

Evaluation of antioxidant, antibacterial and antidiabetic activities of different parts of *Litsea polyantha* extracts

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Abstract: The genus *Litsea* consists of about 700 species of evergreen trees or shrubs and is widely distributed in tropical and subtropical countries of the world. *Litsea polyantha* is used in traditional medicine around the world to cure a variety of illnesses such as influenza, diarrhea, stomach aches, diabetes, vomiting, and central nervous system. The antioxidant, antibacterial, and antihyperglycemic activity of *Litsea polyantha* were investigated. The antioxidant activity was performed by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, and hyperglycemic activity was performed by α -amylase inhibition. The toxicity of plant extracts was assessed by brine shrimp lethality assay (BSLA) using *Artemia salina* as a biological test organism. The total phenolic content was found high in bark extract 182.49 ± 8.18 mg GAE/g and the total flavonoid content was found maximum in the same bark extract 71.23 ± 2.68 mg QE/g. Bark extract and leaf extract showed the highest DPPH radical scavenging activity with an IC_{50} of 36.06 ± 2.94 μ g/mL and 20.46 ± 1.05 μ g/mL. For a methanolic extract of bark, ZOI was observed as 14 ± 0.58 mm, 13 ± 3.18 mm, and 11 ± 0.33 mm against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*. Similarly, for leaf, root, and stem extract ZOI was observed 10 ± 0.57 mm, 11 ± 0.67 mm, and 9 ± 0.58 mm against *Staphylococcus aureus*, *E. Coli*, and *Bacillus subtilis*. Bark extract and leaf extract had IC_{50} of 0.72 ± 0.03 mg/mL and 2.40 ± 0.02 mg/mL respectively. The LC_{50} of 835.35 μ g/mL for the root extract of *Litsea polyantha* was shown to be toxic against brine shrimp. This study showed the different parts of *Litsea polyantha* are rich sources of phenolic and flavonoid content and the potential natural antioxidant and antidiabetic compounds that could be isolated.

Keywords: Antidiabetic; Antimicrobial; Antioxidant; Flavonoid; *Litsea polyantha*; Phenolic; Toxicity.

Introduction

Life and disease have been linked since ancient times. Humans deal with a variety of new illnesses every year that are challenging to treat with traditional medications. Plants are readily available to us and are commonly used as food, medicine shelter, or for other reasons, therefore bioactive compounds isolated from them are thought to be beneficial for human and animal welfare. Plants are found relatively less toxic than the compounds obtained through chemical synthesis¹. The application of natural products has acquired substantial recognition as an alternative and/or supplementary therapeutic method with their vast pharmacological and biological qualities^{2,3}. Since ancient times, plants played a significant role in treating various human-related diseases. Recently modern society also

started using plant extracts as an alternative technique for preventing diseases. Around 2,000 plants are used as medicine around the world, however, around 14000 plants have been identified as medicinal plants. Approximately 80% of the world's population living in developing countries are using such plants against simple to life-threatening diseases in the form of traditional medicine⁴. The secondary metabolite produced by the plants constitutes the number of compounds that can be one of the primary sources for the discovery of new therapeutics. More than 9000 flavonoids have been reported as antimicrobial agents, visual attractors for pollinators, UV protectants against reactive oxygen species, and insect and herbivore feeding repellants, photoreceptors also act as natural antioxidants, anti-tumor, anti-inflammatory, anti-

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allergic, anti-thrombotic, anti-diabetic, Alzheimer's disease, and arteriosclerosis⁵. The phenolic compounds have very wide biological activities such as antioxidants, antimicrobial, anticancer, hepatoprotective, anti-mutagenic, enzyme inhibiting properties, etc. The main reason for the antioxidant property is the presence of phenolic compounds in the crude plant extracts⁶. Reactive oxygen species (ROS) encompasses all highly reactive oxygen-containing molecules including free radicals⁷. The hydroxyl radicals, hypochlorite radicals, superoxide anion radicals, and various lipid peroxide are types of reactive oxygen species. All are capable of reacting with membrane nucleic acids, proteins, lipids enzymes, and other small molecules, resulting in cellular damage. Vitamin C, vitamin E, carotenes, phenolic acids, phytate, and phytoestrogens are all antioxidants found in plants that have been shown to reduce disease risk⁸. Antioxidants can neutralize free radicals, chelate metal ions, suppress lipid peroxidation, have reducing properties, and promote and prevent disease by lowering oxidative stress caused by reactive oxygen or nitrogen species in the human body⁹. Fasting blood glucose levels above or equal to 7 mmol/L are a sign of diabetes¹⁰. Blood glucose levels are raised by the action of digestive enzymes on ingested food¹¹. Major complications such as cardiovascular disorders, nerve damage, retinopathy, and nephropathy can result from untreated hyperglycemia¹². Plant diversity in Nepal has 5833 species of phanerogams, which is about 2% of the total identified species of flowering plants in the world. Out of the total plants 700 species of medicinal plants and 246 species of endemic plants are reported in Nepal¹³. Tanahun district is a part of Gandaki province that is rich in biodiversity and has various medicinal plants. People of the hilly region of Tanahun still use various types of plants for the treatment of various diseases such as food poisoning, ulcers, and gastric. *Litsea polyantha* plants have been employed in ethnomedicine and look to be very promising leads for potential pharmaceutical exploitation, especially now that modern science has made it feasible to identify their potential medical importance with antifungal, anti-inflammatory, antimicrobial, antioxidant, male anti-infertility, anti-HIV, cytotoxic, antidepressant, antibacterial¹⁴. Plants are readily available

to us and are commonly used as food or for other reasons, therefore bioactive compounds generated from them are beneficial and have lower toxicity. Antioxidant and anti-diarrheal qualities have been attributed to *Litsea polyantha's* bark.

The leaves of *Litsea polyantha* have been used for antimicrobial, anti-hyperglycemic, anti-inflammatory membrane stabilization, antibacterial anti-fungal, and also used as purgative and laxative¹⁵. The root of the plant has long been used to treat aches, pains, bruises, and fractures in animals. Alkaloids, sesquiterpenes, lactones, lignans, flavonoids, and volatile oils were produced by the entire *Litsea* species, including the fruit, leaves, stems, and roots¹⁶. This research was focused on phytochemical screening and the biological activity of *Litsea polyantha*.

Materials and methods

Chemicals and reagents

The reagents and chemicals such as ethanol, sodium carbonate, sodium acetate, aluminium chloride, Folin-Ciocalteu reagent, acarbose, quercetin, gallic acid, dimethyl sulphoxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), soluble starch, disodium hydrogen phosphate, monosodium hydrogen phosphate, sodium potassium tartrate, porcine pancreatic amylase (PPA) were purchased from Sigma-Aldrich Company. All reagents and solvents were of analytical reagent grade.

Sample collection and identification

The fresh leaves, bark, stem, and roots of particular plant materials were collected from the forest of the Tanahun district of Nepal by consulting a local traditional healer in June 2078. The herbarium sheet of the plant sample was deposited at the Central Department of Botany, Tribhuvan University from where the voucher specimen no. TUCH 210072 was received.

Sample preparation

The different parts of the plant were thoroughly washed with tap water to remove dust particles then chopped into small pieces, and airdried at room temperature ($25 \pm 5^\circ\text{C}$)



Fig. 1. Photograph of the plant sample used in the study.

under shade. After completely drying, plant parts were ground to a coarse powder. During this process, plant parts were broken down into smaller pieces to expose internal tissues and cells to the solvents. The powdered samples were stored in a clean closed glass container until the extraction of secondary metabolites.

Extraction

The 100 g of fine powder was maintained in an Erlenmeyer flask and submerged in methanol for 72 hours with frequent agitation. The cap of the flask was closed properly to prevent the entrance of air into the flask. The dipped plant samples were filtered with Whatman filter paper No. 1. The filtrate was concentrated at low temperature using a rotatory evaporator. The partially concentrated plant extracts were kept in the water bath at 35 °C for complete evaporation of the solvent. The extract was weighed to determine the percentage yields and the dried extract was stored at 4 °C in the refrigerator for further study.

$$\text{Percentage yield} = \frac{\text{dry weight of plant extract}}{\text{dry weight of plant material}} \times 100$$

Total phenolic contents (TPC)

The total phenolic content of all selected plant extracts was estimated using the Folin-Ciocalteu phenol reagent method with a slight modification of the colorimetric method in which the gallic acid was standard ¹⁷. The experiment was carried out in a 96-well plate reader in

which the initial absorbance was measured after the addition of 100 µL of Folin-Ciocalteu reagent and 20 µL of the standard gallic acid/plant extracts (0.5 mg/mL). Afterward, 80 µL of 1M Na₂CO₃ (sodium carbonate) solution was added to make a final volume of 200 µL. The content was incubated for 30 minutes after that the absorbance was measured at 765 nm with the help of a synergy LX microplate reader. The TPC was quantified using a calibration curve of gallic acid, the outcome data were expressed in milligrams of gallic acid equivalent per gram (mg GAE/g) of dry extract. The triplicate of each measurement was carried out for validation of the experimental results.

Total flavonoid content (TFC)

Total flavonoid content (TFC) was calculated by the AlCl₃ method, based on the formation of a complex between AlCl₃ and flavonoid with a maximum absorbance at 415 nm ¹⁸. The 20 µL of each extract (0.5 mg/mL) was loaded on 96 well plates in triplicate. In each well 110 µL deionized water was added to maintain a final volume of 130 µL. Then, 60 µL ethanol, 5 µL AlCl₃, and 5 µL sodium acetate were added to each well and the reaction mixture was allowed to stand for 30 minutes. The TFC was expressed as milligrams of quercetin equivalent per gram (mg. QE/g) of the dry weight of extracts using the quercetin standard calibration curve.

Antioxidant activity

Antioxidant activity was determined by using a 96-well plate reader and the method was slightly modified from the colorimetric method ¹⁹. A positive control of 20 µg/mL quercetin and a negative control of 50% DMSO were used. The samples, positive control quercetin, and negative control DMSO were loaded (100 µL) in 96 well plates in triplicate. Then 100 µL of DPPH reagent was added to each well, it was incubated for 30 minutes in the dark, and absorbance was taken at 517 nm using a microplate reader 102 (Epoch2, BioTek, Instruments, Inc., USA). The IC₅₀ was calculated by using the software GraphPad Prism. The ability to neutralize the DPPH radical was calculated by using the following equation.

Antimicrobial activity

Collection of test organisms

The bacterial strains included in this study were gram-positive bacteria (*Staphylococcus aureus* KCTC 1916, *Bacillus subtilis* ATCC 66333) and gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031) which were isolated from Research Institute for Bioscience and Biotechnology (RIBB) Saptakhel-9 Balkumari, Chyasal, Lalitpur, Nepal.

In vitro antibacterial screening

The bacterial susceptibility of the plant extracts was assessed by the agar well diffusion method in Mueller Hinton agar plates²⁰. Briefly, an overnight incubated broth culture of entire test organisms was prepared in nutrient broth and diluted with sterile nutrient broth media to maintain the turbidity at 0.5 McFarland standards (10⁶-8 CFU/mL). Then, about 100 µL of inoculum was taken and spread on MHA agar plates. After that, wells were bored aseptically into the agar surface by using a sterile gel puncture of 7 mm diameter and filled with 20 µL of samples (concentration of 50 mg/mL). Finally, the plates were incubated at 37 °C for 24 hours and after incubation, the plates were checked to see if a clear zone had formed around each well which would indicate the antibacterial activity of the plant samples. The zone of inhibition (ZOI) for each sample was measured using a ruler in mm. In this bioassay, ampicillin (1 mg/mL) was taken as a positive control and DMSO as a negative control.

Antidiabetic activity

Screening of plant material for α-amylase inhibitors was carried out in 96 well microtiter plates according to Xiao et al., based on the starch-iodine test with a slight modification²¹.

Pancreatic α-amylase inhibition assay (Starch-iodine color assay)

The α-amylase activity can be evaluated *in-vitro* by hydrolysis of starch in the presence of the α-amylase enzyme. This process was enumerated by using iodine,

which gives a blue color solution with starch. The reduced intensity of the blue color indicates the enzyme-induced hydrolysis of starch into the monosaccharides. In other words, the intensity of the blue color in a test sample is directly proportional to α-amylase inhibitory activity. Thus, inhibition of α-amylase can lead to a reduction in post-prandial hyperglycemia in diabetic conditions.

The total assay mixture composed of 20 µL 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), (porcine pancreatic α-amylase) PPA solution, and 20 µL plant extracts were incubated at 37 °C for 15 min. 1 M (20 µL) HCl was added to terminate the enzymatic reaction, followed by the addition of 100 µL of 5 mM iodine reagent. The color change was noted and the absorbance was read at 620 nm on a 96-well microplate reader. A dark-blue color indicates the presence of starch an absence of starch is shown by a yellow colour while partially degraded starch is indicated by a brown color in the reaction mixture. In the presence of inhibitors from the extracts, the starch added to the enzyme assay mixture is not degraded in the absence of the inhibitor, indicating that starch is completely hydrolyzed by α-amylase.

$$\% \text{ Relative enzyme activity} = \frac{\text{Enzyme activity of test}}{\text{Enzyme activity of control}} \times 100$$

Percentage inhibition in the α-amylase activity = (100 – percentage relative enzyme activity), where, the enzyme activity of test = total starch – remaining starch, the enzyme activity of control = starch only – (starch + enzyme).

Brine shrimp assay

This assay has been successfully employed as an important tool for toxicity screening of plant extract using *Artemia salina* brine shrimp nauplii²². The extract (20 mg) was weighed out and dissolved in 2 mL methanol to make a stock solution of a concentration of 10,000 ppm (µg/mL). From that stock solution of concentrations of 1000 µg/mL, 100 µg/mL, and 10 µg/mL were prepared by serial dilution method. 2 mL solution from each solution (1000 ppm, 100 ppm, and 10 ppm) was transferred to nine different test tubes, three for each concentration. Similarly, 2 mL

methanol was taken in three test tubes (as a blank). After labeling these test tubes, they were kept for 24 hours to evaporate the solvent (methanol). After complete evaporation of the solvent, 5 ml of simulated seawater was added to each test tube and the solution was gently shaken so that the dry compounds diffused adequately in the aqueous solution. The ten matured shrimps were transferred to each test tube. All the test tubes were maintained under illumination. The number of surviving nauplii was counted

after 24 hours. From this data, the percent mortality of the brine shrimp nauplii for each concentration was calculated by using the following formula.

$$\% \text{ Mortality} = \frac{\text{No of nauplii taken} - \text{No of nauplii alive}}{\text{No of nauplii taken}} \times 100$$

The LC₅₀ values were statistically examined using Finney's probit analysis method.

Results and discussion

Table1. Name of the plant, family, parts used for the study, and traditional medicinal uses.

Sample code	Scientific name	Common name	Family	Parts used	Traditional medicinal uses
CLE	<i>Litsea polyantha</i>	Kutmero	Lauraceae	Leaf	Anti-hyperglycemic, anti-inflammatory membrane stabilization, antibacterial, purgative, laxative, arthritis
CBE	<i>Litsea polyantha</i>	Kutmero	Lauraceae	Bark	Diarrhea, pains, skin diseases, fractures in animals
CRE	<i>Litsea polyantha</i>	Kutmero	Lauraceae	Root	Aches, pains, bruises, fractures
CSE	<i>Litsea polyantha</i>	Kutmero	Lauraceae	Stem	Anticancer, stomach ache, sedative, antiseptic

Total phenolic content (TPC)

The quantitative determination of total phenol content in leaf, bark, stem, and root extracts was carried out with Folin Ciocalteu reagent taking gallic acid as standard (The regression equation of constructed standard calibration curve of gallic acid is $y = 0.0023x$, $R^2 = 0.983$).

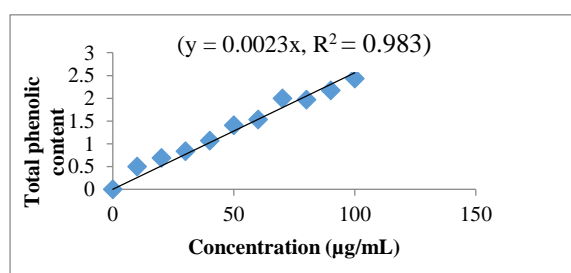


Fig. 2. Standard calibration curve of gallic acid.

The results of TPC are shown in Fig. 3. The results demonstrate that total phenolic content was found highest in *Litsea polyantha* bark of (214.12 ± 2.84 mg GAE/g extract) and lowest in *Litsea polyantha* roots of (76.12 ± 5.21 mg GAE/g extract) while the rest had intermediate values. The results of this study showed that *Litsea polyantha* was found rich in total phenolic content as compared to the bark of the same genus reported as 511.47 ± 22.304 mg GAE/g²³ and 152.69 mg GAE/g²⁴. The phenolic compounds are potent antioxidants that have biological action that can help to prevent diseases such as enzyme inhibition, and bacterial inhibition²⁵. A greater

amount of polyphenol content in plant extract reflects higher antioxidant activities.

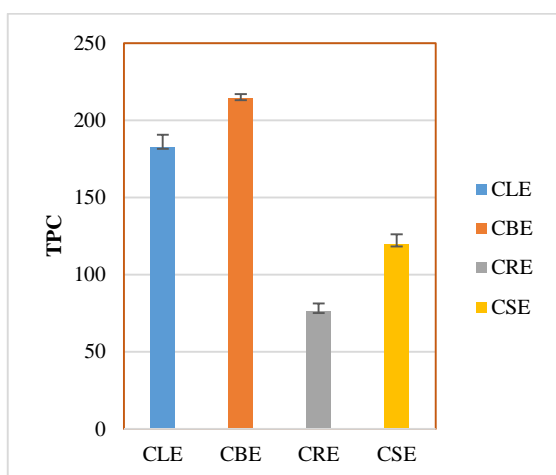


Fig. 3. Comparative TPC of (CLE- crude leaf extract, (CBE- crude bark extract), (CSE- crude stem extract), (CRE- crude root extract).

Total flavonoid content (TFC)

The total flavonoid content (TFC) was measured in terms of quercetin equivalent (mg QE/g) with the help of a standard calibration curve constructed of quercetin ($y = 0.0216x - 0.0243$, $R^2 = 0.9983$).

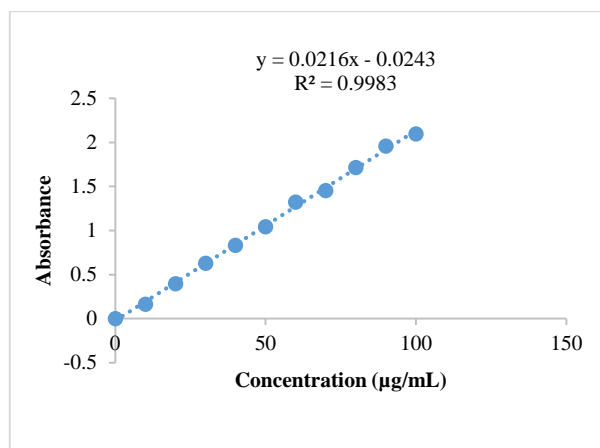


Fig. 4. Calibration curve of standard quercetin.

The results showed that total flavonoid content was highest in bark extract (71.23 ± 2.68) mg QE/g and lowest in stem extract (6.35 ± 1.24 mg QE/g) while the rest of the extracts were found to have moderate values. Ghosh et al. 2015 reported that the total flavonoid content in the same genus of this plant was 230.785 ± 5.439 mg QE/g in the bark extract. The result of the present study showed that *Litsea polyantha* is found rich source of flavonoid content²³

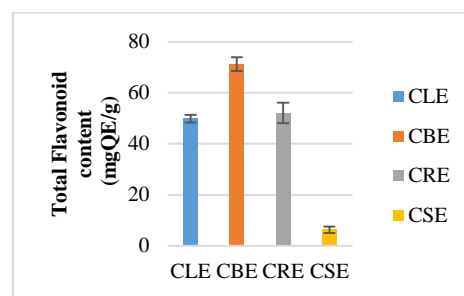


Fig. 5. Comparative TFC of (CLE- crude leaf extract), (CBE- crude bark extract), (CSE- crude stem extract), and (CRE- crude root extract of *Litsea Polyantha*).

Antioxidant screening

The results of antioxidant screening are shown in Table 2.

Table 2. Antioxidant screening of the crude extracts.

Plant samples	Concentration ($\mu\text{g/mL}$)	% Inhibition
Crude leaf extract (CLE)	500	61.95
Crude bark extract (CBE)	500	85.05
Crude root extract (CRE)	500	70.53
Crude stem extract (CSE)	500	76.44

The results showed that the crude bark extract inhibits the highest 85.05% at 500 $\mu\text{g/mL}$ concentration and crude leaf extract inhibits the lowest 61.95% at 500 $\mu\text{g/mL}$ while others showed moderate inhibition.

The bark and leaf extracts showed the highest DPPH radical scavenging activity of IC_{50} 36.06 ± 2.94 $\mu\text{g/mL}$ and 20.46 ± 1.05 $\mu\text{g/mL}$ was found comparable to the values of standard quercetin while the antioxidant potential was found lowest for the root and stem extracts. The results showed that the plant with greater flavonoid and phenolic content was found to have higher antioxidant activities. The antioxidant potential of this plant was found higher as compared to the previously reported results in the same genus of IC_{50} 223.22 $\mu\text{g/mL}$ as compared to ascorbic acid IC_{50} of 27.33 $\mu\text{g/mL}$ ²⁶. It was found that plant-derived antioxidants scavenge free radicals generated in the human body and control oxidative stress-related degenerate effects²⁷.

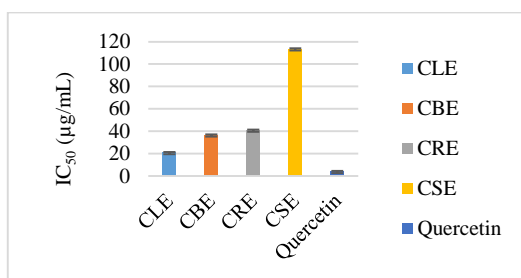


Fig. 6. IC₅₀ of the plant extracts showing the antioxidant activity. (CLE- (crude leaf extract), (CBE- crude bark extract), (CSE- crude stem extract), and (CRE- crude root extract of *Litsea polyantha*).

Antibacterial properties

The microorganisms undertaken were *E. coli*, *Bacillus*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The agar well diffusion method has been used to measure the zone of inhibition. In the agar, well diffusion method the diameter of the zone of inhibition (ZOI) shown by plant extract was measured to know the antibacterial activity of plant extracts. The results of antibacterial activity are shown in Table 3.

Table 3: Zone of inhibition (ZOI) against the various bacterial strains.

Extracts	Bacteria	ZOI (mm)	ZOI (mm) positive control
CLE	<i>E. coli</i>	-	18
	<i>Staphylococcus Aureus</i>	11±0.58	36
	<i>Bacillus subtilis</i>	11±0.58	11
	<i>Klebsiella pneumoniae</i>	10±0.33	23
	<i>E. coli</i>	8 ±0.47	18
CBE	<i>Staphylococcus Aureus</i>	13 ±3.18	36
	<i>Bacillus subtilis</i>	14±0.58	11
	<i>Klebsiella pneumoniae</i>	11±0.58	23
	<i>E. coli</i>	10±0.33	18
	<i>Staphylococcus Aureus</i>	10±0.57	36
CRE	<i>Bacillus subtilis</i>	11±0.67	11
	<i>Klebsiella pneumoniae</i>	9±0.58	23
	<i>E. coli</i>	10±0.58	18
	<i>Staphylococcus aureus</i>	10±0.58	36
	CSE	<i>Bacillus subtilis</i>	11±0.67
<i>Klebsiella pneumoniae</i>		9±0.58	23

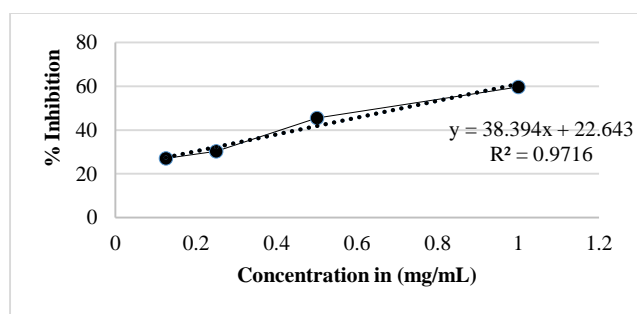
The ZOI shown by the methanolic bark extract is 14 ± 0.58 mm, 13 ± 3.18 mm, and 11 ± 0.33 mm against *Bacillus subtilis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Similarly, the moderate ZOI exhibited by the leaf, root, and stem extract against the *Staphylococcus aureus*, *E. Coli*, and *Bacillus subtilis*. The antimicrobial property was reported in the same genus of ZOI in the range of 10-12 mm in the agar well diffusion antibacterial test. The highest zone of inhibition (12 mm) was obtained against *Staphylococcus aureus*, *Vibro cholera* *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *E. coli* respectively followed by 11, 11, 11 and 10 mm²⁸.

Alpha-amylase inhibition

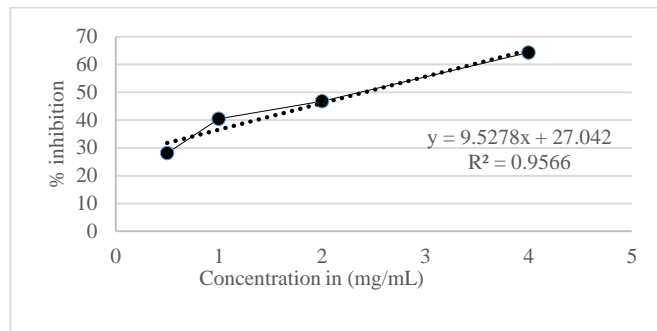
The quantitative starch-iodine method was used to measure the α -amylase inhibitory activity of plant extracts. The α -amylase inhibition activity shown in the solvent extracts is displayed in Table 4.

Table 4. α -amylase inhibition shown by the plant extracts.

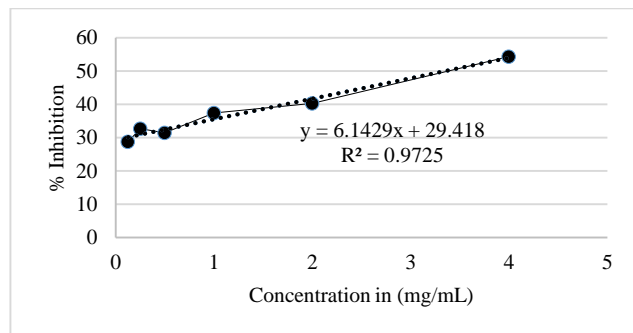
Plant extracts	IC ₅₀ (mg/mL)
CLE	0.72 ± 0.035
CBE	2.40 ± 0.028
CRE	3.35 ± 0.008
CSE	3.14 ± 0.010
Acarbose	0.05 ± 0.023



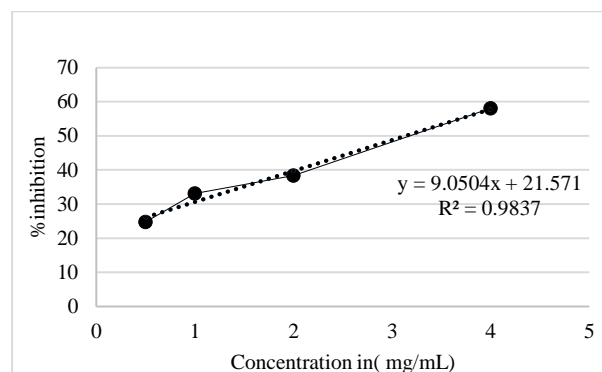
(a)



(b)



(c)



(d)

Fig. 7. α -amylase inhibition against the concentration of (a) leaf extract (b) bark extract (c) root extract and (d) stem extract.

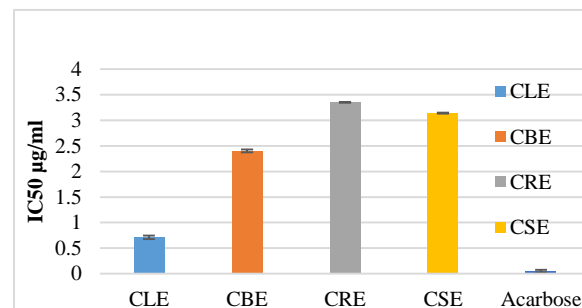


Fig. 8. The IC₅₀ for α -amylase inhibition by the plant extracts.

According to an *in vitro* starch digesting bioassay, it was found that bark extract and leaf extract had IC₅₀ of 0.713 ± 0.035 mg/mL and 2.409 ± 0.028 mg/mL, respectively,

showing good enzyme inhibition activity. This α -amylase inhibition analysis can be related to the previously reported antihyperglycemic activities. At two different doses of 250 and 500 mg/kg body weight, the root extract significantly increased glucose tolerance as compared to the negative control ($p < 0.01$ and $p < 0.001$). At 120 minutes, both of the aforementioned doses of crude extracts in glucose-loaded mice demonstrated the greatest potential for reducing blood glucose ²⁴.

3.5. Brine shrimp toxicity

The brine shrimp lethality experiment was used in this work to determine the lethal concentration (LC_{50}) at which 50% of exposed subjects died, and different doses of plant extracts were tested. The results obtained during these studies are shown in Table 5.

Table 5: Calculation of LC_{50} of different plant extracts.

Plant extracts	Regression equation obtained by plotting the log C against probit values of percentage mortality	LC_{50} ($\mu\text{g/mL}$)
Crude leaf extract (CLE)	$Y = 0.0511x - 1.1278$ $R^2 = 0.99995$	1940.8
Crude bark extract (CBE)	$Y = 0.0265x + 85.4$ $R^2 = 0.4616$	1963.36
Crude root extract (CRE)	$Y = 0.0276x + 80.83$ $R^2 = 0.3522$	835.35
Crude stem extract (CSE)	$Y = 0.024x + 85.317$ $R^2 = 0.4114$	2624.22

The lethality was found to be proportional to extract concentration, and the largest mortality of brine shrimp larvae occurred at a concentration of 1000 $\mu\text{g/mL}$ and the lowest mortality occurred at 10 $\mu\text{g/mL}$. It is supposed that those with LC_{50} less than 1000 $\mu\text{g/mL}$ are pharmacologically active. The LC_{50} value for the root extract of *Litsea polyantha*, which was found to be less than 1000 $\mu\text{g/mL}$, indicated that the extract was toxic to brine shrimps. The *Litsea polyantha* bark, leaf, and stem extracts also produced encouraging results, demonstrating that the material is physiologically active and free of toxicity. This

variance in the results may be caused by differences in the gathered plants' attitudes or by the conditions in the laboratory. Some of the plant extracts were found toxic against the Brine shrimp nauplii showing an LC_{50} less than 1000 $\mu\text{g/mL}$. The ethanolic extract of the root of *Litsea polyantha* the positive control vincristine sulphate showed brine shrimp lethality in a dose-dependent manner and exhibited an approximately linear correlation between the concentration and percentage (%) of mortality. The LC_{50} for the crude extract of the same genus was found to be 0.648 $\mu\text{g/mL}$ and 56.082 $\mu\text{g/mL}$ ²⁴.

Statistical analysis

Each experiment was performed three times and data were expressed as mean \pm standard deviation. The plots were constructed using Microsoft Excel, GraphPad Prism 9, and Sigma Plot.

Conclusions

Natural products derived from plants are now widely used to treat a variety of illnesses and improvement of life span. The evaluation of the antioxidant activity, antibacterial, antihyperglycemic, and cytotoxicity revealed that *Litsea polyantha* extract exhibited good biological activities. The plant is rich in total phenolic and total flavonoid content as secondary metabolites which act against infectious diseases and accomplish the medical service decreasing side effects. This report provides scientific validation for traditional uses of this plant against diabetes. The present study strongly recommended further work to isolate, purify, characterize, and standardize the bioactive constituents from the active extract of *Litsea polyantha*. The *In vitro* and *in vivo* activity of these plant extracts could be performed which will be part of the drug discovery process.

Conflicts of interest

All the authors have no conflict of interest in publishing this manuscript.

Author contribution

SB: Experimental analysis and preparation of the manuscript draft. KRS: Supervised the research,

preparation of the final manuscript, original idea presentation, study supervision, and final approval of the version to be published.

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