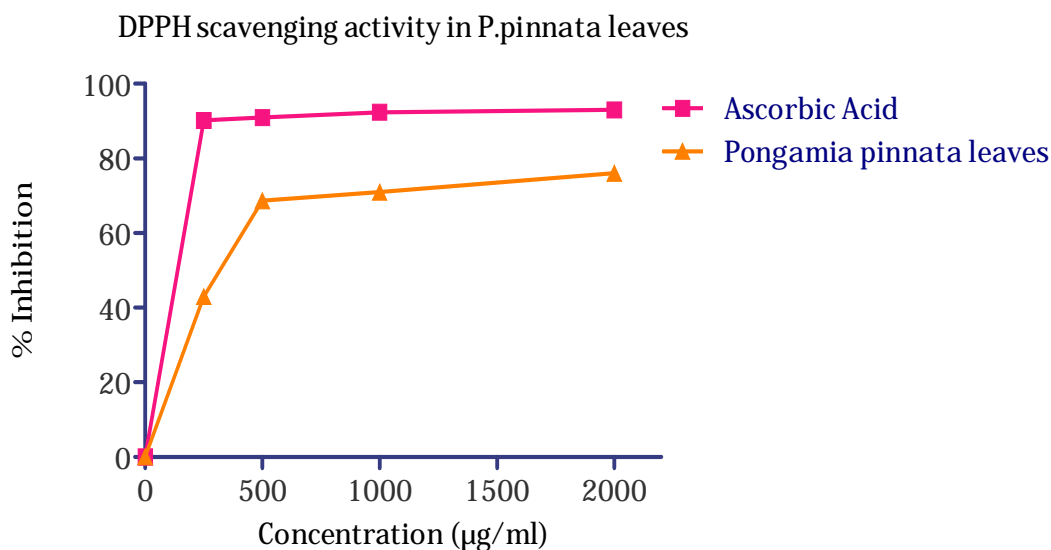


Fig 1:- Study on DPPH scavenging activity in *Pongamia pinnata* leaves at 517 nm

Table 3:- Study on ABTS scavenging activity in *Pongamia pinnata* leaves

Concentration (µg/ml)	Ascorbic acid (% scavenging activity)	<i>P. pinnata</i> (% scavenging activity)
0	0	0
250	68±0.014	62.3±0.013
500	73.4±0.016	80.2±0.015
1000	75±0.015	83.4±0.014
2000	79.2±0.017	84.5±0.014

Values are mean \pm SEM of three separate experiments; Statistical comparison has been done by student's t- test

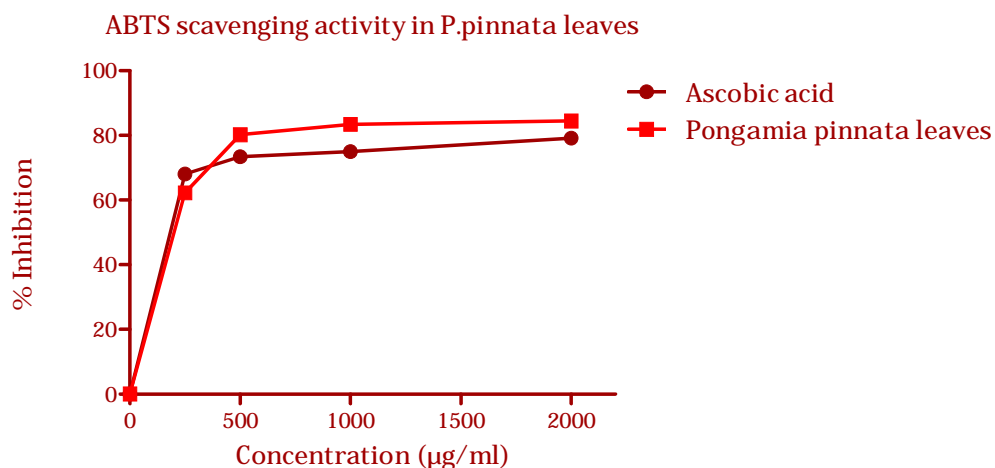
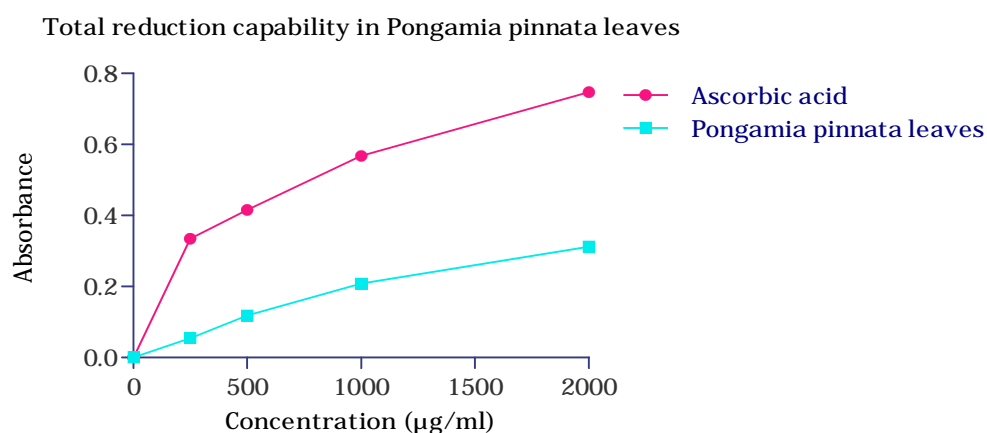


Fig 2: Study on ABTS scavenging activity in *Pongamia pinnata* leaves at 734 nm

Table 4:- Study on Total reduction capability in *Pongamia pinnata* leaves

Concentration ($\mu\text{g/ml}$)	Ascorbic acid (Absorbance)	<i>P. pinnata</i> (Absorbance)
0	0	0
250	0.335 ± 0.002	0.0545 ± 0.001
500	0.4159 ± 0.004	0.1183 ± 0.002
1000	0.5679 ± 0.003	0.2082 ± 0.001
2000	0.7404 ± 0.003	0.3120 ± 0.001

Values are mean \pm SEM of three separate experiments; Statistical comparison has been done by student's t- test

**Fig 3: Study on Total reduction capability in *Pongamia pinnata* leaves 700 nm*****Carrageenin-induced hind paw edema***

The mean increase in paw edema volume was about 0.86 ± 0.13 ml in the vehicle-treated control rats. HAPP (200 and 400 mg/kg, p.o.) significantly ($P < 0.01$) reduced the mean paw edema volume at 3 h after Carrageenin injection. The HAPP (200 and 400 mg/kg, p.o.) exhibited anti-inflammatory activity in a dose-dependent manner with the percent inhibition of paw edema of 27.9, 55.81 and 60.47 respectively, as compared with the control group.

Table:-5 Effect of *P. pinnata* hydro-alcoholic leaf extract on Carrageenan induced hind paw edema

Drugs	Dose (mg/kg)	Carrageenan induced hind paw edema (ml; Mean \pm SEM)	Percent inhibition of edema volume
Control	Distilled Water 0.5 ml	0.860 ± 0.13	0
Standard	Diclofenac Sodium 0.5	0.62 ± 0.11	27.90
HAPP	200	$0.380 \pm 0.02^{**}$	55.81
HAPP	400	$0.34 \pm 0.05^{**}$	60.47

n= 6 in each group; *, P 0.05; **, P 0.01 vs. control group

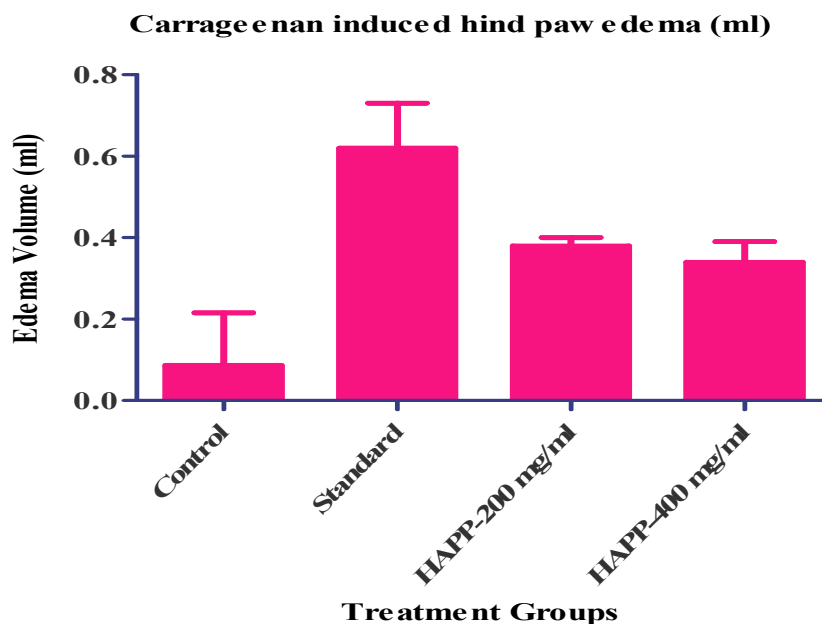


Fig:-5 Effect of HAPP on Carrageenan induced hind paw edema

DISCUSSION

The present study demonstrates the potent anti-inflammatory activity of the hydro-alcoholic extract of HAPP in different models of inflammation-acute exudative (Carrageenan-induced rat paw edema) indicating the possibility of developing HAPP as the cheaper, safer and potent anti-inflammatory therapeutic agent. Although, different parts of *P. pinnata* have been used in the traditional systems of medicine for treating various ailments including febrile and inflammatory disorders and the seeds and roots of this plant have already been reported to possess significant anti-inflammatory and analgesic activities (Singh *et al.*, 1996, 1997), there was no scientific evidence as regards to the anti-inflammatory activity of the leaf models of inflammation, upon challenge by phlogistic stimuli, HAPP showed significant anti-inflammatory activity. The edema and inflammation induced by Carrageenan is shown to be mediated by histamine and 5-HT during first 1 h, after which increased vascular permeability is maintained by the release of kinins up to 2.30 h and from 2.30 to 6 h, the mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site. In conclusion, the present study clearly showed that the hydro-alcoholic extract of *Pongamia pinnata* leaves possessed good anti-inflammatory activity and also scientifically validated the use of this plant for treating inflammatory disorders.

CONCLUSION

The present study clearly indicates that *Pongamia pinnata* can be effectively used against free radical mediated diseases. The results obtained in the study indicate that HAPP possesses significant anti-inflammatory activity suggesting its potential as an anti-inflammatory agent for use in the treatment of various inflammatory diseases mediated through free radicals

REFERENCES

1. Brownlee, G., "Effect of doxycortone and ascorbic acid on formaldehyde-induced arthritis in normal and adrenalectomized rats". Lancet 1950; 1, 157–15
2. Chatterjee A, Prakash SC. "The Treatise on Indian Medicinal Plants". Vol II. Publications and Information Directorate, New Delhi: 1995; 110p
3. Chopra, R.N., Chopra, I.C., Handa, K.L., Kapur, L.D., "Indigenous Drugs of India." Academic Publishers, Calcutta. 1933; p. 388.
4. Di Rosa, M., Giroud, J.P., Willoughby, D.A., "Studies on the mediators of acute inflammatory response induced in rats in different sites of carrageenin and turpentine". Journal of Pathology 1971; 104, 15–29.
5. Essa M.M., Suthakar G., Manivasagam T. and Dakshayani K.B., "Protective influence of *Pongamia pinnata* (Karanja) on blood ammonia and urea levels in ammonium chloride induced hyperammonemia". J Appl Biomed 2005, 3, 133-8
6. Fugh-Berman A. "Herbs and dietary supplements in the prevention and treatment of cardiovascular disease". PrevCardiol 2000; 3:24-32.
7. Gupta V., Sharma M. "Screening of three Indian medicinal plant extracts for antioxidant activity." International Journal of Institutional Pharmacy and Life Sciences July-August 2011; 1(1): 118-137
8. Gupta Y.K. and Briyal S., "Animal models of cerebral ischemia for evaluation of drug's". Indian J Physiol Pharmacol. 2004, 48, 379–94.
9. Harvey J, Paige SM. The Instat Guide to choosing and interpreting statistical tests: A manual for Graph pad Instat, Version 3. San Diego, CA USA. 1998
10. Krishnamurthi A. 1969. The Wealth of India, vol. VIII. Publication and Information Directorate CSIR, New Delhi, India.

11. Kirtikar, K.R., Basu, B.D., Indian Medicinal Plants, Lalit Mohan Basu, Allahabad, India. 1933. vol. I, p. 830.
12. Nadkarni, K.M., Indian Materia Medica, vol. 1. Popular Book Depot, Bombay, India, 1954; p. 1001.
13. Nakamura, H., Motoyoshi, S., Shimizu, M., "Therapeutic effect of anti-inflammatory drugs on hind paw edema induced by kaolin-carrageenin in rats." Japanese Journal of Pharmacology 1978; 28,83.
14. Punitha R. and Manohar S., "Antihyperglycemic and anti-lipid-peroxidative effects of *Pongamia pinnata* (Linn.) Pierre flowers in alloxan induced diabetic rats". J Ethnopharmacol 2006; 105, 39–46
15. Reed LJ, Muench H "A simple method of estimating fifty per cent end points". Am. J. Hyg., 1938; 27: 493–497
16. Singh, R.K., Goel, R.K., "Pharmacological actions of *Pongamia pinnata* roots in albino rats". Indian Journal of Experimental Biology 1997; 35, 831–836.
17. Sofowora A, Medicinal plants in Africa. Spectrum Books, Ibadan. 1993.
18. Swingle, K.F. Anti-inflammatory Agents. Chemistry and Pharmacology, vol. 2. Academic Press, New York, 1974; p. 33
19. Tanaka T, Linuma M, Fujii Y, Yuki K, Mizuno M. "Flavanoids in root, bark of *Pongamia pinnata*". Phytochemistry 1992; 31: 993-98.
20. Trease, C.E., Evans, V.C., Pharmacopoeial and Related Drugs of Biological Origin. Part Five: Pharmacognosy. Saunders, London, 1959; pp. 161–466.
21. V.V. Chopade, N.M. Gowekar, S.R. Bhandari, S.N. Khandake. "*Pongamia pinnata*: Phytochemical constituents, Traditional uses and Pharmacological properties: A review". International Journal of Green Pharmacy. 2008, 72-75
22. Weil, C.S., Tables for convenient calculations of median effective dose (LD50 or ED50) and instruction in their use. Biometrics 1952; 8, 249–263.
23. Wheeler A, "Neurogenic and tissue mediated components of formalin-induced edema: evidence for supraspinal regulation". Agents Action 1991; 34, 264–269.
24. Yin H, Lijuan L, Yang J, et al. "Pongaflavanol: a Prenylated Flavonoid from *Pongamia pinnata* with a Modified Ring A". Molecules 2006; 11: 786-91.