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## A COMPREHENSIVE REVIEW OF PHARMACOLOGICAL PROPERTIES OF *LITSEA POLYANTHA*

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### Keywords:

*Litsea polyantha*, Folklore, antioxidant and anti-diarrheal

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**ABSTRACT:** *Litsea*, an outsized genus comprising 400 species of evergreen trees or shrubs, distributed chiefly in tropical and subtropical Asia, Australia, and therefore the Pacific Islands, belongs to the Lauraceae. *Litsea polyantha* is found throughout North, East, and Central India at an altitude of 1,200 m. It is often planted as an ornamental plant. It is mostly grown as an honest source of fodder for the livestock. It is a little to medium-sized evergreen tree attaining a height of 21 m and a girth of 1.8m. Barks are dark grey or pale brown colour, exfoliating in quite small polygonal corky scales. In Folklore medicines-Bark, Stem, Roots and Leaves are used to treat various diseases and disorders. The bark of *Litsea polyantha* is mildly astringent and used for diarrhea. Powdered bark and roots are used for pains, bruises, contusions and for fractures in animals. Previous chemical investigation of this plant has revealed the presence of beta-sitosterol and actinodaphnine within the bark and an arabinoxylan containing D-xylose and L-arabinose during a molar ratio of 1:2 in leaves. The anti-diarrheal activity of methanol extract of dried bark and aerial parts of *Litsea polyantha* in mice using different models has been evaluated. Various chemical and enzymatic methods carried out the antioxidant activity of phenolic fractions of bark extract.

**INTRODUCTION:** Since past, life and diseases are related to one another. Every year human civilization is facing many new diseases which are repeatedly difficult to treat with conventional drugs.

So, it's an urge to seek out a completely unique alternative that is potent but less toxic. Scientists are continuously dedicated to looking for new synthetic drugs as natural sources like minerals, plants and animals<sup>1</sup>.

As plants are readily available to us and are routinely used as foods or for other purposes, it's believed that bioactive compounds derived from the plant may cause less toxicity<sup>2</sup>. Thus, natural products, especially those derived from plant are drawing interest as alternative therapies. Approximately 25% of prescription drugs

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throughout the planet are derived from diverse plant sources. However, the investigation of plants as a source of the therapeutic lead compound remains impoverished and there are many new windows for identifying the specified potent drug molecules<sup>3,4</sup>. *Litsea polyantha* may be a small to a medium-size evergreen tree.

The anatomical parts belonging to *Litsea polyantha* have an extended history of medicinal use among the normal healers of Chittagong, Chittagong Hill Tracts, Sylhet, and Shal forests of Gazipur, Madhupur, and Dinajpur, Bangladesh<sup>5-9</sup>.

The bark of *Litsea polyantha* is medicinally used as a stimulant, analgesic, nerves, bone tonic, and antiseptic agent and has been given to treat diabetes, diarrhea, dysentery and arthritis.

Moreover, the bark of the plant was reported to possess antidiarrheal activities and antioxidant<sup>10-12</sup>. The leaves of *Litsea polyantha* are traditionally used as purgative and laxative.

The leaves of *Litsea polyantha* have also been reported for antiatherothrombosis, antihyperglycemic, anti-diarrhoeal, antimicrobial, anti-inflammatory membrane stabilization, antibacterial and antifungal activity<sup>13-17</sup>.

*Litsea*, an outsized genus comprising of 400 species of evergreen trees or shrubs, distributed chiefly in tropical and subtropical Asia, Australia and therefore the Pacific Islands, belongs to the Lauraceae. *Litsea polyantha* is found throughout North, East and Central India and to an altitude of 1,200 m. It is often planted as ornamental plant.

It is mostly grown as an honest source of fodder for the livestock. It is a little to medium-sized evergreen tree attaining a height of 21 m and a girth of 1.8m. Barks are dark grey or pale brown, exfoliating in small polygonal corky scales<sup>18-20</sup>.

In Folklore medicines - Bark, Stem, Roots and Leaves are wont to treat various diseases and disorders. The bark of *Litsea polyantha* features an extended history of medicinal use among the traditional healers of Oraon and Munda community of Jharkhand. It is known by the various names of Kakuri, Munga, Pojo and Barkukuchita. The bark of *Litsea polyantha* is mildly astringent and used

for diarrhea. Powdered bark and roots are used for pains, bruises, contusions and for fractures in animals.

Previous chemical investigation of *Litsea polyantha* has revealed the presence of actinodaphnine and beta-sitosterol within the bark and an arabinoxylan containing D-xylose and L-arabinose during a molar ratio of 1:2 in leaves.

The anti-diarrheal activity of methanol extract of aerial parts and dried bark of *Litsea polyantha* in mice using different models have been evaluated.

Various chemical and enzymatic methods carried out the antioxidant activity of phenolic fractions of bark extract. The bark of *Litsea polyantha* features a strong characteristic aroma, but its phytochemical background has not yet been fully elucidated<sup>22</sup>.

**Taxonomic Hierarchy:** The taxonomic hierarchy of *Litsea polyantha* is as follows:

**Kingdom:** Plantae

**Phylum:** Tracheophyta

**Class:** Magnoliopsida

**Order:** Laurales

**Family:** Lauraceae

**Genus:** *Litsea*

**Species:** *polyantha*<sup>23</sup>

**Description:** *Litsea polyantha* may be a small tree up to 18 m tall, up to 60 cm in diameter, with a straight to crooked trunk. The bark is dark greyish, longitudinally fissured, and the inner bark brown mottled.

Alternately arranged elliptic leaves, 4.5-17 cm x 2.5-10 cm, are pointed or blunt, sparsely hairy below, and smooth above.

The midrib is sunken above with 6-13 pairs of secondary veins. The leaf stalk is about 1-2.5 cm long. Yellow flowers are borne in stalked umbellets briefly racemes, with 6 tepals and 9-12 stamens. Fruit is oblong to ellipsoid, 0.7-1.2 cm long, seated on a little flat perianth cup. *Litsea polyantha* is found within the Himalayas, from E Pakistan, Kumaun to NE India, SE Asia, Burma, and SW China, at altitudes below 1500 m. It is also found in South India. Flowering: March-May<sup>24</sup>.

FIG. 1: *LITSEA POLYANTHA* PLANTFIG. 2: *LITSEA POLYANTHA* FLOWERFIG. 3: *LITSEA POLYANTHA* BARKFIG. 4: *LITSEA POLYANTHA* BUD

### Pharmacological Activities:

**Antioxidant Activity Test:** Qualitative Test of Antioxidants (2, 2 – diphenyl – 1 - picrylhydrazyl (DPPH) Free Radical Scavenging Assay). The antioxidant potential of root extract was measured based on the scavenging capacity of a stable DPPH free radical on thin-layer chromatography (TLC) plate. Test sample was suitably diluted and then uniformly spotted on precoated colloid TLC plates. TLC plates were developed in medium polar ( $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$ =5:1), polar ( $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$ :  $\text{H}_2\text{O}$ =40:10:1), and nonpolar (n-Hexane: Acetone=3:1) solvent systems to resolve medium polar, polar and nonpolar components of the

extract. After developing, the plates were allowed to dry in outdoors for a short time and then sprayed with 0.02% DPPH solution in ethanol with the assistance of an atomizer.

The bleaching of the purple colour of DPPH reagent to yellow colour on purple background was assessed for the presence of possible antioxidant potential of the crude extract<sup>25</sup>. In qualitative antioxidant activity assay, a root extract of *Litsea polyantha* bleached DPPH reagent on TLC plate from its deep purple colour to yellow colour on the purple background which revealed the presence of antioxidant components in extract **Fig. 5**<sup>26</sup>.

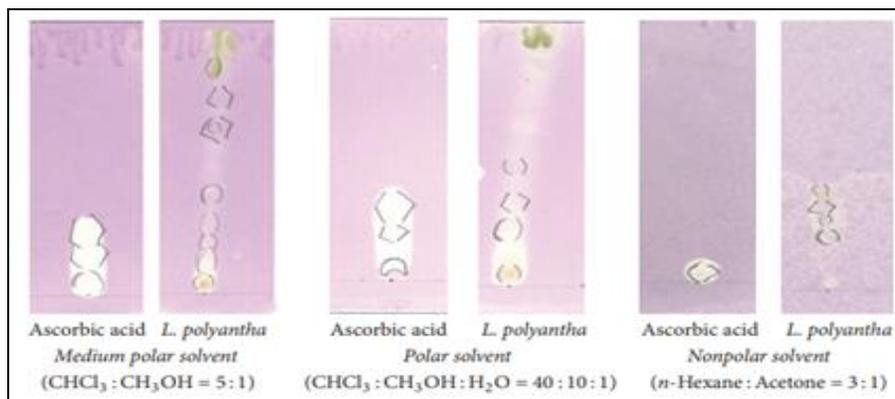
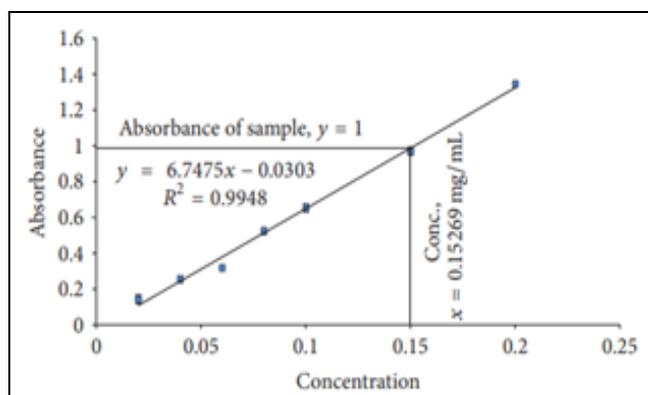


FIG. 5: COMPARISON OF TLC PLATE OF *LITSEA POLYANTHA* WITH STANDARD (ASCORBIC ACID) AFTER APPLYING 0.02% DPPH

**Determination of Total Phenolic Content:** Total phenolic content of the ethanol extract of *Litsea polyantha* was measured consistent with modified colorimetric Folin-Ciocalteu's method<sup>27</sup>. Briefly, a volume of 0.5 mL extract solution in methanol was subsequently mixed with 5 mL 10% (v/v) aqueous Folin-Ciocalteu's reagent and 4 mL of 7.5% w/v aqueous sodium carbonate. The mixture was shaken for 15 seconds and allowed to incubate at 40 °C for half-hour. The absorbance of the mixture was then recorded using an equivalent spectrophotometer at 765 nm wavelength against blank and compared to a typical curve obtained from acid solution of different concentrations in methanol. The absorbance of every concentration was measured 2 times, and the mean was used for further calculation. The total phenolic content value was expressed as mg of acid equivalent (GAE) per gram of dry extract<sup>26</sup>. The crude extract's total phenolic content was calculated by using linear regression equation,  $y = 6.7475x - 0.0303$  ( $R^2 = 0.9948$ ), obtained from a standard gallic acid calibration curve **Fig. 6** and found to be 152.69 mg GAE/g dry extract<sup>26</sup>.

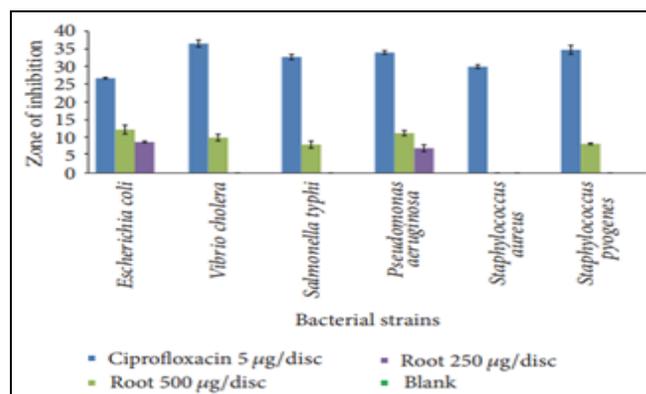


**FIG. 6: DETERMINATION OF TOTAL PHENOLIC CONTENT FROM GALLIC ACID CALIBRATION CURVE**

**Determination of Antimicrobial Activity by the Disc Diffusion Method:** Antimicrobial activity of root of *Litsea polyantha* by ethanol extract was evaluated by disc diffusion method<sup>30</sup>. Nutrient agar media were prepared by adding water and sterilized in a volumetric flask, and cooled to 45–50 °C. The sterilized agar media were poured into sterilized Petri dishes with a diameter of 120 mm and allowed to chill for a jiffy to organize the agar slants. The crude ethanolic extract of *Litsea polyantha* was put at a concentration of 250 and 500 µg/disc on paper discs of 6 mm in diameter.

After that, the impregnated discs were placed over agar plates containing previously inoculated gram-positive and gram-negative test bacteria. The gram-positive bacteria included *Staphylococcus aureus* and *Streptococcus pyogenes*, and gram-negative bacteria included *Escherichia coli*, *Vibrio cholera*, *Pseudomonas aeruginosa* and typhoid bacillus. The Petri dishes were kept at 4 °C for 2 h and then incubated at 37 °C for 16 h. The antibacterial activity of the test agent was decided by measuring the diameter of the zone of inhibition in millimetre with the assistance of slide calipers. Ciprofloxacin at the dose of 5 µg/disc was used as a positive control. The experiment was duplicated, and the average zone of inhibition was used<sup>26</sup>.

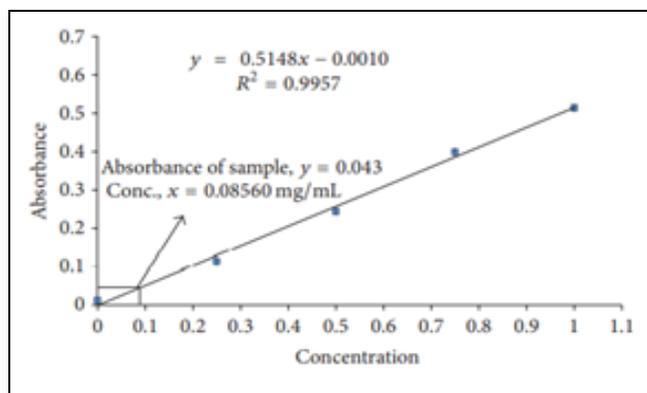
The *Litsea polyantha* root extract exhibited mild to moderate antibacterial potential against six different species of bacteria compared to the control. The root extract at the dose of 250 µg/disc exhibited a zone of inhibition against *Pseudomonas aeruginosa* (7 ± 1 mm) and *Escherichia coli* (8.75 ± 0.25 mm) but displayed no zone of inhibition against *Staphylococcus aureus*, *Vibrio cholera*, *Salmonella typhi*, and *Staphylococcus pyogenes*, whereas, at the dose of 500 µg/disc, the test sample exhibited zone of inhibition against *Escherichia coli* (12.25 ± 1.25 mm), *Vibrio cholera* (10 ± 1 mm), *Salmonella typhi* (8 ± 1 mm), *Pseudomonas aeruginosa* (11.25 ± 0.75 mm) and *Staphylococcus pyogenes* (8.25 ± 0.25 mm) but showed no zone of inhibition against *Staphylococcus aureus* **Fig. 7**<sup>27</sup>.



**FIG. 7: DIAGRAM OF ZONE OF INHIBITION ± SEM OF ROOT EXTRACT AGAINST 6 BACTERIAL STRAINS**

**Determination of Total Flavonoid Content:** The total flavonoid content of ethanol extract of *Litsea polyantha* root was measured by the aluminium trichloride colorimetric method.

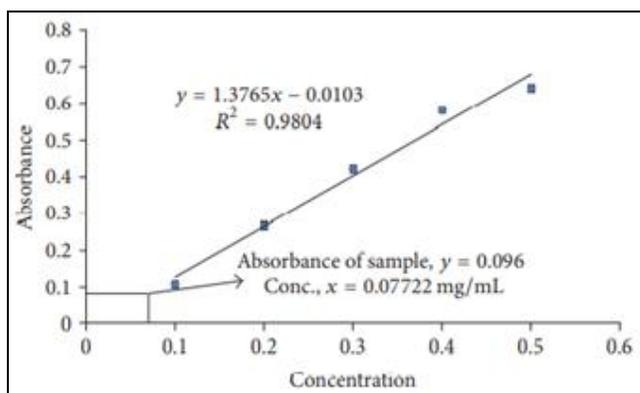
A volume of 1 mL of a known concentration of ethanol extract was added to test tube containing 4 mL distilled water and 0.3 mL 5% (w/v) sodium nitrate solution which was allowed to face for five min. Then, 0.3 mL 10% (w/v) aluminium chloride was added to the mixture and allowed to face for 1 minute. Afterward, 2 mL of 1 M caustic soda solution was aliquoted into the mixture, and therefore the volume of the mixture was adjusted to 10 mL by adding water, shaken for 15 seconds, and allowed to react for a further half-hour. The absorbance of the mixture was recorded at 510 nm against the blank by using an equivalent spectrophotometer. The absorbance of every concentration was measured 2 times, and the mean was used. The measurement was compared with a typical curve of quercetin solution in methanol of various concentrations. The total flavonoid content value was expressed as mg of quercetin equivalent (QE) per gram of dry extract<sup>28</sup>. The crude extract's total flavonoid content was calculated using the rectilinear regression equation,  $y = 0.5148x - 0.0010$  ( $R^2 = 0.9957$ ), obtained from a typical quercetin calibration curve **8** and located to be 85.60 mg QE/g dry extract.



**FIG. 8: DETERMINATION OF TOTAL FLAVONOID CONTENT USING QUERCETIN CALIBRATION CURVE**

**Determination of Total Tannin Content:** The ethanol extract tannins of *Litsea polyantha* were measured using the Folin-Ciocalteu phenol reagents. At first, 0.1 mL of the sample extract was diluted with 7.5 mL of water. Folin-Ciocalteu phenol reagent (0.5 mL) was aliquoted into tube containing diluted solution of extract. Then, 1 mL of 35% washing soda solution was added thereto mixture and adjusted to 10 mL with water. The mixture was shaken and allowed to face for 30 min at temperature. The absorbance of the mixture was

recorded at 510 nm against the blank by using a spectrophotometer and compared to a typical curve of prepared acid solutions in methanol. Each concentration was checked in duplicate, and mean absorption was taken. Total tannin content was expressed as mg of acid equivalent per gram of dry extract<sup>29</sup>. The total tannin content of root extract of *Litsea polyantha* was calculated by using a linear regression equation,  $y = 1.3765x - 0.0103$  ( $R^2 = 0.9804$ ), obtained from a standard gallic acid calibration curve **Fig. 9** and found to be 77.22 mg GAE/g of dried plant extract.



**FIG. 9: DETERMINATION OF TOTAL TANNIN CONTENT USING GALLIC ACID CALIBRATION CURVE**

**Determination of Cytotoxic Activity by Brine Shrimp Lethality Bioassay:** General toxicity of the ethanolic extract of the root of *Litsea polyantha* decided by following the tactic developed by Meyer et al.<sup>31</sup> This test was conveniently performed on *Artemia salina* (*Artemia Salina*). *Artemia salina* nauplii were obtained by hatching *Artemia salina* eggs in simulated sea water prepared by dissolving 38 gm fresh salt in 1 L water. The tank water was continuously saturated by oxygen using a vacuum pump. The bathtub temperature was maintained at 28 °C with the assistance of an electric bulb. The brine shrimps were developed to mature nauplii within 24 h. during this test, 5 clean test tubes were taken for the negative control, and 7 clean test tubes were taken for a sample and positive control. A stock solution of the test sample was prepared by dissolving in water with DMSO to get a concentration 640 µg/mL. Each tube was accurately marked to point to the ten mL volume with the assistance of another tube containing 10 mL of sea water. Then with the assistance of the micropipette, 5 mL samples of every concentration, that is, 10, 20, 40, 80, 160, 320, and 640 µg/mL, were

transferred to 7 test tubes through serial dilution technique and adjusted to 10 mL with saline water to urge final concentration of 5, 10, 20, 40, 80, 160, and 320  $\mu\text{g}/\text{mL}$ , respectively. Stock solution of positive control (vincristine sulphate) in concentration 20  $\mu\text{g}/\text{mL}$  was also subjected to serial dilution to get final concentration of 0.156, 0.312, 0.625, 1.25, 2.5, 5 and 10  $\mu\text{g}/\text{mL}$ . The remainder of test tubes contained only DMSO in 10 mL seawater as a negative control. 10 living nauplii were transferred to each tube with the assistance of a Pasteur pipette. The concentration of DMSO in each tube didn't exceed 10  $\mu\text{L}/\text{mL}$ . After 24 h, numbers of living nauplii were counted using hand glass and noted carefully.

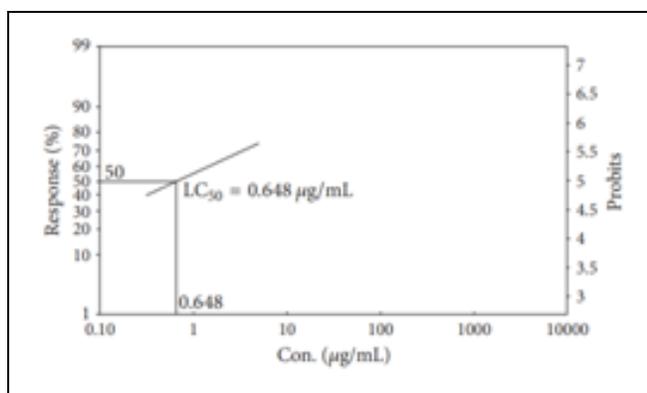


FIG. 10: DETERMINATION OF  $\text{LC}_{50}$  FOR THE STANDARD (VINCRIStINE SULPHATE) BY LDP LINE SOFTWARE

**Determination of Antihyperglycemic Activity by Oral Glucose Tolerance Test (OGTT):** Antihyperglycemic activity of ethanol extract of *Litsea polyantha* root marked by the following method described by Djilani *et al.*<sup>33</sup> with few modifications. In brief, overnight fasted mice of both sexes were divided into four groups of 5 mice each.

The groups were referred as the negative control group, which were given vehicle by oral route (1% tween 80 in water at a dose of 10 mL/kg body weight), the positive control group, which were treated orally with a typical hypoglycemic drug, glibenclamide at the dose of 10 mg/kg weight and test groups 1 and a couple of which were orally treated with the plant extract at two different doses of 250 and 500 mg/kg weight, respectively. At the onset, the blood sugar level of all mice was recorded with the assistance of a glucometer by punching the tail with a sterile needle. Then the

The experiment was performed twice to attenuate error<sup>31</sup>. The ethanolic extract of the root of *Litsea polyantha* and, therefore, the positive control vincristine sulphate showed *Artemia salina* lethality in a dose-dependent manner and exhibited approximate linear correlation between the concentration and percent (%) of mortality. The median lethal concentration as  $\text{LC}_{50}$  expressed in terms of  $\mu\text{g}/\text{mL}$  was ascertained by means of Probit analysis software to gauge the toxic potentiality of the crude ethanolic extract of *Litsea polyantha*. The  $\text{LC}_{50}$  for crude extract and positive control (vincristine sulphate) were found to be 56.082  $\mu\text{g}/\text{mL}$  and 0.648  $\mu\text{g}/\text{mL}$ , respectively Fig. 10 and 11.

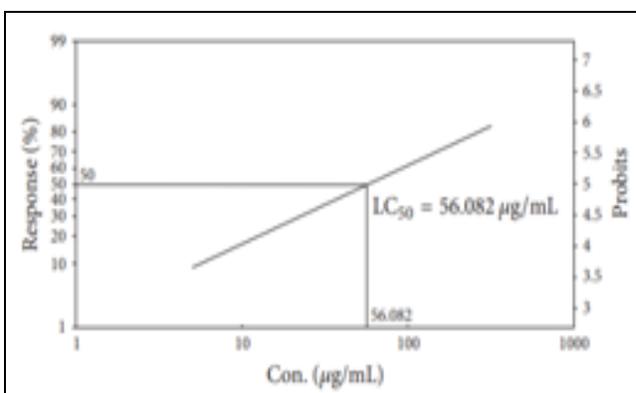


FIG. 11: DETERMINATION OF  $\text{LC}_{50}$  OF ETHANOLIC EXTRACT OF *LITSEA POLYANTHA* ROOTS BY LDP LINE SOFTWARE

extract, negative control, and positive control solution were given by oral route of a feeding needle, maintaining the aforementioned dose level(s). After half-hour, all mice were orally given glucose at a dose of two gm/kg weight through a feeding needle.

Blood glucose levels were again measured employing a glucometer at half-hour, 90 minutes, and 120 minutes of administration of glucose with the assistance of same glucometer in terms of mmol/L. The experiment was matched to minimize the statistical error.

The root extract exhibited distinct improvements in glucose tolerance at two different doses of 250 and 500 mg/kg body weight compared to the negative control ( $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ ). The crude extracts demonstrated highest blood glucose-lowering potentiality in glucose-loaded mice at the point of 120 min at aforementioned doses **Table 1**.

Treatment (oral) group	Blood Glucose level (mmol/L)			
	Fasting state (before treatment)	30 min	90 min	120 min
Negative control	5.70 ± 0.24	13.66 ± 0.71	8.74 ± 0.35	7.16 ± 0.24
Positive control (glibenclamide)	5.96 ± 0.12	6.38 ± 0.19 <sup>c</sup>	3.88 ± 0.21 <sup>c</sup>	2.96 ± 0.081 <sup>c</sup>
Root 250 mg/kg	4.38 ± 0.33	12.34 ± 0.72	7.16 ± 0.39 <sup>a</sup>	5.28 ± 0.26 <sup>b</sup>
Root 500 mg/kg	4.68 ± 0.35	11.32 ± 0.47 <sup>c</sup>	5.48 ± 0.33 <sup>c</sup>	4.96 ± 0.26 <sup>c</sup>

<sup>a</sup>p<0.05, <sup>b</sup>p<0.001 and <sup>c</sup>p<0.001 when compared with negative control.

**CONCLUSION:** The wonder and miraculous tree, *Litsea polyantha*, has proven its wide range of applications in treating many ailments. Though the plant is well known for its medicinal values, controlled clinical trials are required to prove and evaluate its real efficacy. Further exploration for isolation and characterization of compounds may contribute to drug discovery using natural sources.

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**CONFLICT OF INTEREST:** We declare that we have no conflict of interest.

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