



Garcinia lanceifolia Roxb; An Endemic Medicinal Plant of Assam Relieves Pain and Delays Nociceptive Response: An Assay for Its Analgesic and Anti-inflammatory Activity

Nilutpal Sharma Bora*, Bibhuti Bhusan Kakoti, Partha Sarathi Bairy, Barnali Gogoi

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India

ABSTRACT

The stem bark of *Garcinia lanceifolia*, a lesser known species of the genus *Garcinia*, was extracted with methanol after pretreatment with petroleum ether. The resultant extract was evaluated for its analgesic and anti-inflammatory potential using various models. The assays were carried out on adult male Wistar Albino rats. The analgesic activity was assayed by hot plate method and tail flick assay using Morphine sulphate as standard drug at a dose of 5 mg/kg body weight and the results were expressed as mean increase in latency after drug administration $\pm$ SEM. The anti-inflammatory activity was evaluated by Carrageenan-induced rat paw oedema model using Diclofenac sodium as standard drug at a dose of 100 mg/kg body weight. The results were expressed in terms of mean increase in paw volume $\pm$ SEM. The extract of the stem bark was given in doses of 250 mg/kg and 500 mg/kg body weight. All the doses were administered per orally. Results reveal that *Garcinia lanceifolia* Roxb. possesses remarkable analgesic and anti-inflammatory activities.

Keywords: Analgesic, anti-inflammatory, *Garcinia lanceifolia*, methanolic extract, Wistar Albino rats.

INTRODUCTION

Medicinal plants and herbs have been used as therapeutic agent for the relief of pain since time immemorial. Taking into account the present day analgesic; viz. salicylic acid and morphine we can state that plants are also the source of chemical substances with therapeutic analgesic efficacy.^[1] There has been a huge increase in the research of new anti-inflammatory agents from herbal drugs due to the huge and extensive taxa of plants that are being prescribed as traditional remedies for treatment of pain.^[2] At present over 30% of the drugs used therapeutically are derived directly or indirectly from plants or parts of plants, extracts of plants and in homeopathic or ayurvedic medicine.^[3] The pathological response of tissues towards foreign agents, like physical injury, tumour growth, toxic chemicals or infectious organisms which leads to local accumulation of plasma fluid and blood cells is known as inflammation.^[4] There are various side effects, like gastric lesions which have been associated with the used of conventional non-steroidal anti-inflammatory drugs and also that of tolerance and dependence, associated with that of opiates. Renal failure, allergic reactions, occasional ototoxicity and hemorrhage due to reduced platelet function are other side effects associated

with these drugs. Therefore, safer alternatives to present day NSAIDs and opiates are being searched all over the world since the used of these classes of drugs have not been successful in all cases.^[1-3]

Garcinia lanceifolia commonly known as “Rupahi-thejera” (Assamese), “Pelh” (Mizo), “Rupohi tekera” (Mising); belonging to the family (Clusiaceae) is an important and endemic medicinal plant found in Assam. The plant is a handsome, small, evergreen tree. It is glabrous and grows upto a height of 12 feet under the dense shade of other trees. The leaves are about 6-12.5 $\times$ 2-3, lanceolate, long acuminate, fleshy when green. The lateral nerves are about 8-10 on either side of the midrib which meets close to the tetramerous

consisting of male and hermaphrodite flowers. Male flowers are 1-2, terminal, with thick sepals, oblong fleshy with smaller petals, oblique stamens about 40 in number arranged in a glabrous mass which contains four celled anthers. Hermaphrodite flowers are terminal or axillary, larger than male flowers, stamens arranged in 4 bundles of 4-5 each; ovary ovoid, 6-8 stigmatic rays and glandular. Fruits are the size of small palm, ovoid, orange-yellow, 6-8 seeded. It flowers annually between February and March while the fruiting occurs between June to July.^[5] Found previously, in the evergreen forests of Assam and Meghalaya extensively; present day it is facing the danger of extinction in nature and is often cultivated at homestead.^[6]

***Corresponding author: Mr. Nilutpal Sharma Bora,**
Dibrugarh

University, Dibrugarh, 786004, Assam, India; **Tel.:** +91-9864320371; **E-mail:** nilutpalsharma3@gmail.com

Ripe fruits are eaten raw or dried and are considered to be effective in cases of diarrhoea. The fruits and gum resin, called "gamboge" as well as the oil and juice of the plant are used as medicine for fever, jaundice, diabetes and urinary problems. [5] Sweet and mature fruits are eaten raw, young leaves and shoots which are slightly acidic in taste are cooked and eaten by the Karbi & Mishing tribes of Assam. The leaves of *Garcinia lanceifolia* are used as stomachic, diuretic and the fruit is used as a cure for dysentery and diarrhoea. Leaves are also cooked as vegetables and made into pickles. [6] The fruits being acidic are used to prepare juices, pickles and other culinary preparations. [7]

Plants belonging to the genus *Garcinia* belonging to the family Clusiaceae are known to contain bioactive compounds such as xanthenes, biflavonoids, benzophenones, benzoquinones, and triterpenes which have antibacterial, antifungal, antioxidant and cytotoxic effects. [6] Therefore, we can see there is a hard and fast evidence of safe traditional use of this plant as a medicinal and edible agent without any side effects. Hence the present study was undertaken as an elaborate and extensive effort to establish the analgesic and anti inflammatory activity potential of the methanolic extract of the stem bark of *Garcinia lanceifolia*. Results have revealed that the plant *Garcinia lanceifolia* possesses appreciable analgesic and anti-inflammatory activity and were comparable with the values of the standard drugs.

MATERIALS AND METHODS

Animals

Adult Male Wistar Albino rats of uniform weight (180-250 g) were used in this assay. The animals were procured from M/S Chakraborty Enterprises, Kolkata, India and were housed under standard conditions in the Animal House of the Department of Pharmaceutical Sciences, Dibrugarh University. They were acclimatized under laboratory conditions for two weeks and provided with free access of food and water until the time of the experiment. The animals were fasted overnight before the experiment while the water was still provided *ad libitum*. The animals were divided into groups of five animals each. Each animal was used only once. All the protocols were approved by the Institutional Animal Ethics Committee (IAEC), Dibrugarh University, Assam and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals); vide approval number IAEC/DU/60 dated 24/09/2013.

Plant Material, Preparation of Extract and Preliminary Phytochemical Screening

The bark of *Garcinia lanceifolia* was collected in the month of August, 2013 from the campus of Dibrugarh University and neighbouring areas of Dibrugarh, Assam India. The plant was identified and authenticated by Dr. A. A. Mao, Botanical Survey of India, Eastern Regional Centre, Shillong vide identification number BSI/ERC/2014/Plant identification/882. The voucher specimen of the plant was deposited in the Research Lab of the Department for further references. The bark were cut into pieces and washed thoroughly with water. The leaves were then dried partially under sunlight and partially under the shade for a week. The dried bark pieces were then ground in mechanical grinder and stored in airtight containers free from moisture.

The methanolic extract of the stem bark of *Garcinia lanceifolia* was prepared by Continuous Hot Percolation

(Sohxlet Extraction) using 1000 mL of Methanol after pre treatment with petroleum ether. The extracts were concentrated by distilling out the solvent and then vacuum dried using a rotary evaporator. Preliminary phytochemical tests were carried out with all the extracts in order to evaluate for the presence of different phytochemical constituents. The MEGL (Methanolic extract of *Garcinia lanceifolia*) showed the presence of major phytoconstituents like amino acids, carbohydrates, flavonoids, glycosides, lignin, proteins, saponins and phenolic compounds.

Acute Toxicity Test

The acute toxicity study of the methanolic extract of the stem bark of *Garcinia lanceifolia* was carried out in Adult Male Wistar Albino rats as per the guidelines set by the OECD. The rats were fasted for 16 hours and were divided into five animals in each group. Different concentrations of MEGL (250, 500, 1000, 2000 and 2500 mg/kg *p.o.*) were administered to all the animals within the tests groups. The animals were provided with their normal food and water and observed for a period of 48 hours for signs of acute toxicity. The number of deaths within this period was observed. There were no reports of death of any animal during the toxicity studies. Based on the study, two doses 250 and 500 mg/kg body weight were selected for the study.

Treatment

Analgesic Activity

The animals were divided into four groups of 5 animals each. The control group received distilled water (*p.o.*), Test Group 1 was given MEGL 250 mg/kg (*p.o.*), Test Group 2 was given MEGL 500 mg/kg (*p.o.*), and Standard Group was given morphine sulphate 5 mg/kg (*i.p.*). [*p.o.* - per orally, *i.p.* - intraperitoneally].

Hot Plate Method: The hot plate test was used to measure analgesic activity by the method described by Eddy [8] with minor modifications. Rats were exposed to heat on a hot plate having a constant temperature of $55 \pm 1^\circ\text{C}$. The time taken for either paw licking or jumping out of the hot plate was recorded. Each rat was separately placed on the hot plate in order to obtain the animal's response thermally induced pain. Jumping out of the hot plate was taken as an indicator of the animal's response to heat-induced pain. The time taken for each rat to jump out of the plate (i.e., reaction time) was noted and recorded in seconds. Morphine sulfate at 5mg/kg administered intraperitoneally was used as the standard drug while two doses of MEGL at 250 and 500 mg/kg, (*p.o.*) were administered per orally to the test groups. The nociceptive response was measured every 15min interval over a 90-minute period.

Tail-Flick Assay: The Tail flick assay was used to measure analgesic activity by the method described by Amour & Smith, 1941 [9] with minor modifications in the process. A radiant heat automatic tail flick analgesiometer was used to measure response latencies. Only those animals which responded to the basal reaction time test were selected. Basal reaction time of animals was recorded by placing the tip (last 1-2 cm) of the tail on the radiant heat source of the analgesiometer. The withdrawal of the tail from the radiant heat source was taken as end point. To avoid tail damage by heat a cut-off time of 10-15 seconds was imposed. Animals failing to withdraw its tail in 3-5 seconds were rejected from study. Three to five basal reaction times for each rat at an interval of 5 minutes were taken to confirm normal behavior of the animal. Control reaction was recorded twice with 15

minutes time interval between readings. Morphine sulfate at 5 mg/kg administered intraperitoneally was used as the standard drug while two doses of MEGL at 250 and 500 mg/kg, (*p.o.*) were administered per orally to the test groups. The nociceptive response was measured every 15 min interval over a 90-minute period.

Acute Anti-inflammatory Activity

The animals were divided into four groups of 5 animals each. The control group received distilled water (*p.o.*), Test Group 1 was given MEGL 250 mg/kg (*p.o.*), Test Group 2 was given MEGL 500 mg/kg (*p.o.*), and Standard Group was given Morphine sulphate 5 mg/kg (*i.p.*).

[*p.o.* - per orally, *i.p.* - intraperitoneally].

Carrageenan-Induced Paw Edema (Acute Model)

The assay was carried out using the method described by Winter et al., 1963.^[10] Acute inflammation was produced by injecting 1% solution of carrageenan into plantar surface of rat left hind paw at the dose of 0.1mL per 100 g body weight. Control group of rats ($n = 5$) received vehicle. Rats in the test groups received different doses of MEGL (250 and 500 mg/kg, *p.o.*), respectively. Standard group received Diclofenac (100 mg/kg, *p.o.*). After 30 minutes of induction of inflammation by carrageenan the paw volumes were measured by dipping the foot in the Digital Plathysmometer, Model: 520 MR (IITC Life Sciences). The paw volume of the test animals were compared with control animals which received only the vehicle. Measurement was done immediately before, first, second, third, fourth, and fifth hour following carrageenan injection. The oedema inhibitory activity was calculated according to the following formulas:

$$\text{Oedema rate } E\% = (Vt - V0)/V0 \times 100$$

$$\text{Inhibition rate } I\% = ((Ec - Et)/Ec) \times 100$$

Vt = Rate of edema at t hour

$V0$ = Rate of edema at 0 hour

Ec = Edema rate in control group at t hour

Et = Edema rate in test group at t hour.

Statistical Analysis

The results for the analgesic activity were expressed as “mean increase in latency after drug administration $\pm$ SEM” in terms of seconds and values for anti-inflammatory activity were expressed as “mean increase in paw volume $\pm$ SEM.” Statistical evaluation was done using one-way ANOVA followed by multiple comparisons using Dunnett’s Multiple Comparisons Test. Results were considered significant at $p < 0.05$.

RESULTS

Acute toxicity

The animals were administered with different doses of 250, 500, 1000, 2000 and 2500 mg/kg body weight *p.o.* However, it was observed that there were no symptoms of acute toxicity in any animal. Moreover there were no reports of any animal death during the course of this study. Hence, the LD_{50} of the extract was found to be safe up to a dose of 2500 mg/kg body weight of the animal. Since $1/10^{th}$ of the LD_{50} was 250 mg/kg b.w., it was selected as one of the dose for the assay. Another dose which was double the former dose, i.e. 500 mg/kg b.w. was also fixed as the high dose for the assay.

Analgesic Activity

In the above study, the methanolic extract of the stem bark of *Garcinia lanceifolia*, showed considerable in both the models. In the hot plate method, the results obtained were highly significant and the extract was able to reduce the reaction time of the animals to the radiant heat induced pain in a dose dependent manner. The results obtained for MEGL₅₀₀ were comparable to the standard drug, Morphine sulphate and are shown in Table 1. Similarly in the Tail Flick assay the activity of the extract also increased in a dose dependent manner. The effect of the MEGL500 was found to be highest and was at par with the standard drug. All the results that were statistically significant are described in the Table 2.

Table 1: Effects of methanolic extracts of *Garcinia lanceifolia* stem barks and Morphine sulphate on pain induced by Hot Plate Method

Group	Dose (mg/kg)	Reaction time (in sec)				
		0 min	15 mins	30 mins	60 mins	90 mins
Control	-	3.71 $\pm$ 0.009	3.94 $\pm$ 0.020	3.47 $\pm$ 0.123	3.73 $\pm$ 0.009	3.62 $\pm$ 0.050
Standard	5 (<i>i.p.</i>)	3.76 $\pm$ 0.066 ^{ns}	4.01 $\pm$ 0.032 ^{ns}	4.35 $\pm$ 0.047 ^{**}	6.87 $\pm$ 0.009 ^{**}	8.65 $\pm$ 0.069 ^{**}
MEGL ₂₅₀	250 (<i>p.o.</i>)	3.71 $\pm$ 0.009 ^{ns}	3.94 $\pm$ 0.020 ^{ns}	4.91 $\pm$ 0.017 ^{**}	5.89 $\pm$ 0.099 ^{**}	6.56 $\pm$ 0.090 ^{**}
MEGL ₅₀₀	500 (<i>p.o.</i>)	3.84 $\pm$ 0.039 ^{ns}	4.09 $\pm$ 0.033 [*]	5.58 $\pm$ 0.096 ^{**}	6.91 $\pm$ 0.017 ^{**}	7.85 $\pm$ 0.029 ^{**}

Data are expressed as Mean $\pm$ S.E.M., $n = 3$ animals in each group. One-way ANOVA followed by comparisons with Dunnett’s Multiple Comparisons Test was carried out. All Comparisons were made with the control group. Symbols represent statistical significance: * $p < 0.05$, ** $p < 0.01$, ns: not significant

Table 2: Effects of methanolic extracts of *Garcinia lanceifolia* stem barks and Morphine sulphate on pain induced by radiant heat of an Analgesimeter

Group	Dose (mg/kg)	Reaction time (in sec)				
		0 min	15 mins	30 mins	60 mins	90 mins
Control	-	2.30 $\pm$ 0.043	2.27 $\pm$ 0.006	2.34 $\pm$ 0.013	2.31 $\pm$ 0.085	2.86 $\pm$ 0.149
Standard	5 (<i>i.p.</i>)	2.24 $\pm$ 0.043 ^{ns}	3.41 $\pm$ 0.043 ^{**}	3.93 $\pm$ 0.173 ^{**}	6.27 $\pm$ 0.083 ^{**}	8.54 $\pm$ 0.016 ^{**}
MEGL ₂₅₀	250 (<i>p.o.</i>)	2.25 $\pm$ 0.033 ^{ns}	2.54 $\pm$ 0.013 ^{**}	3.75 $\pm$ 0.173 ^{**}	5.75 $\pm$ 0.169 ^{**}	6.52 $\pm$ 0.316 ^{**}
MEGL ₅₀₀	500 (<i>p.o.</i>)	2.25 $\pm$ 0.033 ^{ns}	3.09 $\pm$ 0.013 ^{**}	4.08 $\pm$ 0.033 ^{**}	6.13 $\pm$ 0.017 ^{**}	8.04 $\pm$ 0.033 ^{**}

Data are expressed as Mean $\pm$ S.E.M., $n = 3$ animals in each group. One-way ANOVA followed by comparisons with Dunnett’s Multiple Comparisons Test was carried out. All Comparisons were made with the control group. Symbols represent statistical significance: * $p < 0.05$, ** $p < 0.01$, ns: not significant

Table 3: Effects of methanolic extracts of *Garcinia lanceifolia* stem bark and Diclofenac on Carrageenan-Induced Paw Edema in rats

Group	Dose (mg/kg)	Paw volume increase (in mL)				
		0 h	1 h	2 h	3 h	4 h
Control	-	0.56 $\pm$ 0.006	0.66 $\pm$ 0.035	0.83 $\pm$ 0.018	1.03 $\pm$ 0.037	1.26 $\pm$ 0.047
Standard	5 (<i>i.p.</i>)	0.54 $\pm$ 0.008	0.51 $\pm$ 0.023 ^{**}	0.46 $\pm$ 0.020 [*]	0.34 $\pm$ 0.015 ^{**}	0.23 $\pm$ 0.012 ^{**}
MEGL ₂₅₀	250 (<i>p.o.</i>)	0.55 $\pm$ 0.008	0.50 $\pm$ 0.020 ^{**}	0.44 $\pm$ 0.018 [*]	0.34 $\pm$ 0.016 ^{**}	0.28 $\pm$ 0.016 ^{**}
MEGL ₅₀₀	500 (<i>p.o.</i>)	0.53 $\pm$ 0.009	0.45 $\pm$ 0.027 ^{**}	0.39 $\pm$ 0.021 ^{**}	0.31 $\pm$ 0.017 ^{**}	0.27 $\pm$ 0.017 ^{**}

Data are expressed as Mean $\pm$ S.E.M., $n = 5$ animals in each group. One-way ANOVA followed by comparisons with Dunnett’s Multiple Comparisons Test was carried out. All Comparisons were made with the control group. Symbols represent statistical significance: * $p < 0.05$, ** $p < 0.01$, ns: not significant

Anti-inflammatory assay

In the anti-inflammatory assay of the MEGL, it was observed that the extract was able to reduce the Carrageenan induced inflammation in a dose dependent manner. The results are tabulated in Table 3, and it can be seen that MEGL250 was able to reduce the inflammation in a dose dependant manner and was very similar in action when compared with the standard drug, Diclofenac sodium. All the results obtained were statistically significant when compared with the control group. It was seen that in the control group, the inflammation caused by Carrageenan increased with time to 1.26 ± 0.047 value of the Plathysmometer. However in the groups that were pre treated with the MEGL, there was significant decrease of the inflammation in a dose dependent manner.

DISCUSSION

Garcinia lanceifolia is a rare and potentially unexplored medicinal plant which is indigenous to Assam and neighboring areas of North East India. Till date no study has been taken up to screen the analgesic and anti inflammatory activity of this plant. In this study, the screening of the analgesic and anti-inflammatory effect of the methanolic extract of the stem bark of *Garcinia lanceifolia* Roxb. was done using various models. Pain and inflammation are manifestations of nociceptive stimulation which is mediated by various factors like bradykinins, cytokinins, prostaglandins etc. [2] The MEGL was able to illicit effects similar to those of two non steroidal anti inflammatory drugs, viz. Diclofenac sodium and Morphine sulphate.

In this preliminary screening, it can be observed that the MEGL was able to reduce the rate of paw edema formation in experimental animals which may be due to the prevention of formation of various mediators like prostaglandins. In the same manner it considerably reduced the reaction time of the animals towards pain caused by a radiant source of heat. Therefore it can be concluded from the above study, that the extract has potent analgesic and anti inflammatory effect. Further studies are underway for the isolation and identification of different compounds from this plant which are responsible for the above mentioned activities. Studies shall be undertaken for elucidating the exact mechanism of action of the plant extract and compounds in reducing pain and inflammation. Moreover, initiatives need to be taken up to explore the phytochemical composition and other pharmacological activities of this important and endemic medicinal plant of Assam.

ACKNOWLEDGMENTS

The authors are thankful to the Department of Pharmaceutical Sciences, Dibrugarh University for providing the research facilities to perform this work in the Department. It was supported by AICTE (all India Council for Technical Education), Research Promotion Scheme (RPS) 2011, Grant No. 8023/RI/RPS-10/(NER)/2011-12.

REFERENCES

1. Galani VJ, Patel BG. Analgesic and Anti-inflammatory Activity of *Argyrea speciosa* and *Sphearanthus indicus* in the Experimental Animals. *Global Journal of Pharmacology* 2010; 4 (3): 136-141.
2. Kakoti BB, Pradhan P, Borah S, Mahato K, Kumar M. Analgesic and Anti-Inflammatory Activities of the Methanolic Stem Bark Extract of *Nyctanthes arbor-tristis* Linn. *BioMed Research International* 2013; 1-6. <http://dx.doi.org/10.1155/2013/826295>
3. Hassan MM, Khan SA, Shaikat AH, Hossain ME, Hoque MA. Analgesic and anti-inflammatory effects of ethanol extracted leaves

- of selected medicinal plants in animal model. *Vet World* 6(2): 68-71. doi: 10.5455/vetworld.2013.68-71.
4. Sobota R, Szwed M, Kasza A, Bugno M, Kordula T. Parthenolide inhibits activation of signal transducers and activators of transcription (STATs) induced by cytokines of the IL-6 family. *Biochemical and Biophysical Research Communications* 2000; 267(1): 329-333.
5. Baruah A. Less known Local Fruits of NE India: A Digest with emphasis to ethno-medico-botany and chemical constituents. *Ethnomedicinal Plants of India* by P. C. Trivedi. Edition 1, Aavishkar Publisher Distributors, 2007, pp. 122-135.
6. Chowdhury T, Handique PJ. Evaluation of antibacterial activity and phytochemical activity of *Garcinia lanceifolia* Roxb. *IJPSR*. 2012; 3(6): 1663-1667.
7. Policegoudra RS, Saikia S, Das J, Chattopadhyay P, Singh L, Veer V. Phenolic Content, Antioxidant Activity, Antibacterial Activity and Phytochemical Composition of *Garcinia lanceifolia*. *Indian J Pharm Sci*. 2012 May-Jun; 74(3): 268-271.
8. Eddy NB, Leimbach DJ. Synthetic analgesics: II Dithienyl butenyl and Dithienyl butylamines. *Screening Methods in Pharmacology* 1965; 105-109.
9. D'Amour FE, Smith DL. A method for determining loss of pain sensation. *J Pharmacol Exp Ther*. 1941; 72: 74-79.
10. Winter CA, Risley EA, Nuss GW. Carrageenin induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc Soc Exp Biol Med*. 1962 Dec; 11: 544-547.