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Research Article

EVALUATION OF ANTIOXIDANT, ANTIDIABETIC, AND CYTOTOXIC ACTIVITIES OF *Lilium nepalense* D. DON

Chandra Bahadur Thapa[1,2], Anjana Bhattarai[1], Krishna Kumar Pant[1], Hari Datta Bhattarai[1*], Bijaya Pant[1*]

[1]Central Department of Botany, Institute of Science and Technology, Tribhuvan University, Kirtipur, Nepal
[2]Butwal Multiple Campus, Tribhuvan University, Butwal, Nepal
[*]Correspondence: hd.bhattarai@cdbtu.edu.np; bijaya.pant@cdb.tu.edu.np
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ABSTRACT

Lilium nepalense, a temperate medicinal plant, is used as a diuretic, antipyretic, tonic, flavoring agent, and heart pain treatment. This research aimed to evaluate the *in vitro* antioxidant and antidiabetic activities of the methanol, dichloromethane (DCM), and hexane fractions, and *in vivo* cytotoxic activities of the crude extracts of the bulb. The antioxidant activity was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, antidiabetic activity by α-glucosidase inhibitory assay, and cytotoxic activity in terms of LC_{50} (median lethality concentration) by Brine shrimp assay. The DCM fraction showed the strongest antioxidant activity (IC_{50}=134.99±9.75 µg/mL) and highest antidiabetic activity (IC_{50}=182.01±20.50 µg/mL) than other fractions. Similarly, the DCM fraction had the highest total phenolic contents (243.97±33.78 mg of gallic acid equivalent per gram dry weight) and highest flavonoid contents (7.68±0.85 mg of quercetin equivalent per gram dry weight) than other fractions. Moreover, the crude extract of the bulb was not found to be cytotoxic to the Brine shrimp nauplii (LC_{50}=3.83 mg/mL). It is the first report to date describing the antioxidant, antidiabetic, and cytotoxic properties of *L. nepalense*. This study concludes that the DCM fraction of the bulb could be used as an antioxidant and antidiabetic agent for therapeutic purposes; however, further identification and characterization of bioactive compounds responsible for the antioxidant, antidiabetic, and cytotoxicity is required for further validation.

Keywords: Antidiabetic, Brine shrimp, Bulb, DCM, IC_{50}, LC_{50}

INTRODUCTION

Nepal has a varied physiography ranging from 60m to 8848m above sea level, resulting in a wide range of vegetation belts ranging from tropical to alpine. About 13,067 plant species are described from Nepal (Chaudhary *et al.*, 2020), however only 2,500 species are utilized in traditional medicine to treat various illnesses and disorders (Kuwar *et al.*, 2021). Similarly, the Government of Nepal (GoN) has recognized 148 species of medicinal and aromatic plants (MAPs) in Nepal, which are distributed from lowland Terai to high Himalayan ranges (Bhattarai and Subedi, 2023). Moreover, over 701 species are utilized medicinally, 30 species are prioritized for research and development, and 12 species are prioritized for agrotechnology development in Nepal (DPR, 2017). Therefore, there is a need for modern technology to validate the long-standing use of these significant medicinal plants by analyzing both *in vivo* and *in vitro* bioactivities, as well as their phytochemical analysis for valuable biochemical profiling.

An antioxidant is a molecule that may neutralize radicals that are unstable such as oxygen, nitrogen, and lipidic radicals, and prevents or slows the oxidation of other molecules while also protecting a biological system (Ladaniya, 2023). Strong evidence suggests that the buildup of free radicals contributes to many harmful pathophysiological processes, including cancer, diabetes, cardiovascular, and neurodegenerative illnesses (Gilgun-Sherki *et al.*, 2002; Rahman *et al.*, 2015). The human body, however, contains a sophisticated network of naturally occurring enzymatic and non-enzymatic antioxidant defenses that work to neutralize the negative effects of free radicals and other oxidants. Reactive oxygen species (ROS) in excess can weaken cellular antioxidant defenses, causing oxidative stress, which damages proteins and DNA (Betteridge, 2000; Gilgun-Sherki *et al.*, 2002); cardiac arrest, malignancy, age-related disorders, metabolic disorders, and arterial sclerosis (Ames *et al.*, 1993). By providing electrons to these damaged cells, antioxidants are molecules that stop and stabilize ROS-caused cell damage, minimizing the harmful effects of ROS on human health. It is believed that oxidative stress, which results in oxidative harm to cell constituents such as lipids, proteins, and nucleic acid, is to blame for diabetes long-term complications (Rahimi *et al.*, 2005). A metabolic disorder called diabetes impairs the body's capacity to make, use, or react to insulin. Studies have demonstrated that the polyphenols (flavonoids and phenolic compounds) found in several medicinal plants serve as antioxidants and antidiabetics. Inhibiting carbohydrate hydrolyzing enzymes like α-amylase and α-glucosidase with a drug or diet might delay the synthesis or absorption of glucose, which is one of the therapeutic strategies for lowering postprandial hyperglycemia. Additionally, a workable method for general cytotoxicity testing is crucial as an initial step in the investigation of the bioactive substances found in plants. The Brine shrimp (*Artemia salina* Leach) test provides an assay that can be quick, straightforward, and more significantly, affordable and repeatable (McLaughlin, 1991).

Lilium nepalense D. Don (called Ban lasun or Khiraule in Nepali) is one of the biggest genera of the Liliaceae family, with 110 species scattered throughout the temperate northern hemisphere and into the northern subtropics (Stevens, 2015). In Nepal, *Liium* has ten different species (Press *et al.*, 2000; www.Efloras.org). *L. nepalense* can be found in Nepal in the temperate zone between 2200 and 3400 meters above sea level (IUCN, 2004). It is a 0.6 to 1 m tall erect bulbous plant (Fig. 1) that has large, prominent flowers and has been classified as a Data Deficient (DD) plant (Anonymous, 2001). Locals utilize the bulb as a diuretic, antipyretic, tonic, vegetable, and flavoring agent, and to relieve pain in the area around their hearts (IUCN, 2000). The active chemical components, or secondary metabolites, are found in different plant parts and are responsible for the medicinal characteristics of plants (Pant, 2014). More than 180 different chemicals, including steroidal saponins, phenolic glycerides, polysaccharides, alkaloids, and flavonoids have been extracted and ascertained from the genus *Lilium* (Zhou *et al.*, 2021). To the best of our information, no research has been done on the phytochemical makeup and bioactivities of *L. nepalense*. Therefore, this research aims to evaluate the antioxidant, antidiabetic, and Brine shrimp lethality bioassay of bulb extracts.

MATERIALS AND METHODS

Plant material collection

Lilium nepalense bulb (Fig. 1) was collected in May 2021 at a height of 2200 meters above sea level from Phulchowki Hill in Lalitpur, Nepal. By tallying the herbarium specimens from the National Herbarium & Plant Laboratories (KATH), the collected plant was identified as *L. nepalense*. It was then deposited to the Tribhuvan University Central Herbarium (TUCH), Tribhuvan University, Kirtipur (voucher no. C35).

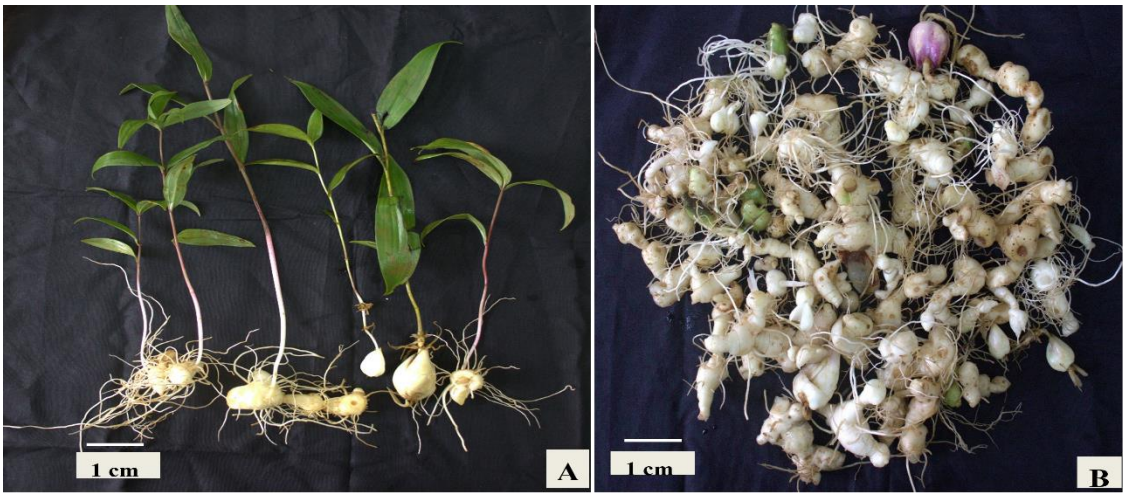

Fig. 1. *Lilium nepalense*: (A) Plants with bulbs and roots, (B) Collected bulbs.

Chemicals, reagents, and enzyme

The chemicals and enzymes utilized in this research were of analytical grade. Methanol, ethanol, dichloromethane (DCM), hexane, ascorbic acid, and gallic acid were of HiMedia Laboratories Pvt. Ltd., while α-glucosidase, 4-Nitrophenyl β-D-glucopyranoside (p-NPG), quercetin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were of Sigma Aldrich Chemicals Pvt. Ltd.

Extract preparation and fractionation of the crude extract

After completely removing all soil particles from the bulb with running water and air drying it, the bulb was ground into powder in an electric grinder. Approximately 350 g of powder was macerated in 90% methanol at room temperature for 48 h before being filtered with the help of filter paper. The filtrate was evaporated in a rotavapor (EYELA Co, Ltd, USA) at a low pressure to produce the crude extract. For fractionation, 50 g of the crude extract was dissolved in 100 mL of distilled water to form a mixture solution. At first, the mixture solution was mixed with hexane in a 1:2 ratio in a separating funnel and shaking well for 3-4 min separated the upper hexane layer from the aqueous layer, and then the aqueous layer was mixed with DCM in a 1:2 ratio to separate the lower DCM layers from the aqueous layer successively. This process was repeated three times in the separating funnel. The hexane and DCM layers were concentrated in a rotary evaporator under low pressure to get hexane and DCM fractions. Moreover, the aqueous layer was concentrated in a rotavapor under reduced pressure to get an aqueous extract. The aqueous extract was dissolved in 100% methanol, which was then concentrated in a rotavapor at a low pressure to get a methanol fraction. They were kept in vials and deposited at 4°C in the freezer for further use.

Antioxidant activity by DPPH assay

The antioxidant activity of the fractions was tested by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay following Blois (1958) and Desmarchelier *et al.* (1997) with some modifications. 100 µL of DPPH solution (0.1 mM of DPPH) was combined with 100 µL of different concentrations of extracts (25 to 400 µg/mL) in a 96-

well plate. A spectrophotometer (Synergy BioTek, Co. Ltd, UK) was used to measure the mixture's absorbance at 517 nm after it had been incubated at ambient room temperature in the dark for 30 minutes. Ascorbic acid-containing reaction mixture and reaction mixtures without the crude extracts served as the corresponding positive and negative controls. The formula below was used to compute the percentage of DPPH radical scavenging activity:

$$\% \text{ DPPH radical scavenging activity} = \frac{Ab_0 - Ab_1}{Ab_1} \times 100 \ldots (1)$$

Ab_1 is the absorbance of the extracts or standard, while Ab_0 is the absorbance of the negative control. After plotting the percentage of inhibition versus concentration, the IC_{50} was determined. At each concentration, the experiment was run in triplicate.

Estimation of total phenolic content (TPC) and total flavonoid content (TFC)

The TPC of the plant extract (1 mg/mL) was evaluated using Folin-Ciocalteau reagent (FCR) comprising gallic acid as standard pursuing Zhang *et al.* (2006) with some changes. The mixture solution containing 20 µL of various plant extract or standard gallic acid, 100 µL FCR (10%) followed by 80 µL Na_2CO_3 (1 M) was incubated in the dark for 15 minutes and absorbance was taken at 765 nm. Using a gallic acid standard curve, the result was reported as milligrams of gallic acid equivalent per gram of dry weight (mg of GAE/g) of the extract.

Similarly, an aluminum chloride complex-forming assay was used to assess the TFC of the fractions comprising quercetin as standard following Chang *et al.* (2002) with some changes. The whole assay mixture containing 130 µL standard quercetin or 20 µL plant extract with 110 µL distilled water, 60 µL ethanol, 5 µL 10% $AlCl_3$, and 5 µL 1 M potassium acetate were incubated in the dark for 30 minutes and absorbance was taken at the 415 nm wavelength. Using the quercetin standard curve, the result was reported as milligrams of quercetin equivalent per gram of dry weight (mg of QE/g) of the extract.

***In vitro* α-glucosidase inhibition assay**

The antidiabetic activity of fractions was determined by measuring the alpha-glucosidase inhibitory activity (van de Laare *et al.*, 2005). A 96-well plate was filled with a mixture of 20 µL of various extract concentrations (50-400 µg/mL), 20 µL of α-glucosidase enzyme (0.5 unit/mL), and 60 µL of phosphate buffer (6.8 pH, 0.1 M). Initial absorbance was taken at 405 nm in a spectrophotometer (Synergy LX) after the mixture solution was pre-incubated at 37°C for 15 min. The mixture solution was then mixed with 40 µL of p-NPG (5 mM), and it was incubated at 37°C for 15 min. By adding 60 µL of Na_2CO_3 (1 M), the reaction process was stopped. In a spectrophotometer, the final absorbance was taken at 405 nm which calculates the volume of p-nitrophenol emitted from p-NPG. Acarbose was used as a positive control and phosphate buffer as a negative control of alpha-glucosidase inhibitors. The average of the three results from each experiment was recorded. The given formula was used to determine the % inhibition of α-glucosidase and the concentration of extract necessary to inhibit 50% of alpha-glucosidase activity (IC_{50}) was calculated using a linear regression equation.

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, A_0=Absorbance of enzyme-substrate reaction with phosphate buffer

A_1= Absorbance of enzyme-substrate reaction with plant extracts

Cytotoxicity test by Brine shrimp lethality assay

The cytotoxicity of crude extract was determined on live Brine shrimp nauplii by following Meyer *et al.* (1982) and Fatope *et al.* (1993) with slight modification. To prepare an artificial seawater solution, 3.5 g of NaCl was initially added to 100 mL of distilled water. Brine shrimp (*Artemia salina*) eggs weighing about 10 mg were incubated in seawater for 48 h with the temperature set at 23°C (using an 80-watt bulb) to hatch eggs into larvae, or nauplii. In 5% DMSO, an extract stock solution (10 mg/mL) was prepared. Then, each concentration of plant extracts such as 5.0 mg/mL, 4.0 mg/mL, 3.0 mg/mL, 2.0 mg/mL, 1.0 mg/mL, 0.5 mg/mL, and 0.25 mg/mL with seawater containing 25 nauplii were added in a 2 mL Eppendorf tube separately. Instead of using plant extract, seawater was utilized as a negative control, while potassium dichromate was used as a positive control. Three duplicates of each experiment were carried out. The tubes were exposed to light for 24 hours and then, all living nauplii were counted. The lethal concentration causing death in 50% (LC_{50}) for each plant extract was determined using the given formula and regression line obtained by plotting the concentration against the mortality percentage on a probit scale.

$$\% \text{ mortality} = \frac{\text{No.of dead larvae (Nauplii)}}{\text{Initial No.of live larvae (Nauplii)}} \times 100 \ldots\ldots (3)$$

Data analysis

For each sample, the experiment was done in triplicate, and results were revealed as mean ±S.D. IC_{50} and LC_{50} values were analyzed using a linear regression equation. Microsoft Excel 2010 was used for all calculations.

RESULTS AND DISCUSSION

Yield of crude extract and fractions

The cold and hot maceration of 350 g powder of *L. nepalense* bulb in 90% methanol yielded 14.28% crude extract (Table 1). The three solvents had quite varied yields, and the polarity-based fractionation showed that methanol fraction had highest yield (31.5%), followed by DCM fraction (0.62%), and then hexane fraction (0.56%) (Table 1). It showed that the polar solvent yields were higher than the non-polar solvent yields. Fractionation of crude extract by liquid-liquid partition method relies on the polarity of the used solvent and the polarity of the components. Plants contain a variety of

bioactive chemicals with varying polarities. The polarity of the target compounds must therefore be complemented by the extraction solvent. Hexane, DCM, and methanol were the three solvents used in this study to examine extraction yields.

Table 1. Yield of crude extract and fractions (Hexane, DCM, and Methanol) in the bulb of *L. nepalense.*

Powder/Crude extract taken (g)	Method applied	Solvent used	Amount of Crude extract/fraction (g)	Yield (%)
Powder (350 g)	Cold & hot maceration	90% Methanol	Crude extract (50g)	14.28%
Crude extract (50 g)	Liquid-liquid partition	Hexane	Hexane fraction (0.289 g)	0.56%
Crude extract (50 g)	Liquid-liquid partition	DCM	DCM fraction (0.31 g)	0.63%
Crude extract (50 g)	Liquid-liquid partition	100% Methanol	Methanol fraction (15.75 g)	31.50%

Antioxidant activity by DPPH assay

The DPPH assay was used to assess the free radical scavenging capacity of the methanol, DCM, and hexane fractions in an *in vitro* condition. Antioxidants' ability to donate hydrogen is largely responsible for their influence on the scavenging of DPPH radicals. Comparing the antioxidant value to the positive control ascorbic acid, which had an IC_{50} of 19.81±0.603 µg/mL, the antioxidant value ranged from 134.99±9.75 to 2510.24±96.54 µg/mL (Table 2). This study demonstrated that, compared to other fractions, the DCM fraction exhibited the strongest free radical scavenging activity (IC_{50}=134.99±9.75 µg/mL) (Fig. 2). It might be because DCM is an intermediate polar solvent, allowing the synergistic effects of both polar and nonpolar types of compounds to manifest themselves (Thapa *et al*., 2023). Similarly, the hexane fraction had the lowest antioxidant activity (IC_{50}=2510.24±96.54 µg/mL), which may be related to the fact that hexane is a nonpolar solvent that dissolves nonpolar compounds having lower antioxidant capacity. The DCM has stronger antioxidant activity than other fractions, according to research done on *Syzygium cumini* leaf (Franco *et al*., 2020), *Apium graveolens* (Emad *et al*., 2022), and *Paris polyphylla* rhizome (Thapa *et al*., 2023). Although the bulbs of the *Lilium* species are frequently used as food and medicine, there have been very few studies examining their antioxidants and other bioactivities. According to Jin *et al.* (2012), six species of *Lilium*, including *L. regale, L. concolor, L. pumilum, L. leucanthum, L. davidii* var. *unicolor*, and *L. lancifolium,* have higher phenolic contents and antioxidant properties. Locals consume the bulbs of *L. nepalense* as food, vegetables, and medicines, therefore thorough investigation into its bioactivities and phytochemical profile may open up new opportunities for the food and drug industries.

Table 2. Antioxidant activity, total phenolic content, total flavonoid content, and α-glucosidase inhibition of various fractions of *L. nepalense*.

Fractions	Antioxidant Activity (IC_{50}: µg/mL)	Total phenolic content (mg of GAE/g dry wt)	Total flavonoid contents (mg of QE/g dry wt)	Antidiabetic activity (IC_{50}: µg/mL)
DCM	134.99±9.75	243.97±33.78	7.68±0.85	182.01±20.50
Hexane	2510.24±96.54	4.58±0.99	0.55±0.48	2767.53±158.72
Methanol	1650±263.91	75.38±10.98	1.03±0.21	1903.85±90.24
Control	19.81±0.603	-	-	125.73±13.54

Total phenolic contents (TPC) and total flavonoid contents (TFC)

Using the gallic acid calibration curve (10-60 µg/mL, Y= 0.018x, R^2 = 0.980), the total phenolic content of the methanol, DCM, and hexane fractions of the *L. nepalense* bulb was calculated by applying the Folin-Ciocalteu Reagent (FCR) concerning gallic acid equivalent (mg of GAE/g of the dry weight of extract). The highest total phenolic content was found in DCM fraction (243.97±33.78 mg GAE/g dry weight), followed by methanol fraction (75.38±10.98 mg GAE/g dry weight) and hexane fraction (4.58±0.99 mg GAE/g dry weight) (Fig. 3).

Similarly, the total flavonoid content of the methanol, DCM, and hexane fractions of *L. nepalense* bulb was determined in terms of mg Quercetin equivalent per gram of dry mass (mg of QE/g dry weight) of extract. The flavonoid content of the various plant extracts was determined by applying the calibration curve that was created using quercetin (10-80 µg/mL) with the line of equation (Y=0.0256x, R^2=0.983). The higher total flavonoid content was reported in the DCM fraction (7.68±0.85 mg of QE/g dry weight), followed by methanol fraction (1.03±0.21 mg of QE/g dry weight) and hexane fraction (0.55±0.48 mg of QE/g dry weight) (Fig. 4).

This study demonstrated a positive relationship between the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant (free radical scavenging) activity of the tested extracts, i.e., the fraction with better antioxidant activity (less IC_{50}) had a higher total phenolic content and total flavonoid contents. Therefore, the total phenolic and total flavonoid contents of the extracts/plants might determine their antioxidant activity.

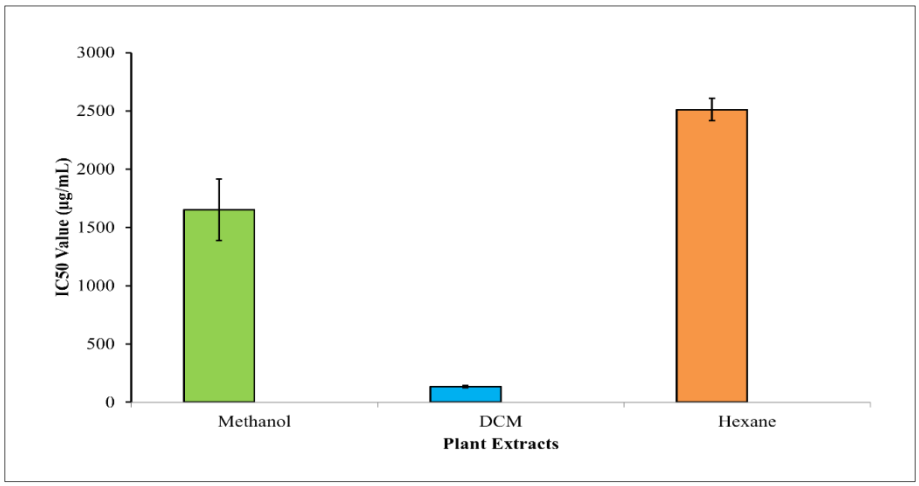

Figure 2. Antioxidant activity (IC_{50}) of various fractions of *L. nepalense* (Note: Error bars represent the standard deviation of three independent measurements).

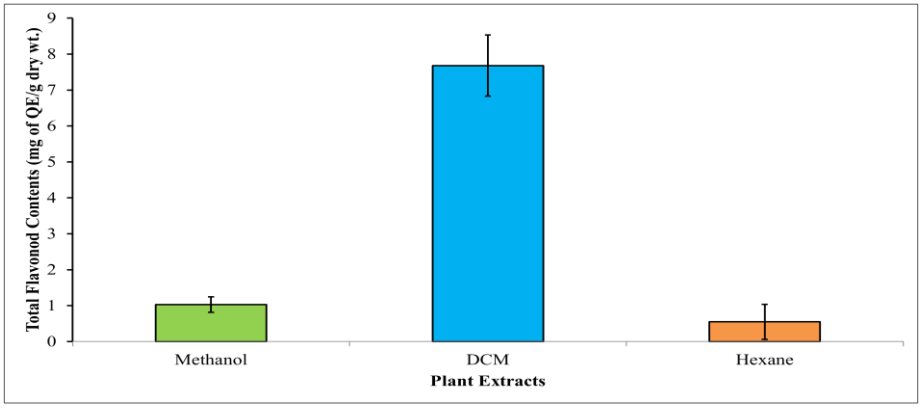

Figure 3. Total phenolic contents (TPC) of various fractions of *L. nepalense* (Note: Error bars represent the standard deviation of three independent measurements).

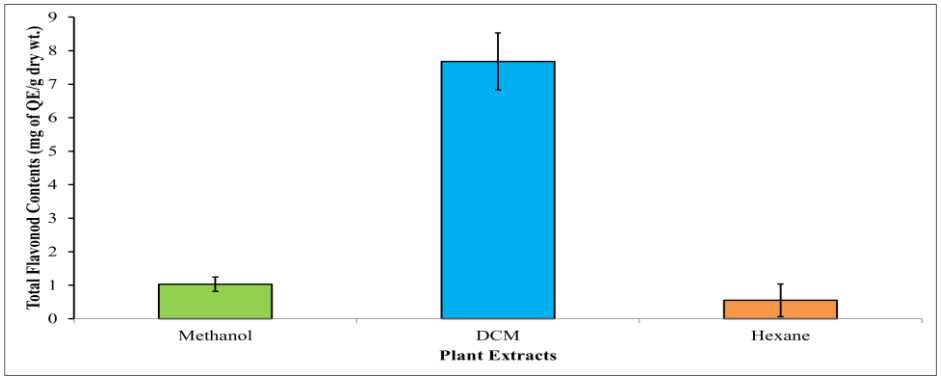

Figure 4. Total flavonoid contents (TFC) of various fractions of *L. nepalense.* (*Note: Error bars represent the standard deviation of three independent measurements*).

In vitro α-glucosidase inhibition assay

The antidiabetic activity of the DCM, methanol, and hexane fractions was assessed by alpha-glucosidase inhibition assay. The highest alpha-glucosidase inhibitory activity was found in the DCM fraction (IC_{50}=182.01±20.50 µg/mL), followed by methanol fraction (IC_{50}=1903.85±90.24 µg/mL) and hexane fraction (IC_{50}=2767.53±158.72 µg/mL) in comparison to the positive control acarbose (IC_{50}=125.73±13.54 µg/mL) (Table 2 & Fig. 5). However, Mir *et al.* (2020) reported that the water extract of *Lilium polyphyllum* had the highest inhibition percentage on α-glucosidase than ethanol and DCM crude extracts. The DCM fraction had stronger antidiabetic activity than other fractions, according to research done on the olive mill plant waste (Mwakalukwa *et al.*, 2020), *Clerodendrum volubile* (Erukainure *et al.*, 2018), and *Syzygium cumini* leaf (Franco *et al.*, 2020). The level of α-glucosidase inhibitory

compounds in the DCM fraction may be higher than in other fractions. Furthermore, there are a few reports on antidiabetic research on the bulb of *Lilium* species. *In vivo* and *in vitro* studies have demonstrated possible hypoglycemic action in extracts of various *Lilium* species, encompassing *L. lancifolium* and *L. brownii* var. *viridulum* (Zhou *et al*., 2021).

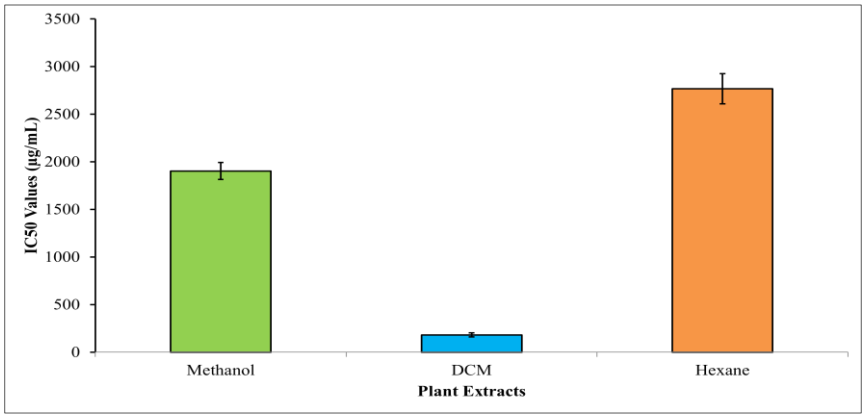

Figure 5. Antidiabetic activity (IC_{50}) of various fractions of *L. nepalense.* *(Note: Error bars represent the standard deviation of three independent measurements).*

The conversion of starch into simple sugars is facilitated by the alpha-glucosidase enzyme. These enzymes help in the breakdown of the carbohydrates and starches that make up our diets into glucose, which is absorbed through the intestines and increases blood sugar levels. In type 2 diabetic patients, α- glucosidase is thought to be one of the potential targets for lowering post-prandial glucose levels. However, α-glucosidase inhibitors prevent the small intestine from absorbing carbohydrates. They block enzymes that change complicated non-absorbable carbohydrates into simple absorbable carbohydrates through competitive inhibition. Numerous phytoconstituents with α-glucosidase inhibitory action have been identified from various plants, including 103 flavonoids, 61 terpenes, 8 steroids, 37 alkaloids, 37 phenols, 49 quinines, 73 phenylpropanoids, 43 additional chemicals (Kumar *et al*., 2011; Yin *et al*. 2014).

The antioxidant and antidiabetic activities of the tested extracts/plants were found to be positively correlated in this study, meaning that the fraction with stronger antioxidant activity also had better α-glucosidase inhibitory activity. As a result, the extracts' or plants' antioxidant activity may affect how effective they are at preventing diabetes.

Cytotoxicity test by Brine shrimp lethality assay

The Brine shrimp (*Artemia salina*), a simple zoological creature, has been used by many researchers to test the lethality of plant extracts (Fig. 6). It is an easy and affordable bioassay used to assess the preliminary toxicity screenings for plant extracts and for screening different chemical components present in different bioactivities. This study found that no nauplii died below a concentration of 500 µg/mL and that only 4% of nauplii died at a concentration of 1 mg/mL of crude extract after 24 h observation. It can be because the plant extracts have fewer toxic metabolites present in lower concentrations for nauplii. The degree of mortality was discovered to be equivalent to the concentration of the extracts (Table 3).

According to this study, the crude extract of the bulb showed less cytotoxicity on nauplii and had an LC_{50} of 3.83 mg/mL in comparison to the positive control potassium dichromate (LC_{50}=134.34±15.66 µg/mL). Any plant extract with an LC_{50} of less than 1000 µg/mL is regarded as pharmacologically active (Meyer *et al*., 1982). Moreover, it is a quick and thorough test that uses a large number of organisms for statistical validation, requires no specialized equipment, and only a small amount of material for bioactive compounds of either natural or synthetic origin (Kwon *et al*., 2007).

Table 3. Brine shrimp lethality test in the crude extract of *L. nepalense.*

Extract concentration (mg/mL)	No. of surviving Nauplii			Average No. of surviving Nauplii	% Mortality	LC_{50} (mg/mL)
	T_1	T_2	T_3			
0.25	25	25	25	25	0	3.83
0.5	25	24	25	25	0	
1.0	25	23	24	24	4	
2.0	20	19	21	20	20	
3.0	14	16	15	15	40	
4.0	13	12	11	12	52	
5.0	9	7	8	8	68	
Negative control	25	25	25	25	0	

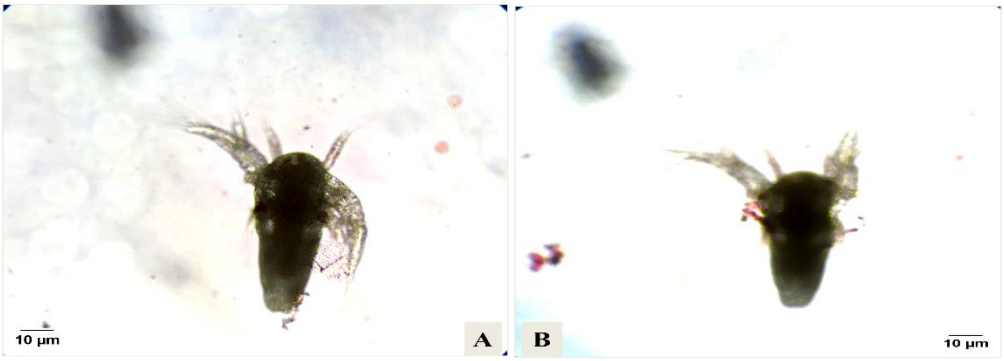

Figure 6. Microscopic images of Brine shrimp larvae (nauplii) after 48 h incubation at 23°C in artificial sea-water solution.

CONCLUSIONS

This study revealed that the DCM fraction of the *L. nepalense* bulb might be used as a source of antioxidants and anti-diabetic agents for therapeutic purposes. Therefore, it might be suitable for consumption and might serve as a source of antioxidant and anti-diabetic agents from the diet. Although, it seems less toxic to Brine shrimp, it might be a good idea to thoroughly explore its anticancer or cytotoxic properties against many types of human cancer cell lines. It is also necessary to explore bioactive compounds responsible for antioxidant, antidiabetic, and cytotoxic activities.

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AUTHORS CONTRIBUTION

Conceptualization: CBT, BP & HDB; Fieldwork: CBT and AB; Laboratory work: CBT & AB; Supervision: HDB & BP; Writing original drafts: CBT; Review and editing: HDB, BP, and KKP.

CONFLICT OF INTEREST

The authors do not have any conflict of interest in this research work.

DATA AVAILABILITY

The raw data used in this work can be obtained upon request from the corresponding author.

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