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Assessing the antioxidant antibacterial and toxic potential of *quercus floribunda* lindl bark

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Abstract

Since ancient times, herbal medical practitioners have been treating a wide range of conditions, from basic to life-threatening, with traditional or folk remedies. Phytochemicals are incredibly helpful in defending against numerous diseases in human beings. Due to their affordability and lack of side effects, herbal medicines have replaced synthetic drugs in many countries. The goal of the present study is to analyze the total phenolic content (TPC) and total tannin content (TTC) by the Folin-Ciocalteu method, total flavonoids content by the Aluminum chloride method, DPPH radical scavenging activity, evaluation of antimicrobial activity by agar-well diffusion method and evaluation of cytotoxic activity of crude extract. The dried bark of the Quercus floribunda Lindl was powdered, and then the extract was prepared in different solvents chosen based on their polarity using the cold maceration technique. Among different crude extracts, ethyl acetate extract has a high TPC (127.23 ± 1.65 mg GAE/g), and Dichloromethane (DCM) has the lowest TPC (9.25 ± 0.69 mg GAE/g). Also, hexane extract shows high TFC (61.72 ± 4.01 mg QE/g), and ethanolic extract shows the least TFC content (3.97 ± 1.03 mg QE/g). Furthermore, ethanolic extract had the highest TTC which is 49.91 ± 1.06 mg TAE/g, and least TTC value in hexane extract which is 5.51 ± 1.3 mg TAE/g. Along with this, methanolic extract showed good antioxidant ability with IC_{50} of 12.70 ± 0.37 µg/ mL. In the case of the antimicrobial susceptibility test, the ethanolic and ethyl acetate extract of bark was found to be effective against Escherichia coli (ATCC 25912) with a zone of inhibition of 15 mm, equal to positive control, and neomycin. Ethyl acetate extract is also found effective against Shigella sonnei (ATCC 25931) with a zone of inhibition of 22 mm. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also estimated in two different bacterial strains. For methanolic extract, the MIC value for Gram-positive Staphylococcus aureus (ATCC 43300) was 3.125 mg/mL, and Gram- negative Shigella sonnei was 3.125 mg/mL, and the MBC was 25 mg/mL for both strains. The methanolic extract shows good cytotoxicity against brine shrimp nauplii with LC50 of 40.55 µg/mL. Thus, from the overall study, the bark of Quercus floribunda Lindl could be used as a natural source to isolate antibiotics and antioxidants.

Keywords

Quercus floribunda Lindl, Antimicrobial, Antioxidant, Cytotoxicity, Phytochemistry.

Article information

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1 Introduction

Nature is a remarkable indicator of the numerous phenomena of coexistence. The foundation for treating human illnesses is natural items derived from plants, animals, and minerals. Right now, there is a growing demand for and acceptance of medicinal herbs. Plants undoubtedly contribute significantly to ecosystems by offering vital services [1]. Almost every region in the world has a remarkable history of using medicinal plants to benefit humankind. This amazing knowledge has been passed down from generation to generation by traditional healers. The deep understanding of this natural medicine paradigm has remained unchanged despite modernization and cultural changes. A new revolution in the treatment of diseases might undoubtedly be brought about by phytochemical research combined with pharmacological thinking in light of traditional uses [2].

Significant medications like atropine, codeine, digoxin, morphine, quinine, and vincristine are derived from plants and the secondary metabolite components of those plants. Plants have a long history of usage in both modern "Western" medicine and some traditional medical systems [3]. Humans could choose which plant tissues to consume based on their observation that certain plant tissues, such as fruit, leaves, or roots of certain species, improved their state of mind. Aromatic and medicinal plants are a valuable source for creating novel medications and treating physical and mental health issues [4]. The pharmaceutical industry developed various commercial synthetic medications, the past was referred to as the "synthetic era". Continuous usage of synthetic medications over time resulted in serious side effects and microbial resistance. Large populations cannot pay the high cost of synthetic pharmaceuticals to profit from them. A worldwide movement has emerged in recent decades that has centered on green medicines because of their low side effects and affordability. The discovery of medicinal plants was crucial to the creation of modern herbal remedies because allopathy is unable to fully treat several illnesses, including cancer, liver disorders, and arthritis. When there are no adequate anti-diabetic, anti-inflammatory, anti-arthritic, or chemotherapy drugs available, the bioactive components of medicinal plants are utilized [5].

Plant-based products are expected to be worth $83 billion globally, and this sector is still expanding. Moreover, it is estimated that up to 60% of anticancer medications and about 25 % of contemporary drugs are derived from natural sources. The WHO estimates that between 65%-80% of people in underdeveloped nations currently use medicinal plants as a form of treatment. Since just 15% of the world's 300,000 plant species have been assessed to establish their pharmacological potential. Research highlighting the value and effectiveness of therapeutic plants is being conducted across the globe in a variety of nations at different stages of development [6]. According to the World Health Organization, a plant is considered medicinal if it contains chemicals that have therapeutic value or serve as precursors for the semi-synthesis of chemotherapeutic medications (WHO). Plants consist of several parts, including leaves, stems, rhizomes, bark, seeds, flowers, and fruits. These plant sections consequently contain chemical substances that are useful for medicinal purposes [7].

According to Bhattarai and Ghimire, 143 species of commercial Medicinal Aromatics Plants (MAP_S) were evaluated from the Himalayan gradient [8]. A total of 161 species of medicinal plants have been reported to be used by the Tamang community in Makwanpur district [9]. The Meche people have been reported to use 64 plant species from the Jhapa district [10]. *Quercus floribunda*, also known as the Tilonj oak, green oak, Moru, or Mohru oak, is a species of oak that is endemic to Afghanistan, Pakistan, Nepal, and the western Himalayas of India. It is usually found at elevations between 2,000-3,000 meters (6,600- 9,800 feet) above sea level. The tree is an evergreen and an important species for fuelwood and fodder, with a dense crown that reaches

up to 30 meters (98 feet) [11]. *Quercus floribunda* has a straight trunk that may grow up to 45 meters in height and 2 meters in diameter. It also has a dense crown. As the bark ages, it becomes dark grey or dark reddish brown and exfoliates in uneven woody scales. The bright green, 4-8 cm long, lanceolate to elliptic moru leaves can have smooth or spiky edges. Catkins in male inflorescences are 8 cm long. The length of the female spikes is 4 cm. The fruit is an ovoid or oblong, brown, 2 cm long acorn with a sharp tip that grows alone on the branches from the previous year [12].

Quercus species, commonly referred to as oaks, are a significant genus within the Fagaceae family. It is found extensively in tropical climates and temperate woodlands in the northern hemisphere. Numerous constituents have been employed in conventional medicine to address and prevent a range of human diseases, including diarrhea, ulcerative gastritis, asthma, hemorrhoids, and wound healing. Bioactive chemicals, including triterpenoids, phenolic acids, and flavonoids, have been linked to a variety of biological activities, including anti- inflammatory, antibacterial, hepatoprotective, antidiabetic, anticancer, gastroprotective, antioxidant, and cytotoxic properties [13].

The decoction or infusion include styptic, hemostatic, antibacterial, antifungal, and antiseptic ef-

fects. It is taken orally to treat conditions like severe diarrhea, dysentery, and hemorrhages. It is used topically on the outside to treat burns, wounds, and a range of skin disorders, hemorrhoids, and irritation of the anal, vaginal, and oral mucosa. It can also be used as a mouthwash to treat gum disease and toothaches. Plant extracts can be used in lotions and ointments to aid in the healing of wounds [14]. The *Quercus* species yields the well-known acorn, which is used in traditional medicine together with bark and leaves. These parts of the plant are used as antiseptics or to treat gastrointestinal issues. Both people and animals eat acorns because of their nutritional value. Oak wood is valuable for its color, durability, and resistance to fungal deterioration in the wood industry as well as for wine maturation in oak barrels [15]. The analysis of phytochemicals and evaluation of biological properties of bark extracts, including antioxidant, antimicrobial, and toxicity against brine shrimp nauplii, have not been well reported yet. So, the proposed research work plays a significant role in fulfilling the research gap. The major phytoconstituents previously reported from the genus Quercus are shown in **Figure 1**

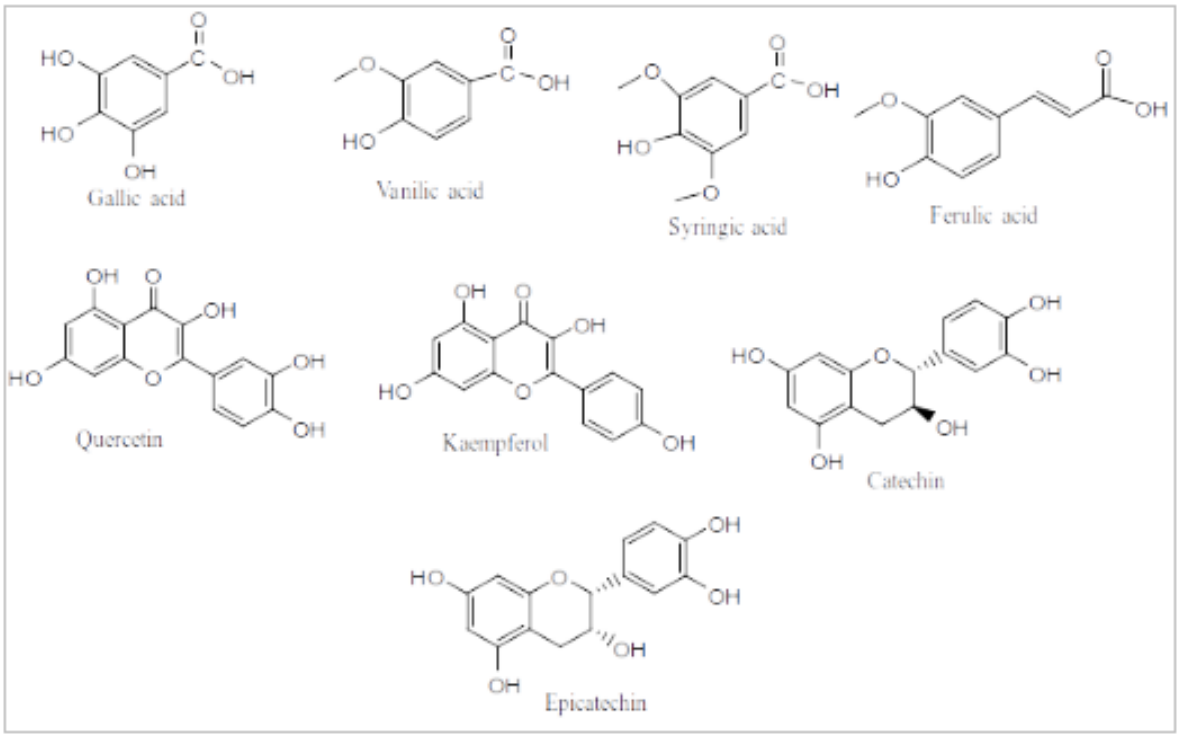

Figure 1: Structure of different phenolic compounds previously reported from Quercus species.

2 Materials and Methods

2.1 Chemicals

Ethyl acetate, hexane, DCM, ethanol, and methanol solvents were purchased from Merck in Germany and Thermo Fisher Scientific in India. Similarly, Gallic acid and Folin Ciocalteu (FC reagent) and other chemicals from Loba Chemie. Dimethyl sulfoxide (DMSO) (Silico Research Laboratory, India), acetic acid (Control Drug House, Gujarat, India), Quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Srichem, Maharashtra, India), potassium acetate (Loba Chemie, PVT Ltd., Mumbai, India), sodium carbonate anhydrous, tannic acid, ferrous nitrate, Müller Hinton broth (MHB), Muller Hinton Agar (MHA), and Nutrient Agar (NA) Growth Nutrient Agar were purchased from HiMedia Lab, Mumbai.

2.2 Collection and identification of the plant

Based on the indigenous knowledge from the local user and literature survey, the plant was collected from the Himali Gaupalika Bajura district at 2500m, latitude 29.53°N and longitude

81.67°E. After plant collection, the plant parts needed to be processed for the herbarium. The herbarium was created once the plant had fully dried and was taken to the National Herbarium and Plant Laboratories in Godavari, Lalitpur, for identification. The plant was identified as *Quercus floribunda* Lindl by the National Herbarium and

Plant Laboratories. The voucher code is provided as KATH163578. The photograph of the herbarium and collection sites of *Q. floribunda* is shown in **Figures 2** and **3.**

Figure 2: Photograph of the herbarium of *Q. floribunda*.

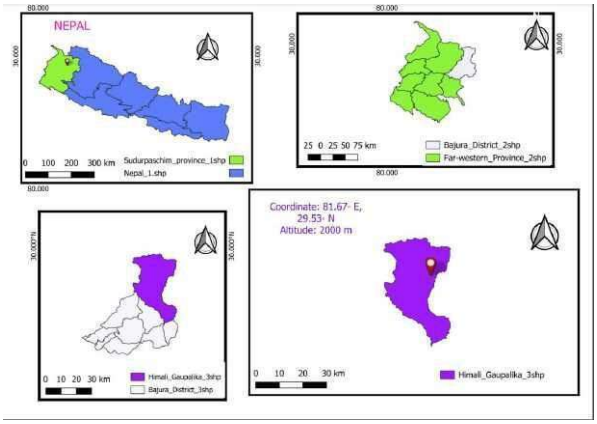

Figure 3: Maps of sample-collecting sites..

The phytochemicals flavonoids, gallotannin, and ellagitannin were found in the aqueous bark extract of *Quercus acutissima*. This plant could be used to block 5-reductase activity and testosterone-induced sebum synthesis in rats; it decreased androgen-related pathogenes of acne, testosterone conversion, and sebum synthesis [16]. The methanolic extract of the same species contains phenolic acids and could show anticancer activity against breast cancer cell lines, cervical cancer cells, human T lymphocyte cells, human colon cancer cell lines, and human embryonic kidney cells [17]. The ethanolic leaf extract of *Quercus salicina* Blume depicts a vasodilatation effect on porcine coronary artery endothelium and good antioxidant activity [18]. The *Quercus sideroxyla* shows antihyperglycemic activity, maintaining the glucose levels at more stable levels by inhibiting the -amylase enzyme [19]. The aqueous extract of the same species consists of gallic acid, catechin, epicatechin, procyanidins, and proanthocyanidins, and these phytochemicals show antioxidant activity [20].

2.3 Preparation of extract

The bark of this plant was collected, cleaned, shade dried, sliced into small pieces, and dipped in six different solvents based on polarity: Aqueous, methanol, ethanol, ethyl acetate, DCM, and Hexane. After 72 hours, filtration was done using filter paper, and the solvents were re-suspended for 48 hours and consecutively for 24 hours one last time. Every time, the filtrates were collected and concentrated under a vacuum in a rotatory evaporator at 40 °C. The extracts were collected in glass vials and stored at 4 ^{o}C for future analysis. The following formula was utilized for the calculation of the yield percentage of the crude extract.

2.4 Qualitative phytochemical analysis

The presence of secondary metabolites, alkaloids, flavonoids, polyphenols, lignins, tannins, steroids, and saponins was determined by observing a color change in the chemical process, and phytochemicals found in different extracts were qualitatively identified by following the standard protocol [21].

2.5 Estimation of total phenolic content (TPC)

The Folin-Ciocalteu colorimetric method was used to estimate the total phenolic content of plant extracts [22–24]. In triplicate, 96-well plates were loaded with 20 µL of plant extract, 100 µL of 10% FC reagent (1:10), and 80 µL of 1M Na2CO3. Before an intense blue color was noticed, the reaction mixture was left to incubate at room temperature for 30 minutes. Finally, absorbance at 765 nm was measured using a spectrophotometer. The total phenolic content (TPC) was measured in milligrams of gallic acid equivalent (mg GAE/g) per gram of extract dry weight, and the standard curve was created, which is the standard curve for gallic acid (7.5- 100 µg/mL).

2.6 Estimation of total flavonoid content (TFC)

The total flavonoid content was estimated by using the aluminum chloride method as described by Ahmed et al. [24, 25]. In triplicate, 96-well plates

were loaded with 20 µL of plant extract, 100 µL of distilled water, and 60 µL of ethanol, followed by 10 µL of 10% AlCl3 solution and 10 µL (1M) CH3COOK solution. At room temperature, the reaction mixture was incubated for half an hour. Then, using a spectrophotometer, absorbance was measured at 415 nm. A standard calibration curve for quercetin (10-100 µg/mL) was developed and quantified in milligrams of quercetin equivalent per gram of the dry weight of the extract (mg QE/g).

2.7 Estimation of total tannin content (TTC)

The Folin-Ciocalteu colorimetric method was used to estimate the total tannin content by applying the standard protocol [26]. Tannic acid concentrations ranged from 7.5 to 100 µg/mL, and 10 µL of plant extract was added to 96-well plates. Then, 50 µL of 10% FC reagent and 70 µL of distilled water were added, and a microplate reader was used to capture the initial reading at 725 nm. After that, 70 µL of 35% Na2CO3 was injected following the first measurement. The ultimate absorbance of the 96-well plate was measured at 725 nm after it had been incubated for 30 minutes. TTC was expressed as mg TAE/g.

2.8 Evaluation of antioxidant activity

The antioxidant activity of crude plant extract was evaluated by following a standard protocol [27, 28]. The crude plant extract was diluted serially up to the required concentrations, but the positive control quercetin was serially diluted up to 0.625 µg/mL from 20 µg/mL concentrations. In triplicate, 100 µL of plant extracts and a positive control were added to 96-well plates. The first reading was then obtained at 517 nm. After that, each well received 100 µL of DPPH reagent, which was then incubated for 30 minutes. At 517 nm, the ultimate absorbance was measured. Because methanol and 50% DMSO were employed as negative controls. The following formula was used to evaluate radical scavenging activity:

$$\text{Radical scavenging capacity} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100 \quad (1)$$

where, $A_{control}$ = absorbance of control, A_{sample} = absorbance of sample.

Inhibitory concentration (IC_{50}) was calculated by using GraphPad Prism (version 8.0.2.263).

2.9 Evaluation of antimicrobial activity

The agar well diffusion method was applied to perform an antibacterial activity using Mueller-Hinton Agar (MHA) plates [29, 30]. The test microorganisms, *Escherichia coli*, *Klebsiella pneumonae*, *Staphylococcus aureus*, and *Shigella sonnei*, were cultivated in Mueller-Hinton Broth (MHB) and incubated for 24 hours at 37 ºC. Its turbidity was maintained at 0.5 McFarland. 50 µL of plant extract, 50% DMSO as the negative control, and 50% neomycin as the positive control were added to each well created by a cork borer. The Petri plates were incubated for 18 to 24 hours at 37 °C after being left for 15 minutes to allow for diffusion. After incubation, the zone of clearing was measured and tracked.

2.10 of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration were determined by following the standard protocol as described [31]. A final concentration of 106 CFU/mL was obtained for the bacterial inoculum by diluting the 0.5 McFarland turbidity culture in MHB 1:100. Each well of the 96-well plates received an injection of 5 µL of bacteria. The positive control was a popular drug called neomycin. A sterile lid was placed over the plate, and it was incubated at 37 °C for 20 to 24 hours. 0.003% resazurin was added to the microtiter plate wells, and the mixture was incubated at 37 °C for three to four hours. While the wells without infection remained blue, the wells with bacterial growth became pink. The lowest concentration at which bacterial growth is inhibited was determined to be the extract's minimum inhibitory concentration (MIC). The MIC and MBC of the crude plant extracts were ascertained by streaking the well contents onto nutrient agar plates and then incubating them at 37 °C for over 18 hours.

2.11 Brine shrimp lethality assay (BSLA)

The toxicity of plant extracts was determined by following a standard protocol [32]. By adding 1M NaOH, the pH of the artificial sea salt water was kept between 8 and 8.5. Different quantities of the plant extract, including 1000, 800, 500, 250, 100, and 10 µg/mL, were diluted. Each test tube was then filled with 4 mL of artificial seawater. 10 nauplii and 500 µL of sample were then added in triplicate to each test tube. The positive control was a potassium dichromate solution, while the negative control was artificial sea salt water. After 24 hours, the number of dead nauplii in a test tube was counted, and the following formula was used to calculate the % mortality of nauplii:

Using the Probit value table, the linear equation can be obtained as Y = mx+c, where Y is the Pro-

bit value at 50% mortality, m is the variable, and c is the intercept. X is the lethal concentration, and finally, the lethal concentration (LC50) was calculated.

2.12 Statistical Analysis

The data was collected by using a Gen5 Microplate reader. Data analysis was carried out by using Microsoft Excel. TPC, TFC, and TTC were reported as the mean ± standard deviation. Mean ± standard error reported for antioxidants. Half maximal inhibitory concentrations (IC_{50}) were calculated by using Graph Pad Prism (version 8.0.2.263). The comparisons were made by using a one-way ANOVA test. Values with $p < 0.05$ were considered statistically different.

3 Results

3.1 Percentage Yield

The highest yield percentage was found in the aqueous extract, which is 15.24% followed by methanol (11.29 %), Ethanol (9.53 %), Ethyl acetate (3.35 %, DCM (0.71 %), and the lowest yield percentage of 0.4% in hexane extract. This is because the aqueous solvent is more polar than other solvents, and the bark might contain highly polar compounds. The bar diagram of the % yield of different solvent extracts is shown in **Figure 4.**

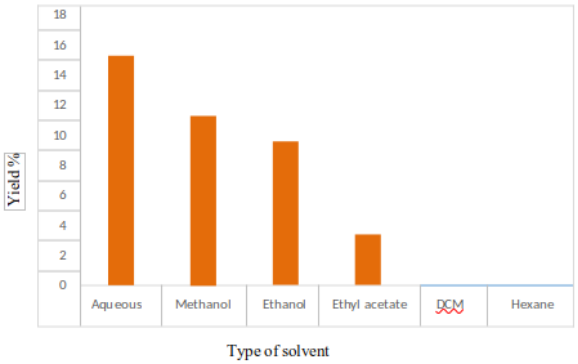

Figure 4: Showing the yield percentage in different solvent extracts.

3.2 Qualitative phytochemical analysis

Qualitative phytochemical screening of different solvent extracts of *Q. floribunda* is shown in Table 1.

Table 1: Qualitative phytochemical screening of plant extracts.

Phytochemicals	Test	Aqueous	Methanol	Ethanol	Ethyl acetate	DCM	Hexane
Alkaloids	Dragendorff's test	+	+	+	+	-	+
Carbohydrates	Molish's test	+	+	+	+	+	-
Protein test	Biuret test	-	-	-	-	-	-
Flavonoids	Alkaline reagent test	+	+	+	+	-	-
Terpenoids	Salkowski's test	+	-	+	+	-	+
Tannins	Braymer's test	+	+	+	+	-	-
Phenolic test	$FeCl_3$ test	+	+	+	+	-	-
Anthraquinones	Borntrager's test	+	+	+	+	-	-

Note: (+) = present, (-) = absent.

3.3 Total phenolic content (TPC)

The TPC of this plant ranges from 127.23 ± 1.65 mg GAE/g to 9.25 ± 0.69 mg GAE/g (Figure 5). The bark of *Quercus floribunda* Lindl showed the highest TPC value in ethyl acetate extract (127.23 ± 1.65 mg GAE/g) followed by aqueous (123.23 ±4.28 mg GAE/g), methanol (111.13 ± 1.55 mg GAE/g), ethanol (93.65 ± 2.23 mg GAE/g), and hexane (9.29 ± 0.26 mg GAE/g), and least value in DCM (9.25 ± 0.69 mg GAE/g). The TPC values are significantly different from each other at $p < 0.05$. The calibration curve is shown in **Figure 6.**

3.4 Total flavonoid content (TFC)

The TFC of *Q. floribunda* ranges from 61.72 ± 4.01 mg QE/g to 3.97 ± 1.03 mg QE/g (Figure 7). The plant *Quercus Floribunda* Lindl possesses the highest flavonoids in hexane extract (61.72 ± 4.01 mg QE/g), followed by DCM (50.21 ± 0.93 mg QE/g), aqueous (6.98 ± 0.93 mg QE/g), ethyl acetate (6.12 ± 1.47 mg QE/g), methanolic (4.08 ± 0.67 mg QE/g), and the lowest value in ethanol extract (3.97 ± 1.03 mg QE/g). Values are significantly different from each other at $p < 0.05$. The calibration curve of quercetin is shown in **Figure 8.**

3.5 Total Tannin content (TTC)

The TTC of *Quercus floribunda* Lindl ranges from 49.91±1.06 mg TAE/g to 5.51±1.3 mg TAE/g. The maximum TTC was found in ethanolic extract (49.91±1.06 mg TAE/g), followed by methanolic extract (49.51±3.33 mg TAE/g), ethyl acetate (47.35±1.69 mg TAE/g), aqueous (37.27±2.96 mg

TAE/g), DCM (14.78±1.39 mg TAE/g), and the least content in hexane extract (5.51±1.3 mg TAE/g) (Figure 9). Values are significantly different from each other at $p < 0.05$. The calibration curve of tannic acid is shown in **Figure 10.**

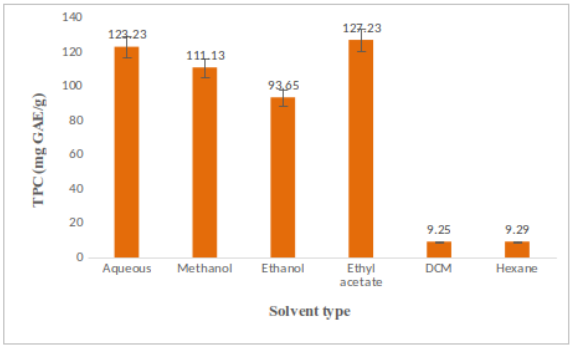

Figure 5: Standard calibration curve of gallic acid..

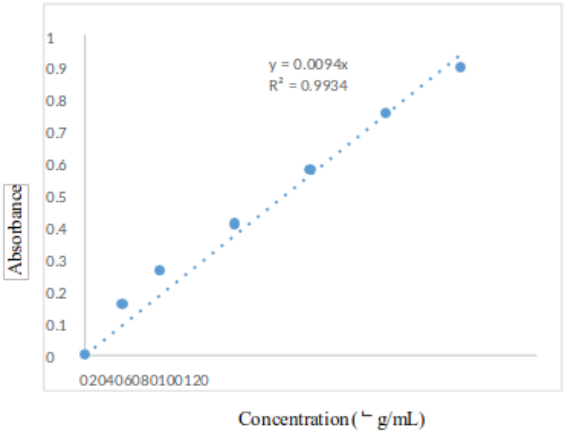

Figure 6: Total Phenolic Content in the various solvent extracts.

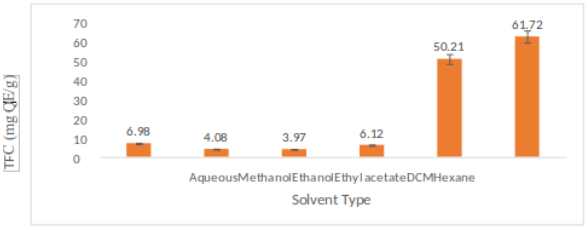

Figure 7: Standard calibration curve of quercetin.

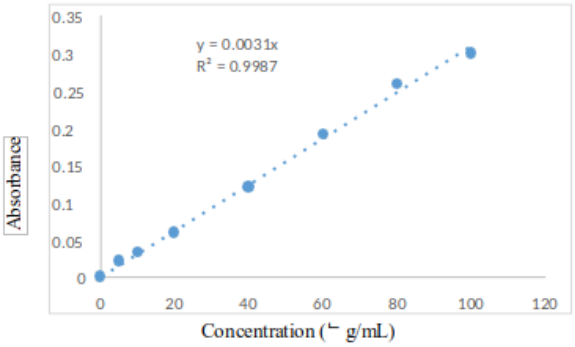

Figure 8: Total flavonoid content in various extracts.

3.6 Antioxidant activity

The DPPH free radical inhibition by various plant extracts was shown through a graphical representation (**Figures 11 and 12**). Based on the result obtained in the bark of *Quercus floribunda* Lindl possesses good antioxidant activity. Among the different extracts, the methanolic extract shows good antioxidant activity with an IC_{50} of 12.70 ± 0.37 µg/mL, followed by ethanolic extract (17.08 ± 0.48 g/mL), ethyl acetate (21.38 ± 0.66 g/mL), and Aqueous extract had the least antioxidant potential, with its IC_{50} of 29.31±1.24 µg/mL. The antioxidant potential of DCM and hexane extract is weak (> 500). Antioxidant values are significantly different from each other at $p < 0.05$. The bar diagram of IC_{50} of different solvent extracts of this plant is shown in **Figure 13.**

3.7 Antimicrobial activity

The result showed the minimum zone of inhibition for the DCM and hexane extract, which confirmed their least anti-bacterial activity. In comparison to the positive control (neomycin), the aqueous extract also does not show a good zone of inhibition. The aqueous extract showed a good zone of inhibition for *E. coli* 10 mm and *Staphylococcus aureus* 15 mm. Methanolic (14 mm), ethanolic (15 mm), and ethyl acetate (15mm) extracts showed a good zone of inhibition in *E. coli,* which is exactly the same as a positive control (15mm). Methanolic and ethanolic extracts showed the same zone of inhibition (20mm) against *Staphylococcus aureus,* whereas for the same bacteria, the positive control showed 25 mm of ZOI. For *Klebsiella pneumoniae,* methanol, ethanol, and ethyl acetate showed comparable ZOI of 15 mm, 16 mm, and 16 mm, respectively, and the positive control showed 28 mm of ZOI for the same bacteria. In the same manner for *Shigella sonnei,* ethyl acetate extract showed a good ZOI of 22 mm, whereas the positive control showed 25 mm. Also, methanolic and ethanolic ex-

tracts showed comparable ZOI of 19 mm and 20 mm, respectively. The antibacterial activity of different solvent extracts in terms of ZOI is shown in **Table 2. Figures 14 and 15** are the test slides against different microorganisms.

Table 2: Zone of inhibition (ZOI mm) shown by various extracts of *Quercus floribunda* Lindl against different bacteria and neomycin as a positive control.

Crude extract	Shigella sonnei	Klebsiella pneumoniae	Staphylococcus aureus	Escherichia coli
Aqueous	14	9	15	10
Methanol	19	15	20	14
Ethanol	20	16	20	15
Ethyl acetate	22	16	18	15
DCM	12	12	11	8
Hexane	9	9	9	9
Positive control	25	28	25	15
Negative control	-	-	-	-

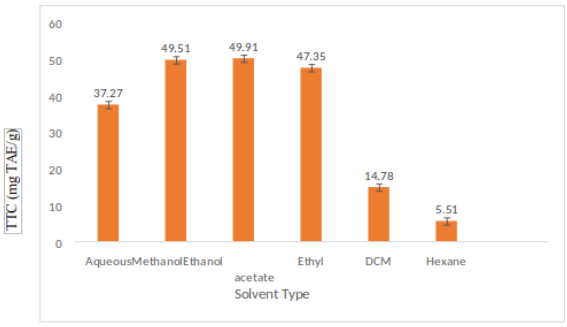

Figure 9: Standard calibration curve of Tannic acid.

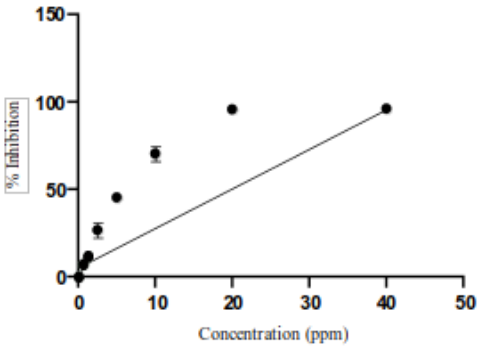

Figure 11: Standard curve of DPPH inhibition by quercetin.

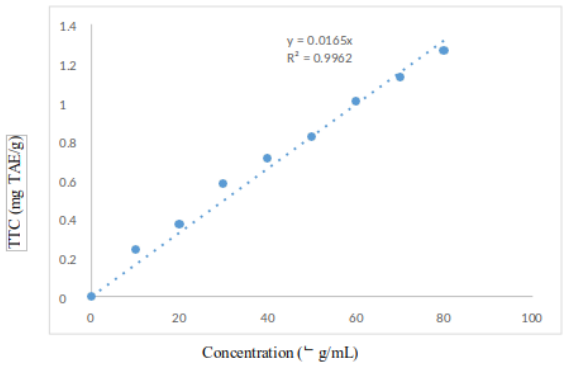

Figure 10: Total tannin content in various extracts of *Quercus floribunda* Lindl.

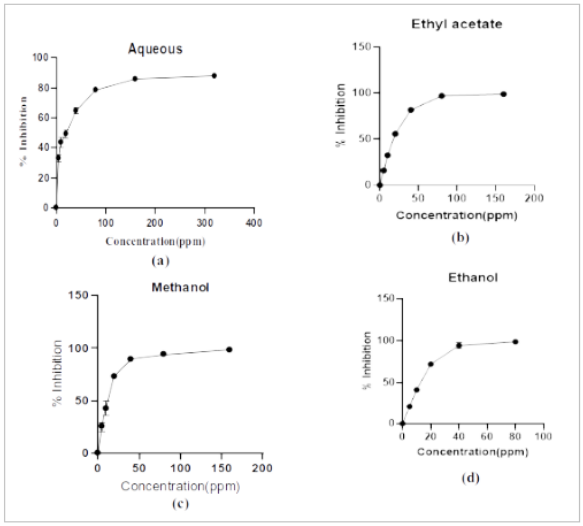

Figure 12: Plot of DPPH inhibition against the concentration of (a) aqueous extract, (b) ethyl acetate extract, (c) methanol extract, and (d) ethanol extract.

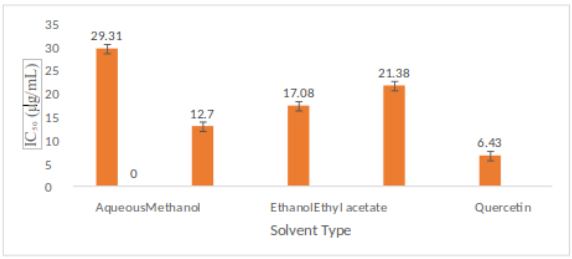

Figure 13: Bar diagram showing the antioxidant potential of bark crude extracts in different solvents at various concentrations.

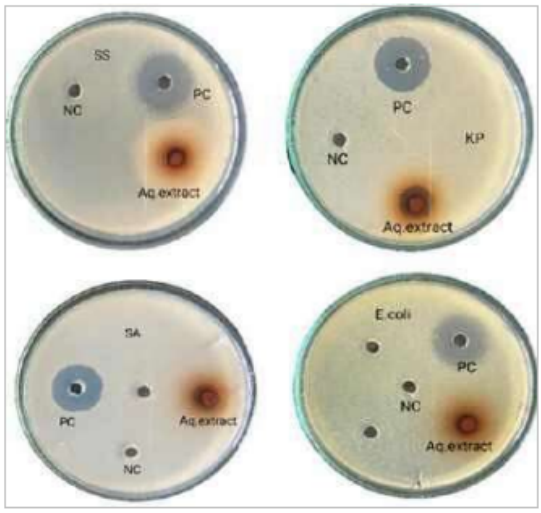

Figure 14: The ZOI shown by the aqueous extract against the bacterial strains.

SA = S*taphylococcus aureus*, KP = *Klebsiella pneumoniae*, E. coli = *Escherichia coli*, SS = *Shigella sonnei*

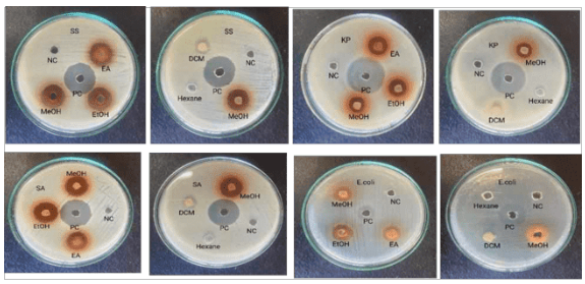

Figure 15: ZOI of methanol, ethanol, ethyl acetate, DCM, and hexane extract against different bacterial strains.

MeOH = Methanol extract, SS = *Shigella sonnei,* EtOH = Ethanol extract, SA = *Staphylococcus aureus* EA = Ethyl acetate extract, KP = *Klebsiella pneumoniae,* DCM = Dichloromethane extract, *E.coli* = *Escherichia coli,* PC = Positive control, NC = Negative control

3.8 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Methanolic extract showed the MIC and MBC value for both bacterial strains (3.12 mg/mL and 25 mg/mL, respectively, where the positive control (neomycin) shows MIC 0.78 mg/mL for both bacterial strains. The MBC value was 6.25 mg/mL for *Shigella sonnei* and 3.12 mg/mL for *Staphylococcus aureus* for neomycin. Ethanolic extract showed MIC against *Staphylococcus aureus* and *Shigella sonnei* was 1.65 mg/mL, but MBC was different for *Staphylococcus aureus,* 25 mg/mL, and more than 25 mg/mL for *Shigella sonnei.* The MBC was more than 25 mg/mL for *Shigella sonnei* by ethanolic extract because the bacterial growth in the petri dish was not completely inhibited or killed, so more than 25 mg/mL will be the required concentration to kill the bacteria completely. Similarly, for ethyl acetate extract, it was found that 3.125 mg/mL. MIC value for *Shigella sonnei* and 1.652 mg/mL for *Staphylococcus aureus,* and for both bacterial strains, the MBC value was 25 mg/mL. **Table 3** depicts the MIC and MBC of different solvents against two bacteria. The results of MIC and MBC are shown in Figure 16.

Table 3: MIC and MBC values of different extracts for gram-positive (*Staphylococcus aureus*) and gram-negative (*Shigella sonnei*).

Extract type	MIC (mg/mL)		MBC (mg/mL)	
	Shigella sonnei	***Staphylococcus aureus***	***Shigella sonnei***	***Staphylococcus aureus***
Methanol	3.125	3.125	25	25
Ethanol	1.562	1.562	>25	25
Ethyl acetate	3.125	1.652	25	25
Positive control	0.781	0.781	6.25	3.125

3.9 Brine shrimp lethality activity (BSLA)

The percentage mortality along with the LC_{50} of the methanolic extract is shown in **Table 4**. The methanolic extract had good lethal potential against brine shrimp nauplii. From BSLA, the LC_{50} value for the methanolic extract was found to be 40.55 µg/mL. A plot of probit against Log C is shown in **Figure 17**.

Table 4: Brine shrimp activity for the methanolic extract of the plant sample.

Extract type	Concentration (μg/mL)	Surviving nauplii after 24 h	Mortality %	LC_{50} value (μg/mL)
Methanol	1000	24	80.00	40.55
	800	17	56.66	
	500	22	73.33	
	250	20	66.66	
	100	16	53.33	
	10	12	40.00	

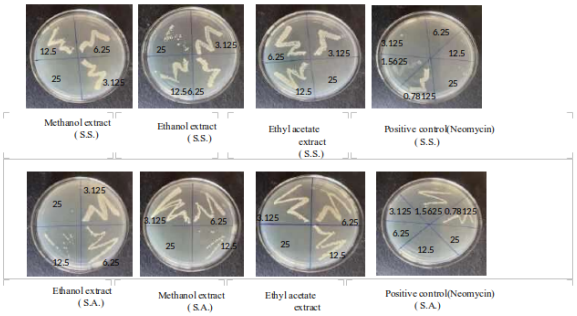

Figure 16: Showing MIC and MBC values of three different extracts (methanol, ethanol, and ethyl acetate) along with the positive control (Neomycin).

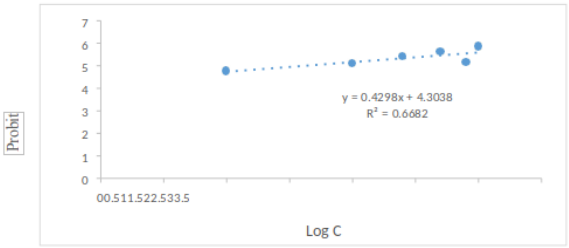

Figure 17: A plot of probit against Log C.

4 Discussion

All the species of *Quercus floribunda* Lindl are useful medicinal plants. The different parts of Quercus species are used in various treatments, where the bark of this species is used as an astringent, antiarthritic, and for other disorders [13]. Since there is no more work in pharmacognosy of this species, my research mainly focuses on the bark of the *Quercus floribunda* Lindl plant, which is widely distributed in the range of the Himalayas (2500-3000 m), Nepal.

Different extraction solvent shows different yield percentages. The aqueous yield is maximum, showing maximum polar constituents in the bark of this plant, and minimum in Hexane, showing minimum presence of non-polar compounds in the bark. This study found the same result, where the yield was maximum in the polar solvent. According to this study, to improve extraction efficiency, a combination of polar and nonpolar solvents should be used [33]. In this species, there is no specific research related to this work, so the data are correlated with other species of the same genus.

In phytochemical screening of the bark of *Quercus floribunda* Lindl, it was found that the bark of the plant is highly rich in different classes of bioactive compounds, alkaloids, flavonoids, steroids, tannins, carbohydrates, and saponins. This result can be correlated to the study carried out in different species of the *Quercus* genus by different researchers [34]. The bark of *Quercus gluca* contains tannins, carbohydrates, saponins, phenols, and quinones, which make it a medicinally active plant. Also, according to [13], the *Quercus* genus contains various classes of compounds such as glycosides, terpenoids, flavonoids, phenolic acids, fatty acids, sterols, and tannins.

The bark of *Quercus floribunda* Lindl had the highest TPC value in ethyl acetate extract (127.23 ± 1.65 mg GAE/g), with the lowest value in DCM (9.25 ± 0.69 mg GAE/g). There is no more significant difference in TPC value between the DCM and hexane extracts. Also, the study finds ethanol extract had the lowest TFC value (3.97 ± 1.03 mg QE/g), and hexane had the maximum value (61.72 ± 4.01 mg QE/g). The bark of *Quercus floribunda* Lindl shows the maximum TTC in aqueous extract (37.27±2.96 mg TAE/g) and the least in hexane extract (5.51±1.3 mg TAE/g). There is no specific research conducted on this species within this solvent and bark, so it is difficult to correlate the data. Thus, the data were correlated with different species of the same genus and another part of the bark in the same plant. A study conducted by Ahmad et al. 2023 [35] on aerial part leaves and galls found the maximum TPC value and TFC value in acetonic extract, 66.9 ± 0.05 g GAE/mgE

and 38.4 ± 0.72 g QE/mgE, respectively. Thus, the bark of *Q. floribunda* Lindl has more phenolic and flavonoid content than the aerial part. A study conducted in the bark of *Q.faginea* by Ferreira et al. 2018 [36] found maximum TPC (630.3 mg GAE/g), TFC(207.7 mg catechin equivalents (CE)/g), and Tannin (220.7 mgCE/g) in ethanol-water extract. This shows that the bark of *Q. faginea* contains more phenolic compounds, flavonoids, and tannins.

Among six solvent extracts, the methanolic extract shows good antioxidant activity with an IC_{50} of 12.70 ± 0.372 µg/mL, followed by ethanol, ethyl acetate, and aqueous. The IC_{50} value for the crude extracts of DCM and hexane was greater than 500 µg/mL, indicating a low level of antioxidant activity in these extracts. According to the research carried out by Ferreira et al. 2018 [36] in the bark of *Q. faginea* IC_{50} was 2.6 µg/mL in polar ethanol-water extract, showing more antioxidant activity in comparison to *Q. floribunda* Lindl. This variation in results is due to climatic conditions, altitude variation between the collected samples, and correlated species from the literature. The value reported by Sánchez-Burgos et al., 2013 [37] for anti-oxidant activity in leaves of different species (*Q. resinosa, Q. grisea, Q. laeta, and Q.obtusata*) ranges from 150 – 450 µg/mL, which is more than compared to aqueous extract (29.31±1.24 µg/mL) of *Quercus floribunda* Lindl. This data shows the bark of *Quercus floribunda* Lindl is more antioxidant than the above-mentioned plants.

From the antibacterial study in the bark of *Quercus floribunda* Lindl, it was found that the gram-negative *E. coli* was inhibited equally by ethanol and ethyl acetate, with a ZOI of 15 mm, exactly as a positive control. This shows the bark of this plant is a potent source of antibiotics against *E. coli.* For gram-positive *Staphylococcus aureus,* methanolic and ethanolic extracts show a ZOI of 20 mm, a minimal zone of inhibition for hexane extract and DCM, confirming their low levels of antibacterial activity. A poor zone of inhibition is also seen in the aqueous extract when compared to the positive control (neomycin). The bark of *Quercus floribunda* Lindl shows antibacterial activity. There is no exact paper to correlate the anti-microbial result.

Of this species, but it was found by Sarwar et al., 2015 [38] that the leaves of *Q. incana* have significant antibacterial activity for both Gram-positive and negative. The n-butanol extract of leaves shows 32 mm and 28 mm ZOI against *A.niger* and *A. flavus,* respectively also 19 mm for *Micrococcus leuteus.* This data shows that the Quercus genus has good antibacterial properties. This supports the bark of *Quercus floribunda* Lindl also acts as a good source of antibiotics. Similarly, according to Iqbal et al., 2023 [33]' the bark of *Q. glauca* shows good antibacterial properties, whereas the methanolic extract shows a maximum ZOI of 13.33mm against *Klebsiella pneumoniae,* which is similar to the methanolic extract of the bark of *Quercus floribunda* Lindl. (15 mm). MIC and MBC values were determined for gram-positive (*Staphylococcus aureus*) and gram-negative (*Shigella sonnei*) bacteria. For both bacterial strains, methanolic extract demonstrated the same MIC values and MBC values for both strains (3.125 mg/mL and 25 mg/mL, respectively). Ahmad et al., 2023 [35] found that nuts of *Quercus floribunda* Lindl are moderately cytotoxic against brine shrimp nauplii. This investigation revealed that methanolic extract from the bark of *Quercus floribunda* Lindl exhibits good toxicity against brine shrimp nauplii with an LC50 of 40.55 µg/mL.

5 Conclusion

Phytochemical screening in the bark of *Quercus floribunda* Lindl revealed that the bark extract included phenol, flavonoids, tannins, terpenoids, carbohydrates, and alkaloids. It was based on the result of yield percentage and phytochemical screening for the extraction process; methanol, ethanol, and ethyl acetate are good solvents. The results of the comparison of TPC, TFC, and TTC of six different crude extracts found the highest TPC in ethyl acetate and the lowest in DCM. The TFC was highest in hexane and lowest in ethanolic extract. Likewise, the TTC value was highest in the aqueous extract and the lowest in the hexane extract. On the evaluation of the antioxidant activity of various crude extracts of *Quercus floribunda* Lindl's bark, the methanolic extract shows good antioxidant properties, followed by ethanol, ethyl acetate, and aqueous extract. As a consequence, this finding has demonstrated that the presence of phenols' antioxidant activity is directly related. DCM and hexane extract were found to be less potent for scavenging free radicals. So, from the above-mentioned results of methanolic, ethanolic, and ethyl acetate extracts, it was concluded that these extract shows good antimicrobial activity and toxicity assay. Ethanol and ethyl acetate showed a good zone of inhibition for *E. coli*, which is equal to the positive control, neomycin. Methanolic and ethanolic extracts showed a maximum zone of inhibition for *Staphylococcus aureus,* about the positive control, neomycin. For *Klebsiella pneumoniae,* all three extracts showed moderate ZOI in comparison to the positive control, neomycin. Ethyl acetate extract showed maximum ZOI for *Shigella sonnei.* These extracts showed comparable zones of inhibition for different bacteria. From the calculation of MIC and MBC of these three extracts against *Shigella sonnei* and *Staphylococcus aureus,* it was

concluded that for the methanolic extract, the minimum required concentration to inhibit was 3.125 mg/mL for both bacteria, and to kill the bacteria completely, the required concentration bacterial growth. The MIC and MBC values are also comparable for these three extracts. From all these results, it was concluded that the methanolic extract is more potent for the toxicity assay. Therefore, further research is necessary to completely comprehend this plant's medical potential. This plant has a wide range of biological properties, so this plant has great biological importance and could be used as a source of natural antimicrobial and antioxidant agents.

Abbreviations

DMSO: Dimethyl sulfoxide

GAE/g: Gallic acid equivalent per gram QE/g: Quercetin equivalent per gram

TAE/g: Tannic acid equivalent per gram TPC: Total phenolic content

TFC: Total flavonoid content TTC: Total tannin content

IC_{50}: Half-maximum inhibitory concentration DPPH: 2,2-diphenyl-1-picrylhydrazyl

ZOI: Zone of inhibition

MIC: Minimum inhibitory concentration MBC: Minimum bactericidal concentration LC50: Lethal concentration 50%

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Conflicts of Interest

The authors declare that there are no conflicts of interest associated with the publication of this research paper.

Author's Contribution

- **Govinda Bhattarai:** Performed laboratory work, writing, review, editing, and original draft.
- **Dipak Raj Jaishi:** Writing, reviewing, formal analysis, and editing
- **Khaga Raj Sharma:** Writing, reviewing, editing, supervision, and conceptualization

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