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Phyto-physicochemical, acute and subacute toxicity studies of *Garcinia lanceifolia* Roxb.- A rare ethnomedicinal plant of Assam, India

Nilutpal Sharma Bora*, Bibhuti Bhusan Kakoti, Priyanka Yadav, Barnali Gogoi and Sudarshana Borah

*Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam India,786004

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Garcinia lanceifolia; a small, glabrous, evergreen tree found in Northeast India belonging to the Clusiaceae family; has long, acuminate, lanceolate and fleshy leaves; tetramerous and polygamous inflorescence with both male and hermaphrodite flowers. A recent study has claimed that the bark contains antinociceptive, antihyperglycemic, and membrane stabilising activities. However, no reports on the pharmacognostic details and toxicity of the bark are reported. The bark was collected, dried and subjected to conventional organoleptic, microscopic evaluation, physicochemical evaluation and TLC methods. The powdered crude drug was examined for its physicochemical, fluorescence and microscopic characteristics. The extracts obtained after hot Soxhlet extraction were screened for their phytochemical constituents and the TLC fingerprints were also established. The toxicological profile of the hydroalcoholic extracts of *G. lanceifolia*, through acute and subacute toxicity tests, were performed. Male and female rats (Wistar) received 5000 mg/kg of hydromethanolic extract of *G. lanceifolia* (HAEGL) for the acute toxicity test and 500, 1000, 1500 or 2500 mg/kg of HAEGL for subacute toxicity test. This is the first study for the bark of *G. lanceifolia* which will serve as a standard for quality control and assurance thereby promoting further insights and conclusive studies on this plant.

Keywords: Acute toxicity, Assam, Ethnomedicinal, *Garcinia lanceifolia*, Pharmacognostic, Phytochemical, Subacute toxicity.

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Introduction

Garcinia lanceifolia is a small, evergreen tree found in northeast India, particularly Assam and Meghalaya. Locally known as "*Rupahi-thekera*" (Assamese), "*Pelh*" (Mizo) and "*Rupohi tekera*" (Mishing) the plant is from the Clusiaceae family and is an important and endemic medicinal plant found in Assam[1]. The plant is glabrous and grows up to a height of 12 ft under the dense shade of other trees. Dimensions of the leaves are about 6.0 cm–12.5 cm in length and 2 cm–3 cm in breadth, lanceolate, long, acuminate, and fleshy when green. The lateral nerves are 8–10 in number and present on either side of the midrib meeting close to the margin. The inflorescence consists of both male and hermaphrodite flowers and is tetramerous, polygamous. Male flowers (1-2 cm in length) terminal with thick sepals, oblong-shaped fleshy and small petals. The stamens are oblique (about 40 in number) and arranged in a glabrous mass with four-celled anthers. Hermaphrodite flowers are terminal or axillary and larger than male flowers. Its staminoids are arranged in 4 bundles of 4-5 each; the ovoid shaped ovary has 6–8 stigmatic rays and is glandular in structure. Fruits are palm-sized, ovoid, orange-yellow coloured and contain 6–8 seeds. The flowering and fruiting seasons occur annually in February-March and June-July respectively[2]. Often cultivated at the homestead, this once abundant plant in the evergreen forests of Assam and Meghalaya is facing the danger of extinction[3.]

The fruits and leaves are reported to be edible by the locals. The fruits are eaten in both raw, dried or cooked forms. It is considered to be effective in cases of diarrhoea. The oil, fruits, juice of the leaves and the gum resin of the plant, called "*Gamboge*" are said to be effective in cases of fever, jaundice, diabetes, and urinary problems[1]. The Karbi and Mishing tribes of Assam, are reported to eat the cooked young leaves and shoots which are slightly acidic in taste. The leaves of the plant are reportedly used as stomachic and diuretic, and the fruit is a cure for dysentery and diarrhoea. Leaves are also cooked as vegetables and made into pickles[3]. The acidic taste of the fruits

*Correspondent author
Email: nilutpalsharma3@gmail.com

enables them to be made into pickles, juices and other culinary preparations[4].

Scientific research done on this plant is very limited, and only a few pharmacological activities are reported. The plant is also reported to contain antibacterial, anti-inflammatory and anthelmintic properties[2,5,6]. Till date, there are no reports of systematic pharmacognostic and toxicology parameters on the bark of this plant. The present work is designed to study the detailed macroscopical, physicochemical, microscopical, and chromatographic characteristics of *G. lanceifolia* along with its acute and sub-acute toxicity profiling. This may serve as a standard reference for detection of adulterants, authentication and identification of the plant and also establish the safety profile of the plant.

Materials and Methods

Plant material

The fresh bark of *G. lanceifolia* was collected during August 2013, from the campus of Dibrugarh University, Dibrugarh, Assam, India (N 27°27’00’’, E 94°53’42’’, Height: 108 m). The taxonomical identification was done by Dr A.A. Mao of Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya, India; vide identification number BSI/ERC/2014/Plant identification/882. The voucher specimen of the plant bearing number DU/NSB/ 2013/04 was deposited in the research lab of the department for further references. The bark was washed thoroughly, cut into pieces and dried partially under sunlight and partially under shade for a week. The dried bark pieces were then pulverised to a coarse powder and stored in airtight containers free from moisture.

Preparation of extract

The stem bark of *G. lanceifolia* was subjected to successive soxhlet extraction by packing 1250 g of the powdered drug in a Soxhlet extractor using 1000 mL solvent. The extracts were prepared using the solvents in the following order; petroleum ether, ethyl acetate, chloroform, and 70 % methanol in water. The extraction was carried out until the crude drug material was exhausted of their phytochemical contents in each solvent. The extracts were filtered using a muslin cloth and concentrated using a rotary evaporator at a temperature of (40±5 ºC), model Rotavapor® R II, BUCHI, Switzerland to yield a dark red gummy residue. The yields (% w/w) of the extracts were 6.96, 8.88, 7.76 and 15.84 respectively for petroleum ether, chloroform, ethyl acetate and hydroalcoholic extract. All the extracts were stored in sealed glass containers away from direct sunlight and moisture until further use.

Preliminary phytochemical analysis

Preliminary phytochemical tests of all the extracts were carried out to validate the presence of different phytochemical constituents[7,8].

Animals

Healthy adult male Wistar rats (150-200 g) were procured from M/S Chakraborty Enterprise, Kolkata (Regd. No.1433/TO/11/CPCSEA). The animals were allowed to stabilise to standard lab conditions for seven days before experimentation and maintained in the Animal House of the Department of Pharmaceutical Sciences, Dibrugarh University. Polypropylene cages lined with husk in standard environmental conditions (temperature 25±2 ºC), relative humidity 55±10 % and 12:12 light:dark cycle) were used for housing of animals. The rats were fed on a standard pellet diet (Hindustan Lever, Mumbai, India) *ad libitum* and provided with free access to water. Before the commencement of the experiments, the protocols were scrutinised and approved by the Institutional Animal Ethics Committee (No. 1576/GO/a/11/ CPCSEA) vide approval no. IAEC/DU/60.

Reagents and chemicals

All chemicals used in this study were of analytical grade and were purchased from Sigma-Aldric, St. Louis, Missouri, United States; Hi-Media Laboratories, Mumbai, Maharashtra, India and Rankem/Avantor Performance Materials India Limited, Patalipada Thane, Maharashtra, India. Commercial diagnostics test kits for serum biochemical parameters were purchased from Beacon India Private Limited, Navsari, Gujrat, India.

Organoleptic evaluation

The sensory characteristics, i.e. the appearance, odour, taste and touch define the macroscopy of the plant crude drug. The macroscopical characterisation of *G. lanceifolia* was done. Special structural features were perceived using a simple microscope of 10X magnification[9].

Microscopic analysis of fresh bark

Young, fresh and tender bark of *G. lanceifolia* was collected and cleaned with water. Both longitudinal and transverse sections of the bark were cut and

boiled in 10 % potassium hydroxide solutions to remove the fatty matters, colouring pigments etc. The sections were stained with safranin dye and observed under a light microscope. Photographs of the sections were taken with a photomicrograph unit; model Leica EC3, Wetzlar, Germany[10-13].

Characterization of powdered crude drug

The dried bark was subjected to mechanical grinding for size reduction until a uniform powder was obtained. The powder was sieved to a 40 mesh size and mounted on a glass slide. The slides were observed under 5×10X magnification of a microscope model Leica EC3, Wetzlar, Germany. Various preliminary tests, fluorescence analysis and the reaction of the powder towards various chemicals were examined.

Quantitative standards

The determination of the quantitative standards, namely total ash, acid-insoluble ash, water soluble ash, extractive values, foaming index and loss on drying were examined as per the methods described in the Indian Pharmacopoeia[13,14]. Each study was performed in triplicate, and the mean values with the standard error of mean were calculated.

Fluorescence analysis

The response of the powdered crude drugs to different chemical reagents and their fluorescent characteristics were observed both under visible daylight and ultraviolet (254 and 366 nm)[15,16]. The fluorescence produced by the crude drug when treated with different chemicals, like sodium hydroxide, hydrochloric acid, nitric acid and ferric chloride etc., can be quantified using the help of a spectrofluorimeter[11]. In this study, the instrument used was Ultraviolet Fluorescence Analysis cabinet UV-Viewer, MAC®, Macro Scientific Work.

TLC fingerprinting of *G. lanceifolia* bark extracts

The extracts were subjected to Thin Layer Chromatography. The TLC was done using Silica gel G as the stationary phase. Glass plates of 15×20 cm were coated with Silica Gel G (HiMedia Laboratories, Mumbai, India) with the help of a spreader to a layer thickness of 0.25 mm. The plates were then air dried and later activated at 110 ºC for 15 min. The plates were cooled and stored in desiccators until required for further use[17-19]. Mobile phase was selected as follows; methanol:ethyl acetate (7:3), acetone:petroleum ether (8:2), chloroform: methanol:glacial acetic acid (6:3:1), ethyl acetate:acetone (5:5) and toluene:diethyl ether:formic acid (5:2.5:0.5).

Toxicity

Acute and subacute toxicity studies were based on the OECD (Organization for Economic Cooperation and Development) – Guidelines 425 and 407[20,21].

Acute toxicity

The hydroalcoholic extract of the bark of *G. lanceifolia* at a dose of 5000 mg/kg b.w. (suspended in 0.5 % carboxymethyl cellulose) to one nulliparous female [20] under fasting for 8 hrs by oral gavage. Then, every 48 h, the same dose was administered to four females; totalling to five treated animals in one group (Group HAEGL 5000 mg/kg). Parallel to this, five females were administered 0.5 % carboxymethyl cellulose solution which established the negative control group.

The animals were periodically observed for the first 24 h after administering the doses and then once a day for the next 14 days. The animals were observed for the five parameters of the Hippocratic screening[22]. These include (1) the maintainance of a conscious state in the animals (characterised by general activity of the animals); (2) activity and coordination of motor system and muscle toning (response to touching and gripping of tail, straightening of the tail, strength to grab or hold different items); (3) reflexes (which include the reflexes of the cornea and setting of the head of the animal at different positions); (4) activities on the central nervous system (tremors, convulsions, straub, sedation, anesthesia and ataxia) and (5) activities on the autonomic nervous system (lacrimation, cyanosis, ptosis, salivation and piloerection). The water and feed consumption and body weight were also recorded daily[20]. On the 15th day, all the animals were anaesthetised using ketamine hydrochloride and xylazine, 25 and 10 mg/kg, respectively and euthanised by cervical dislocation. The organs like heart, lung, liver, kidney and spleen removed, weighed and examined macroscopically.

Sub-acute toxicity

The animals were divided into four experimental groups of 10 animals each (five males and five females). Four different doses of HAEGL (500, 1000, 1500, and 2500 mg/kg) were administered per group, via oral gavage, daily for 28 consecutive days. The control group received only vehicle (0.5 % CMC solution).

Another group (Satellite group) received the maximum dose of 1000 mg/kg of HAEGL for 28 days and left untreated for 14 more days. This is performed for the observation of delayed occurrence of toxic

effects, reversibility or persistence related to the administration of the test substance. These doses were chosen based on Guideline 407 from OECD (Repeated Dose 28-Day Oral Toxicity Study in Rodents) [21].

During this treatment, daily food and water consumption, body weight and possible signs of toxicity were observed and recorded, following the Hippocratic screening. Clinical examination was performed once daily. At the end of the observation period, all animals were anaesthetized (ketamine hydrochloride and xylazine, 25 and 10 mg/kg, respectively). Blood samples were collected by cardiac puncture for subsequent haematological and biochemical analysis.

The biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), Total cholesterol (TC) and triglyceride level (TG); were assessed using commercial diagnostic kits and Merck 300 Semi Automated Clinical Chemistry Analyzer, Merck, ELITech Group, France[23].

After collecting blood, the vital organs (kidney, liver and spleen) were weighed. Samples of all organs were fixed in 10 % buffered formalin. The organ tissues were treated with Bouin's solution (mixture of 75 mL of saturated picric acid, 25 mL of 40 % formaldehyde and 5 mL of glacial acetic acid) for 12 h embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin and finally mounted in diphenyl xylene[23]. The histological analysis aimed to assess tissue integrity of the organs. The parameters analysed were: necrosis, fibrosis, leukocyte infiltration, degeneration, congestion, extravasation of blood and apoptosis[24].

Statistical analysis

Results have been expressed as mean±standard error of the mean (SEM). Student's t-test was used for comparison between two experimental groups (acute toxicity). The differences between groups of sub-acute toxicity were determined by analysis of variance (one-way ANOVA) followed by Dunnett's test. Differences were considered significant at $p < 0.05$.

Results and Discussion

Organoleptic evaluation

The bark was collected from the trunk of the tree by stripping method which yielded hard quills of 6.25-11.78 cm in length, 1.20-1.87 cm in thickness. The collected bark showed a greenish brown colour on the outer surface in a fresh form which after drying showed a dark brown colour. The inner surface showed a pale brown colour in the fresh form which after drying retained the same colour. The bark had a characteristic wood odour and taste. The outer surface of the bark was rough with signs of mould growth due to the typical high moisture of its biological location. The inner surface was smooth in texture. The photographs of the collected bark and prepared herbarium are shown in Plate 1.

Microscopic analysis

The transverse section of the bark showed the presence of periderm, cork cambium, secondary phloem, vascular cambium and latewood xylem as shown in Plate 2a. The longitudinal section of the bark showed the presence of vascular cambium, sieve tubes and vessel elements as shown in Plate 2b.

Powder microscopy

The microscopic observation of the crude drug powder revealed the presence of various characteristic features which are shown in Plate 3.

Quantitative standards

Quantitative standards are important for the evaluation of the crude drug in powdered or whole form. These standards are numerical and are measures for the quality and efficiency of the crude drugs. The results are tabulated in Table 1.

Foaming Index

The height of the foam from the 9th tube was measured, and the foaming index value was found to

Plate 1 — a) The leaves arrangement of *Garcinia lanceifolia*, b) Young fruits of *G. lanceifolia*, c) The prepared herbarium of *G. lanceifolia*, d) The interior and exterior surface of the collected bark of *G. lanceifolia*.

be 92.11±0.704. This denotes that the drug contains saponins and can form froth or foam. Along with this the acid value, saponification value and ester value were also calculated which is tabulated in Table 2.

Fluorescence analysis

Powdered drug was treated with different reagents and was observed under daylight and ultraviolet light of 254 nm and 364 nm. The results are tabulated in Table 3.

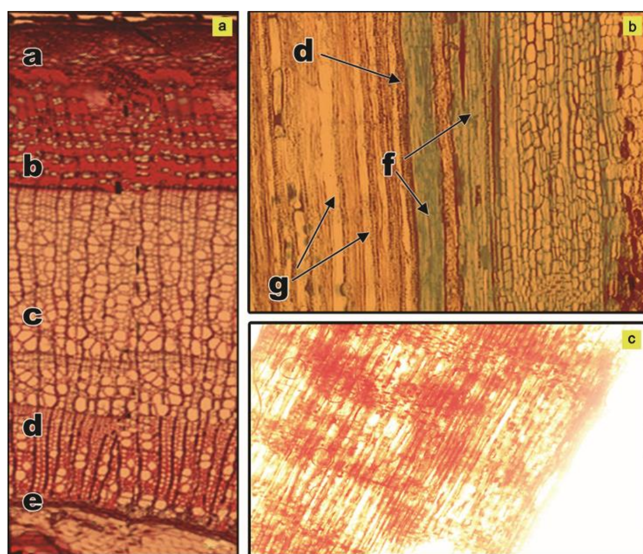

Plate 2 — a) The transverse section of the fresh bark of *Garcinia lanceifolia* (40X), b) longitudinal section of the fresh bark of *G. lanceifolia* (40X), c) Phloem fibers in 100X magnification. Legends: (a) Periderm or Cork; (b) Cork cambium; (c) Secondary phloem; (d) Vascular cambium; (e) Xylem (Late wood); (f) Seive tubes; (g) Vessel elements.

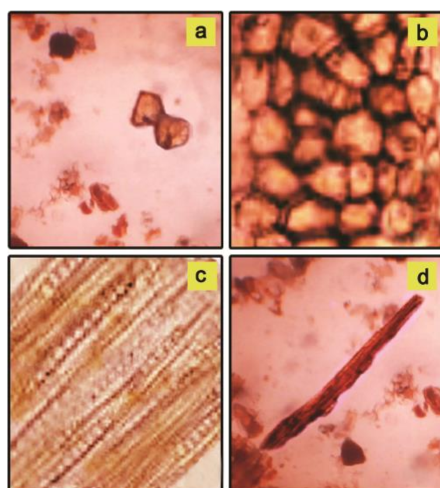

Plate 3 — The microscopy of the powdered bark of *Garcinia lanceifolia*; (a) Parenchymatous tissue; (b) Xylem vessels; (c) Fibres; (d) Calcium oxalate crystals.

Preliminary phytochemical screening

The extracts that were obtained after successive extraction were of petroleum ether, ethyl acetate, chloroform and 70 % methanol. All the extracts were semi-solid in nature having a characteristic woody odour. The different extracts obtained by successive solvent extraction were tested separately for the presence of various phytoconstituents viz., alkaloids, amino acids, carbohydrates, fats and fixed oils, flavonoids, glycosides, saponins, gums, lignins, proteins, steroids, triterpenoids, tannins and phenolic compounds[7,13]. The presence or absences of the specific phytochemicals are tabulated in Table 4. Lignins which are polymeric substances present in the plant are rarely soluble in organic solvents. However, its presence in ethyl acetate extract can be justified from the fact that low molecular weight lignins (less than 10 kDa) are highly soluble in the aforementioned solvent[25].

TLC fingerprint profile

The petroleum ether, chloroform and methanol extracts of *G. lanceifolia* were subjected to thin layer chromatography using five different solvent systems which were selected via a series of trial and error methods. The results are tabulated in Table 5 and chromatograms are depicted in Plate 4.

Toxicity evaluation

Acute toxicity studies

The acute toxicity effects of the HAEGL was determined where the test limit dose of 2000 mg/kg body weight was used. No treatment-related adverse

Table 1 — Ash values, extractive values and loss on drying of powdered *G. lanceifolia* bark

S. no.	Parameters	Mean* % ± Standard error of mean
1	Total ash	8.99±0.069
2	Acid insoluble ash	2.04±0.083
3	Water soluble ash	6.43±0.120
4	Water soluble extractive	6.33±0.196
5	Alcohol soluble extractive	2.01±0.049
6	Loss on drying (fresh bark)	80.42±0.271

*n= 3; The values are from three independent replicates.

Table 2 — Acid value, saponification value and ester value of powdered bark of *G. lanceifolia*.

S. no.	Parameters	Castor oil	*G. lanceifolia* bark
1	Acid value	3.34±0.087	0.18±0.027
2	Saponification value	125.67±1.763	9.33±0.881
3	Ester value	122.00±2.645	0.33±1.763

Values are mean % ±Standard error of mean. The values are from three independent replicates.

Table 3 — Fluoresence analysis of powdered bark of *G. lanceifolia*.

S. no.	Treatment	Daylight	UV Light 254 nm	366 nm
1	Powder as such	Dark brown	Green	Greenish brown
2	Powder + Acetic acid	Pale brown	Whitish brown	Pale grey
3	Powder + 5 % Ferric chloride	Blackish brown	Brown	Black
4	Powder + Conc. Hydrochloric acid HCl (5N)	Greyish brown	Brown	Pale brown
5	Powder + Conc. nitric acid	Reddish brown	Brown	Black
6	Powder + Conc.sulphuric acid	Reddish black	Black	Bluish
7	Powder + Iodine solution (1 %)	Brownish black	Brown	Dark brown
8	Powder + Methanol	Reddish brown	Deep brown	Blackish brown
9	Powder + Picric acid	Yellowish brown	Deep greenish yellow	Blackish brown
10	Powder + NaOH solution(1N)	Reddish yellow	Yellow	Blackish yellow
11	Powder + Distilled water	Deep brown	Brown	Greenish brown
12	Powder + Liquid ammonia (NH_3)	Blackish yellow	Yellow	Black
13	Powder + Conc. HNO_3+ NH_3	Yellow	Reddish	Yellowish black
14	Powder + Dil. HNO_3	Reddish brown	Brown	Blackish
15	Powder + 10 % Potassium dichromate solution	Deep brown	Brown	Black brown
16	Powder + Benedict's reagent	Yellowish blue	Yellowish	Greenish black
17	Powder + Acetone	Brown	Light brown	Blue

N= 3; The values were from three independent replicates.

Table 4 — Phytochemical screening of *Garcinia lanceifolia* bark extracts

Plant Constituents	Test	Petroleum-ether extract	Chloroform extract	Ethyl acetate extract	Hydro alcoholic extract
Alkaloids	Dragendorff's test	-	+	-	-
	Mayer's test	-	+	-	-
Amino acids	Ninhydrin test	-	-	+	+
Carbohydrates	Molisch test	-	-	+	+
	Fehling's test (for reducing sugars)	-	-	+	+
	Benedict's test (for non-reducing sugars)	-	-	+	-
Fats and Oils	Saponification test	+	-	-	-
Flavonoids	Shinoda test	-	+	+	+
	Alkaline reagent test	-	+	+	+
Anthraquinone	Borntrager's test	-	+	+	+
glycosides	Modified Borntrager's test	-	-	+	+
Cardiac glycosides	Legal's test	-	-	-	+
	Liebermann's test	-	-	-	+
	Keller–Killiani test	-	-	-	+
Saponin glycosides	Foam test	+	-	+	-
Coumarin glycosides	Alkaline treatment of alcoholic extract	-	-	-	-
Gums	Sample hydrolysis followed by Benedict's test	+	+	-	-
Lignins	Sample+alcoholic phluroglucinol+HCl	-	-	+	-
Proteins	Biuret test	-	-	+	-
	Million's test	-	-	+	-
Steroids	Liebermann–Burchard's test	+	+	-	-
	Salkowski test	+	+	-	-
Triterpenoids	Noller's test	+	-	+	+
Saponins	Foam test	+	-	+	+
Tannins and	Sample+5 % $FeCl_3$ Solution	-	-	+	+
Phenolic Compounds	Sample+Dilute HNO_3	-	-	+	+

+, present; –, absent; n= 10; The values were from three independent replicates

effects or mortality was found when animals were administered with a single dose of 5000 mg/kg body weight of the hydroalcoholic extracts. No drug-related changes in behaviour or metabolism of the animals were observed in both short period observation (24 h) and long period observation (14 days). Therefore, the extract is supposed to be safe for oral administration to animals at a dose level of 5000 mg/kg, and the LD_{50} was considered be >5000 mg/kg.

Table 5 — Thin layer chromatography (TLC) of bark extracts of *Garcinia lanceifolia* Roxb.

Chromatography solvents	Extracts	Number of spots detected	R_f values	Visualising agents used
Methanol:Ethyl acetate (7:3)	Petroleum ether	2	0.23, 0.55	Iodine
	Hydroalcoholic	2	0.76, 0.88	Iodine
Acetone:Petroleum ether (8:2)	Petroleum ether	1	0.37	Iodine
	Chloroform	2	0.24, 0.45	Iodine
Chloroform:Methanol:Glacial acetic acid (6:3:1)	Petroleum ether	1	0.40	Iodine
	Hydroalcoholic	3	0.20, 0.35, 0.80	Iodine
Ethylacetate:Acetone (5:5)	Petroleum ether	1	0.25	Iodine
	Hydroalcoholic	4	0.75, 0.55, 0.30, 0.60	Iodine
Toluene:Diethyl ether:Formic acid (5:2.5:0.5)	Petroleum ether	2	0.38, 0.50	Iodine
	Hydroalcoholic	1	0.70	Alcoholic $FeCl_3$

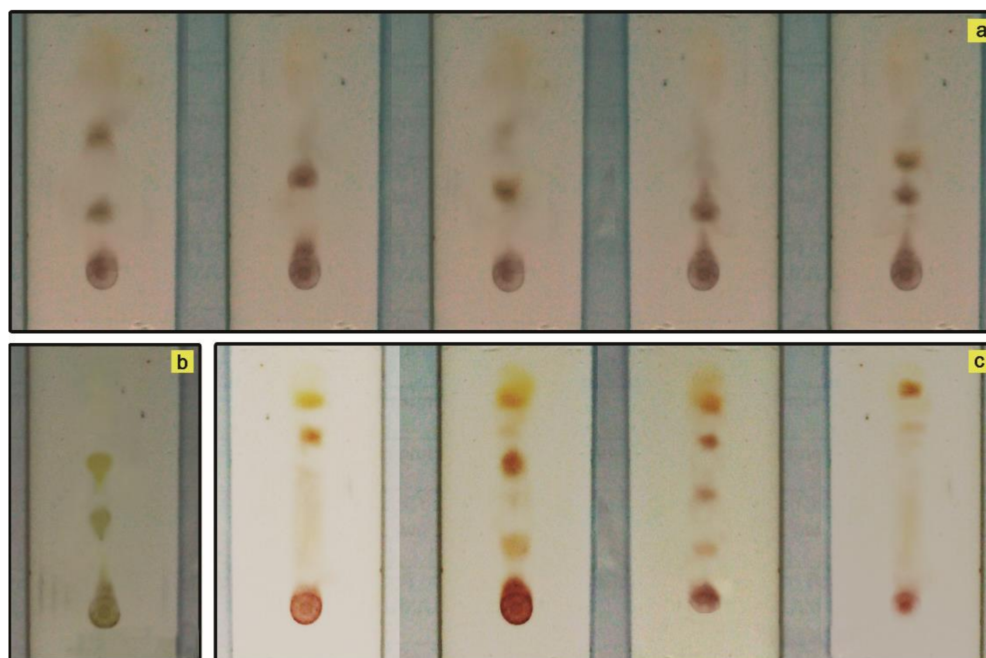

Plate 4 — TLC chromatograms of different extracts of *Garcinia lanceifolia*; a) Petroleum ether extract; b) Chloroform extract, c) Hydroalcoholic extract.

Body weight and relative organ weight are a clear indication of any adverse effects caused by the exposure of the animals to the test substance. The Hippocratic screening provided a general idea that the test substance did not cause any toxic effects related to pharmacological nature. The females which were administered with the test drug showed no signs of behavioural change as well as in the amount of food and water intake, results of which are shown in Table 6. Also at necropsy, the vital organs did show any signs of abnormality. Therefore, the extract is a Class 5 compound (a substance with oral lethal dose (LD_{50}) higher than 2000 mg/kg) according to OECD guidelines[20].

Sub-acute toxicity studies

After subacute exposure, the animals were active and responded to external stimuli, with no clinical signs of local or systemic toxic effects. There was no mortality among the animals, and the behaviour of animals remained normal. The consumption of water and food for all the groups treated with HAEGL, at all doses diminished when compared to the control group. Although the mean value for all parameters differed statistically, no biological importance was given to this, since statistical significance between the control and other groups was not observed (Table 6). Similarly, in the present study, the relative weights of all organs examined did not vary significantly among groups

(Table 7), supporting the hypothesis of the low toxicity of the extract after subacute exposure. Biochemical blood parameters did not show any abnormal values (Table 8). The histopathological analysis of the vital organs of the animals did not reveal any overt signs of toxicity or adverse effects (Plate 5). All the organs retained their normal architecture when compared with the control groups. This further proved that the oral administration of the extract did not have any adverse effects on the vital organs of the animals.

The macroscopic analysis of the treated animals' vital and reproductive organs did not yield any qualitative changes. Similarly, there were no histopathological

Table 6 — Variation in body weight, food intake and water consumption of rats treated orally with methanolic extracts of *Garcinia lanceifolia*

	Acute toxicity		Subacute toxicity				
	Control	2000 mg/kg	Control	125 mg/kg	250 mg/kg	500 mg/kg	Satellite
Female							
Initial weight (g)	195.63±4.95	177.77±4.39	199.28±2.91	177.18±0.81	181.06±0.75	176.39±0.74	195.43±2.44
Final weight (g)	202.46±6.90	205.18±7.47	244.22±4.98	214.49±2.14	218.68±2.33	218.30±1.76	230.69±4.13
Food intake (g/day)	131.66±1.56	128.61±1.52	123.28±1.80	110.79±3.36	111.00±3.00	112.49±3.59	112.79±1.70
Water intake (mL/day)	203.14±2.35	207.10±7.73	199.05±5.73	186.48±4.23	181.82±2.23	183.38±1.53	196.11±3.74
Male							
Initial weight (g)			259.63±3.10	253.52±2.04	250.19±2.79	256.33±2.11	
Final weight (g)			342.16±5.95	316.63±7.45	320.16±4.94	332.52±6.75	246.33±0.92
Food intake (g/day)			151.68±2.80	138.84±2.56	148.08±2.43	152.89±2.83	26.99±0.56
Water intake (mL/day)			245.66±7.18	211.08±3.74	244.32±8.44	239.00±4.39	151.09±2.13

All values expressed as mean±Standard error of mean, n = 5 animals/group. $p > 0.05$ (ANOVA/Dunnet's test).

Table 7 — Relative organ weight (g/100 g of body weight) of rats treated orally with methanolic extracts of *Garcinia lanceifolia*

	Acute toxicity		Subacute toxicity				
	Control	2000 mg/kg	Control	125 mg/kg	250 mg/kg	500 mg/kg	Satellite
Female							
Heart	0.41±0.01	0.39±0.02	0.36±0.01	0.366±0.01	0.36±0.01	0.36±0.01	0.376±0.02
Lung	0.50±0.02	0.58±0.02	0.54±0.01	0.550±0.01	0.52±0.02	0.55±0.01	0.572±0.03
Spleen	0.19±0.02	0.20±0.02	0.22±0.01	0.170±0.01	0.21±0.01	0.17±0.01	0.194±0.02
Liver	4.63±0.05	4.63±0.06	3.95±0.08	3.888±0.08	3.48±0.01	3.73±0.06	3.736±0.03
Kidney	0.46±0.03	0.45±0.03	0.40±0.01	0.402±0.01	0.41±0.01	0.40±0.01	0.382±0.02
Male							
Heart			0.36±0.02	0.36±0.02	0.42±0.02	0.40±0.02	0.38±0.02
Lung			0.42±0.01	0.44±0.02	0.51±0.02	0.50±0.01	0.45±0.02
Spleen			0.17±0.01	0.16±0.01	0.15±0.01	0.16±0.01	0.15±0.01
Liver			3.57±0.12	3.64±0.10	3.60±0.14	3.82±0.17	3.83±0.05
Kidney			0.56±0.07	0.52±0.04	0.52±0.06	0.52±0.03	0.51±0.03

All values expressed as mean±SEM, n= 5 animals/group. $p > 0.05$ (ANOVA/Dunnet's test).

Table 8 — Biochemical parameters of rats treated orally with methanolic extract of *Garcinia lanceifolia*

	Subacute toxicity				
	Control	125 mg/kg	250 mg/kg	500 mg/kg	Satellite
Aspartate aminotransferase (U/L)	79.46±1.63	75.33±2.67	69.62±5.37	79.22±2.29	85.22±3.29
Alanine aminotransferase (U/L)	41.37±4.03	40.56±1.85	49.46±3.81	49.7±2.71	43.57±3.98
Total cholesterol (mg/dL)	69.99±3.38	72.79±5.68	67.87±4.73	76.81±5.23	67.59±3.30
Triglyceride	216.71±9.19	256.10±24.57	232.29±19.15	276.41±20.45	222.31±8.91

All values expressed as mean±Standard error of mean, n= 5 animals/group. $p > 0.05$ (ANOVA/Dunnet's test).

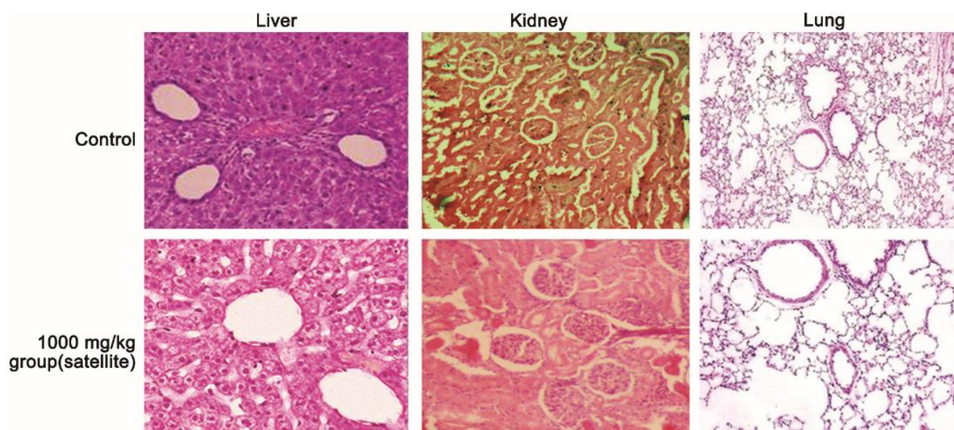

Plate 5 — Histopathological photomicrographs of organs treated with HAEGL (100X view)

changes which suggest the non-toxicity of the HAEGL. These results proved to be at par with biochemical analyses, confirming the safety of *G. lanceifolia* extract.

Conclusion

Pharmacognostic and phytochemical parameters provide a standard reference for authentication, identification and detection of adulterants in any crude drug. Along with the identification of its organoleptic characteristics, the histological feature of the bark of this plant has been established for the first time in this study. Preliminary phytochemical screening of the bark of *G. lanceifolia* has revealed the presence of various phytochemicals in different extracts, viz. alkaloids, carbohydrates, flavonoids, glycosides, saponins and tannins and phenolic compounds. The powder characteristics and fluorescence behaviour were also established which may predict the presence of different phytochemicals and functional groups present in the bark. These parameters may help in the proper identification and determination of quality and purity of the crude drug. The TLC of the different extracts established R_f values which are predicted to be helpful in the isolation of the phytoconstituents in the future. Identification of the isolated compounds is possible by relating the R_f values with their corresponding position in the chromatogram. This study has established the pharmacognostic standards for the possible identification and authentication of the bark of *G. lanceifolia* for the first time which will be helpful in the future for the any further studies including the safety of the drug for clinical use and promote the further development of drug formulations.

The HAEGL was found to be a low toxic compound with its LD_{50} higher than 5000 mg/kg., which is evident from the absence of acute and subacute toxicity. This provides us with a relevant and concrete data for a plant which is widely used for its antioxidant and food values. However, other studies based on protocols elaborated by regulatory agencies should be performed (such as studies of chronic toxicity, reproductive toxicity, and others) to evaluate the total safety of this plant in humans.

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