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RESEARCH ARTICLE

Phytochemical Analysis and Biological Activities of *Zingiber officinale* Grown in Different Geographical Regions of Nepal

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Highlights

- Methanol extract & essential oils of ginger were obtained by cold percolation and hydrodistillation method, respectively
- Methanolic extract of ginger grown in Sunsari district exhibited the highest antioxidant potential
- Essential oil obtained of ginger grown in Kathmandu district exhibited the highest antioxidant potential
- All ginger methanolic extract and essential oils were inactive against *E. coli* and *F. oxysporum*
- All methanolic extract & essential oils were effective against *k. pneumoniae, S. aureus* and *S. typhi, A. flavus, C. albicans* and *C. parapsilosis*

Abstract

Zingiber officinale, an important spice cash crop is an herbaceous perennial aromatic, medicinal plant that belongs to the family Zingiberaceae. Five samples of gingers were collected from Kaski (G1), Kanchanpur (G2), Makwanpur (G3), Sunsari (G4), and, Kathmandu (G5) districts. Ginger extracts were extracted by cold percolation method and essential oil was extracted by hydrodistillation using Clevenger apparatus. Ginger extract G4 showed the highest TPC (total phenolic content) of 140.91±2.93 mg GAE/g and G3 showed the lowest 43.31±6.80 mg GAE/g TPC value. Sample G5 showed the highest TFC(total flavonoid content) of 25.67 ± 1.16 mg QE/g and, G1 showed the lowest of 14.57±1.07 mg QE/g. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to determine the antioxidant activity of the ginger samples. The ginger sample G4 shows potent antioxidant activity with an IC_{50} of 162.73±3.22 µg/mL. The extract of sample G1 showed less radical scavenging activity with an IC_{50} value of 279.63±14.68 µg/mL. The essential oil of sample G5 shows potent antioxidant activity with an IC_{50} value of 68.98±7.24 µL/mL and, that of G4 showed less scavenging activity with an IC_{50}330.40±8.70 µL/mL. The diameter of the zone of inhibition (ZOI) produced by ginger extracts and essential oil on particular bacteria and fungi was measured for the estimation of their antimicrobial activity. Methanolic extracts and essential oils of ginger from all districts are ineffective against E. Coli but displayed variable ZOI against Klebsiella pneumoniae, P. aeruginosa, S. aureus, and Salmonella typhi. Ginger extracts of all samples show a zone of inhibition in Candida ablicans, Candida parapsilosis, and Aspergillus flavus, but Fusarium oxysporum does not show any zone of inhibition. Essential oil has more antifungal activity than methanolic extracts. The LC_{50} value of the methanolic extract of sample G1 was found to be 1×10^5 µg/mL and the least from the methanolic extract of sample G2 with an LC_{50} value of 3.0×10^3 µg/mL, indicating that the methanolic extract of ginger was non-toxic.

Keywords: *Zingiber officinale*, Essential oil, Antioxidant, Antimicrobial**.**

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Introduction

Due to the diverse geography from Terai to the Himalayan region, Nepal is blessed with numerous natural products. Nepal's abundance of medicinal plants has been a valuable resource for the scientific community. In the context of Nepal, many people rely on traditional plant remedies due to diverse terrains, and seasonal changes, leading to abundant medicinal plant diversity[1]. While Nepal covers not more than 0.1% of the global land area, it boasts almost 2% of the world's flowering plants, out of which 690 species are considered medicinal plants.[2, 3] In Nepal these medicinal plants are consumed by around 125 ethnic groups and have their medicinal knowledge transferred from one age to another[4].

Among the many, Zingiberaceae is well well-documented medicinal plant in Nepal. Zingiberaceae family, belonging to the suborder Scitaminae, consists of around 49 genera and 1300 species[5–7]. Especially the species *Z. officinale*, known as ginger, is the most important and extensively cultivated in tropical countries[6]. *Z. officinale* is a native perennial plant originating in Southeast Asia and cultivated worldwide[7]. On a global level, Asia contributes 81% of the *Z. officinale* rhizome yield, and according to the Food and Agriculture Organization (FAO), Nepal ranks as the world's fourth-largest producer and exporter of *Z. officinale* rhizome[8]. The rhizome of *Z. officinale* serves as food and supplement and is reported to boast diverse attributes such as pain relief, antiviral, anti-inflammatory, and anticancer effects, with extensive traditional use[9]. Ginger is abundant in secondary metabolites like phenolics, terpenes, organic acids, and lipids, with its biological activities attributed to the presence of phenolic compounds[10]. Likewise, volatile compounds present in ginger's essential oil, such as monoterpenes, sesquiterpenes, and their oxygenated derivatives, along with certain aldehydes and alcohols, have been reported to display noteworthy pharmacological properties, including antibacterial, anticancer, antifungal, anti-inflammatory, and antioxidant effects[11]. The secondary metabolites reported from the *Z. officinale* are 6-gingerol, 8-gingerol,10-gingerol, β-bisabolene, α-curcumin, zingiberene, kaempferol, catechine, α-farnesene, and β-sesquiphellandrene[11–13].

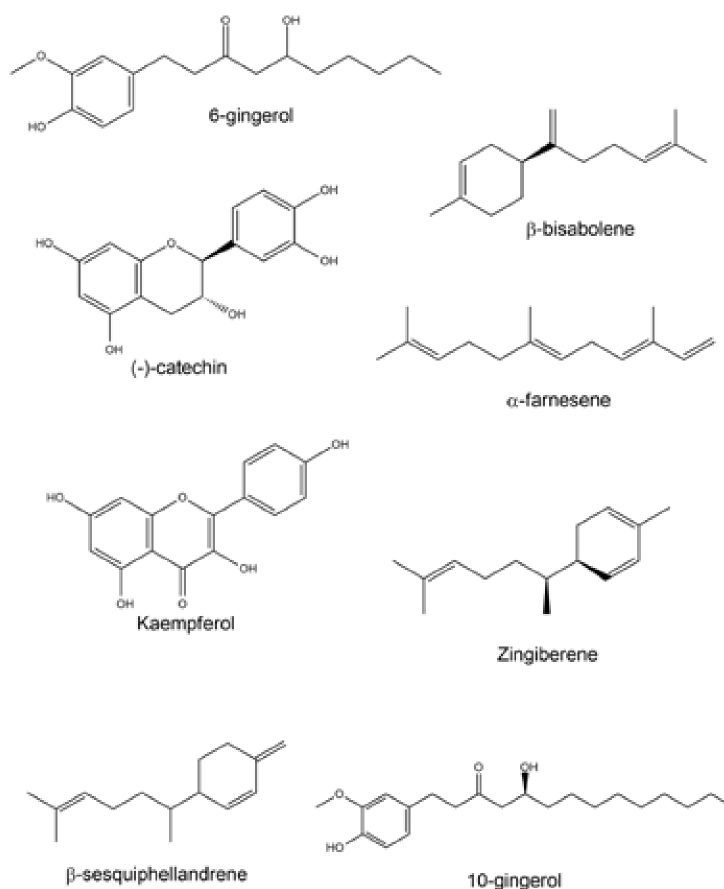

Fig 1. Secondary metabolites reported from Zingiber officinale

Based on the literature mentioned earlier, extensive pharmacological research has been conducted on *Z. officinale*, showcasing its effectiveness for various purposes. The present study highlights the phytochemistry, biological activities, and toxicity study of *Z. officinale* growing in different geographical regions of Nepal.

Materials and Methods

Chemicals

Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, dimethyl sulphoxide (DMSO), disodium hydrogen phosphate, sodium dihydrogen orthophosphate, and other chemicals were purchased from Fisher Scientific (India**).** All reagents and solvents used were of analytical quality.

Collection and Identification of Plant Samples

The ginger rhizomes were gathered from various districts in Nepal, including Kaski, Kanchanpur, Makwanpur, Sunsari, and the Kathmandu district. The collection of ginger samples was guided by both local ethnobotanical knowledge and a comprehensive literature review. The plant specimens that were collected underwent identification at the National Herbarium & Plant Laboratories located in Godavari, Nepal.

Table 1. Ginger samples collected from different regions of Nepal

S. N	Ginger collected districts	Ginger samples	Variety
1	Kaski	G1	Domesticated
2	Kanchanpur	G2	Domesticated
3	Makwanpur	G3	Domesticated
4	Sunsari	G4	Domesticated
5	Kathmandu	G5	Domesticated

Extracts Preparation

The collected ginger rhizomes were cleansed with tap water to eliminate contaminants. Subsequently, they were dried under shade, and finely ground into a powder. The phytochemicals within the powdered leaves were extracted using the cold percolation method with methanol as the solvent. A quantity of 100 g of powdered samples was accurately measured using a digital balance and placed in a clean and dry conical flask. To the flask, 250 mL of methanol was added, and the mixture was allowed to stand for 48 hours with frequent agitation. After thorough maceration, the mixture was decanted and filtered using a cotton plug. The solvent from the resulting filtrate was removed by evaporation, employing a rotary evaporator set at 40 ºC. The concentrated filtrate was transferred to a 100 mL beaker with a known weight, which was then covered with aluminum foil featuring multiple small perforations to facilitate solvent evaporation. The extracts were stored at 4 ºC until to perform the biological activities and qualitative and quantitative analysis. The percentage yield of the extract was calculated as,

$$\text{Percent inhibition} = \frac{\text{Acontrol - Asample}}{\text{Acontrol}} \times 100\ \%$$

Extraction of Essential Oils from Ginger Rhizome

To extract the essential oils, 300 g of small ginger rhizome pieces were placed in a 1-liter conical flask and connected to the Clevenger apparatus. Subsequently, 500 mL of distilled water was added to the flask and heated to the boiling point. The steam, carrying the essential oils, was then distilled into a graduated cylinder over 3 hours. This process was repeated three times to ensure thorough extraction. The oil, obtained through this method, was then subjected to drying using dry sodium sulfate, and the quantity of oil obtained was measured. The resulting oil was stored at 4 ºC for subsequent analysis. The same procedure was replicated for the remaining samples as well[13].

Determination of Total Phenolic Content (TPC)

The quantitative determination of total phenol was carried out using the Folin-Ciocalteu reagent[14, 15]. Initially, 20 µL of different concentrations of the gallic acid standard were loaded in triplicate onto a 96-well plate. Subsequently, 20 µL of a plant

sample with a concentration of 500 µg/mL was also loaded onto the 96 well plates in triplicate. Following this, in each well containing both standard and sample, 100 µL of F-C reagent was added, followed by the addition of 80 µL of Na_2CO_3. The plate was then kept in the dark for 15 minutes, after that the absorbance was measured at 765 nm using a microplate reader (Synergy LX, BioTek Instruments, Inc., USA) equipped with Gene 5 software. For establishing the standard curve (10-80 µg/mL), gallic acid was used, and the total phenolic content (TPC) within the extracts was expressed as milligrams of gallic acid equivalent per gram of dry weight (mgGAE/g) of the extract, employing the gallic acid standard curve.

Determination of Total Flavonoid Content (TFC)

The total flavonoid content of the extracts was determined using a modified 96-well plate method based on the colorimetric method[16]. Initially, 130 µL of various concentrations of quercetin standard were loaded onto a 96-well plate in triplicate. Subsequently, 20 µL of the plant sample was loaded onto the 96 well plates in triplicate. To each well containing the plant sample, 110 µL of distilled water was added, maintaining a final volume of 130 µL. Next, in each well containing both the standard and plant sample, 60 µL of ethanol, 5 µL of $AlCl_3$, and 5 µL of potassium acetate were added. The plate was then placed in darkness for 30 minutes to allow the reaction to complete. After this incubation period, absorbance was measured at 415 nm using a microplate reader (Synergy LX, BioTek Instruments, Inc., USA). The total flavonoid content (TFC) within the extract was quantified and expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g) of the extract.

Evaluation of Antioxidant Activity

The antioxidant activity of the extracts was assessed using a DPPH assay[17]. The capacity to induce a color change from purple to yellow in the DPPH radical, accomplished through the reaction with the extract, was tracked spectrophotometrically at 517 nm. For the DPPH assay, quercetin was considered a positive control, while a negative control consisting of 50% DMSO in water was employed. In the 96 well plates, 100 µL of the positive control quercetin, negative control DMSO, and various concentrations of plant extract were loaded in triplicate. Subsequently, 100 µL of DPPH reagent was introduced to each well. The plate was then incubated for 30 minutes in darkness to allow the reaction to reach completion. After this 30-minute incubation period, absorbance readings were taken at 517 nm using a microplate reader.

The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\text{Percent inhibition} = \frac{\text{Acontrol - Asample}}{\text{Acontrol}} \times 100\ \%$$

Where A is the absorbance of the sample and control.

Evaluation of Antibacterial Activities

The antibacterial activity of ginger extract and ginger essential oil was assessed using the agar well diffusion method[18]. In this approach, the effectiveness of the antibacterial properties was determined by measuring the zone of inhibition (ZOI), a clear area around the wells where bacterial growth is inhibited. To begin, a stock solution of 25 mg/mL of methanolic extract and 25 µL/mL (100 % DMSO) of essential oil was prepared. The test organisms, including gram-positive *S. aureus* and gram-negative *E. coli, Salmonella typhi, Klebsiella pneumoniae, and P. aeruginosa* bacteria. Standard culture inoculums were created by transferring the organisms from primary culture plates into the nutrient broth, which was then incubated at 37 ºC overnight. For the qualitative screening and evaluation of antibacterial activity, sterile MHA plates were dried and labeled, and bacterial inoculums were swabbed onto the plates. Wells were created using a sterile cork borer, and the test samples, including the plant extract, negative control (DMSO), and positive control (neomycin), were loaded into separate wells. The plates were incubated at 37°C for 24 hours. Following incubation, the presence of a clear zone around the wells indicated bacterial growth inhibition. The diameter of the zone of inhibition was measured to express the antibacterial activity. The absence of a zone implied no activity. This method provided valuable insights into the antibacterial potential of ginger extract and ginger essential oil against the tested bacterial strains.

Evaluation of Antifungal Activity

The antifungal potential of ginger extracts and essential oil was evaluated by the method suggested by K.R. Sharma and S. Adhikari [19]. To begin, a stock solution of 50 mg/mL of methanolic extract (in 50 % DMSO) and 50 µL/mL (100 % DMSO)

of essential oil was prepared. The fungal strains for testing were inoculated in Potato Dextrose Broth (PDB) at 37 °C to achieve turbidity of 0.5 McFarland.For qualitative screening and evaluation of antifungal activity, sterile MHA plates were labeled, and the test organisms were swabbed onto the plates. The wells were created using a sterile cork borer, and the test samples plant extract, negative control (DMSO), and positive control (Amphotericin B) were loaded into separate wells. Following an incubation period at 37°C for 24 hours, the plates were observed for the presence of a clear zone around the wells, indicating inhibition of fungal growth. The diameter of the zone of inhibition was measured, providing a metric for antifungal activity.

Brine Shrimp Toxicity

The toxicity assay was conducted using a brine shrimp assay, following the well-established protocol by Meyer et al. (1982), which is renowned for its simplicity, speed, and cost-effectiveness[20]. This method assesses the toxicity of bioactive compounds towards brine shrimp larvae, wherein compounds with LC_{50} values below 1000 ppm are considered potentially pharmaceutically active. For the test, artificial seawater was meticulously prepared, and approximately 10 mg of brine shrimp eggs were sprinkled into a beaker filled with artificial seawater. The setup was illuminated with a 60-watt table lamp for 48 hours, maintaining a temperature of 30ºC. Subsequently, freshly hatched brine shrimp larvae (Artemia salina) were introduced to solutions of crude plant extracts and their fractions. After incubating under controlled conditions for 24 hours, survivor counts were recorded to calculate the percentage of mortality. These results were then utilized to determine LC_{50} values, enabling potency comparisons.

Data Analysis

All the experiments were performed in triplicates and data were presented in standard error of the deviation. The TPC, TFC, and antioxidant assay results were processed by using Gen5 Microplate Data Collection and analysis by MS Excel 2007. The IC_{50} was calculated using GraphPad Prism software version 8.

Results and Discussion

Yield Percentage

There was variation in the yield of ginger extract and essential oils obtained from ginger samples collected from different districts. The highest yield of methanolic extract was observed in the G1 sample collected from the Kaski district, yielding 8 g in 500 g of dry sample whereas the lowest yield was found in the G2 sample collected from Kanchanpur, yielding 5.2 g in 500 g dry sample. Similarly, The highest yield was achieved with the G2 ginger rhizome from the Kanchanpur district, yielding 2.5 mL of essential oils while the lowest yield was observed in the G4 of 1.6 mL per 300 g of dry ginger powder. Table 2 displays the methanolic extract and essential oils yields from all the ginger samples.

Table 2. Yield of ginger extracts and essential oils of the samples

Ginger sample	Methanolic extract of ginger		Essential oils of ginger	
	The dry weight of ginger powder (g)	Extract yield (g)	The dry weight of ginger powder (g)	Oils yield (mL)
G1	500	8	300	1.9
G2	500	5.2	300	2.5
G3	500	6.4	300	2.3
G4	500	7.3	300	1.6
G5	500	5.9	300	2.0

Total Phenolic and Flavonoid Content

Phenolic and flavonoid contents were measured using a gallic acid calibration curve for Total Phenolic Content (TPC) and a quercetin calibration curve for Total Flavonoid Content (TFC). Among the samples, ginger extract G4 displayed the highest TPC of 140.91 ± 2.93 mg GAE/g, while ginger extract G3 exhibited the lowest TPC value of 43.31± 6.80mg GAE/g. The total phenolic content for samples G2, G5, and G1 was measured as 88.56 ± 3.91 mg GAE/g, 88.43 ± 1.87 mg GAE/g, and 61.98 ± 9.60 mg GAE/g, respectively. The results obtained in this study are comparable to the results reported by Aliet. al., which was 60.34 ± 0.43 mg GAE/g[21].

For Total Flavonoid Content, the extract of G5 collected in the Kathmandu district demonstrated the highest TFC of 25.67 ± 1.16 mg QE/g, while the G1 exhibited the lowest TFC of 14.57 ± 1.07 mg QE/g. TFC for samples G4, G2, and G3 were measured as 24.13 ± 0.23 mg QE/g, 21.57 ± 0.35 mg QE/g, and 14.57 ± 0.81 mg QE/g, respectively. Table 4 provides the TPC and TFC values for all ginger samples used in the study. The result obtained in this study is found slightly low as compared to the reported by Ali et. al., and they found a TFC value of 40.25±0.21 mg QE/g[21]. The variation observed in both TPC and TFC values could be attributed to various factors, including differences in plant genetics, geographical origin, environmental conditions, and extraction methods.

Table 3. Total phenolic and flavonoid content of ginger methanolic extracts

Ginger samples	TPC (mg GAE/g)	TFC (mg QE/g)
G1	61.98±9.60	14.57±1.07
G2	88.56 ± 3.91	21.57±0.35
G3	43.31± 6.80	14.57 ± 0.81
G4	140.91± 2.93	24.13 ± 0.23
G5	88.43± 1.87	25.67 ± 1.16

Antioxidant Potential

DPPH was used to assess the antioxidant activity of ginger methanolic extracts, with potent antioxidant extracts expressed in terms of IC_{50} values. The antioxidant potential bears an inverse relation to the IC_{50} value, where a lower IC_{50} value indicates higher antioxidant potential. Sample G4 displayed remarkable scavenging ability in comparison to other samples. Results were compared against the standard quercetin, exhibiting an IC_{50} value of 6.3 ± 1.0 µg/mL. G4 demonstrated robust antioxidant activity with an IC_{50} value of 162.73 ± 3.22 µg/mL. Extract from ginger sample G1 exhibited significantly lower scavenging activity, about 44-fold less than the standard, with an IC_{50} of 279.63 ± 14.68 µg/mL.IC_{50} values for G2, G3, and G5 were determined as 222.97 ± 8.21 µg/mL, 190.10 ± 7.62 µg/mL, and 250.53 ± 10.45 µg/mL, respectively. The present result exhibited low antioxidant potential as compared to about 19 to 30 folds less with Ali et. al., and they found a value of 8.29 ±1.73µg/mL[21].

Similarly, the essential oil from sample G5 showcased substantial scavenging ability compared to other samples. Essential oil of G5 demonstrated potent antioxidant activity with an IC_{50} value of 68.98 ± 7.24 µL/mL, around 10-fold less compared to the standard quercetin. Conversely, G4 displayed lower scavenging activity, with an IC_{50} value of 330.40 ± 8.70µL/mL, approximately 50 times less than the standard quercetin. The IC_{50} values for samples G1, G2, and G3 were 111.60 ± 3.72 µgL/mL, 170.10 ± 7.98 µL/mL, and 120.60 ± 5.05 µL/mL, respectively. The results of antioxidant activity for the extracts and the essential oils are shown in Table 4.

Table 4. IC_{50} of ginger methanolic extracts, essential oils, and standard quercetin

Ginger samples	IC_{50} (µg/mL) of methanolic extracts	IC_{50} (µL/mL) of essential oils
G1	279.63± 14.68	111.60 ± 3.72
G2	222.97 ± 8.21	170.10 ± 7.98
G3	190.10 ± 7.62	120.60 ± 5.05
G4	162.73 ± 3.22	330.40 ± 8.70
G5	250.53 ± 10.45	68.98 ± 7.24
Quercetin	6.3 ± 1.0	6.3± 1.0

Antibacterial Activity

The outcomes of the assessment of the antibacterial activity of the methanolic extract and essential oil of ginger samples against various bacterial strains are summarized in Tables 6 and 7. For the methanolic extracts of ginger samples displayed different ratios of inhibition against bacteria. Notably, for *Klebsiella pneumoniae*, the highest Zone of Inhibition (ZOI) was recorded for G2 at 11 mm, followed by G4 (9.7 mm), G5 (7.6 mm), and G1 (7 mm). However, no inhibition was observed for G3. Similarly, *Pseudomonas aeruginosa* displayed no inhibition for any of the samples except G5, which exhibited a ZOI of 6 mm. Concerning

Staphylococcus aureus, G4 demonstrated the most potent inhibition with a ZOI of 17.3 mm, followed closely by G5 (16.3 mm), G3 (13.2 mm), G2 (11 mm), and G1 (15.8 mm). For *Salmonella typhi*, G4 displayed a ZOI of 13.3 mm, while G2 (12.4 mm), G3 (11.8 mm), G5 (11 mm), and G1 showed a ZOI of 9 mm. No inhibition was observed against *Escherichia coli* by any samples.

Similarly, the essential oil of ginger collected from different districts showed variable inhibition against different bacterial strains. For *Klebsiella pneumoniae*, sample G2 exhibited the highest ZOI (19.9 mm), followed by G5 (14.7 mm) and G4 (14 mm), while G1 displayed the lowest ZOI (11.4 mm). *Pseudomonas aeruginosa* showed inhibition of 9 mm for G3 and 6.4 mm for G4, while G2 and G5 exhibited no inhibition. Regarding *Staphylococcus aureus*, G1 showcased the largest ZOI (20.2 mm), followed by G2 (19 mm) and G5 (17.9 mm), with G3 and G4 having ZOIs of 17.4 mm and 15.9 mm, respectively. Additionally, *Salmonella typhi* displayed ZOIs of 12.4 mm for G2, 11.8 mm for G3, and 13.3 mm for G4, with G1 and G5 having ZOIs of 9 mm and 11 mm, respectively. The present result is comparable with the results reported by Norajit et. al., the result obtained was 16 mm for *S. aureus* and no inhibition for *E. coli* in the aqueous extract[22]. These results appear to be consistent with recent findings.

Table 5. Antibacterial activity of ginger methanolic extracts

Bacteria	G1 ZOI (mm)	G2 ZOI (mm)	G3 ZOI (mm)	G4 ZOI (mm)	G5 ZOI (mm)	PC
Klebsiella pneumoniae	7	11	-	9.7	7.6	20
P. aeruginosa	-	-	-	-	6	16
S. aureus	15.8	11	13.2	17.3	16.3	18
Salmonella typhi	9	12.4	11.8	13.3	11	22
E. coli	-	-	-	-	-	18

Note: ZOI Zone of Inhibition in millimeters. "PC" Positive Control. "-" No inhibition.

Table 6. Antibacterial activity of essential oils of ginger samples

Bacteria	G1 ZOI (mm)	G2 ZOI (mm)	G3 ZOI (mm)	G4 ZOI (mm)	G5 ZOI (mm)	PC
Klebsiella pneumoniae	11.4	19.9	13.3	14	14.7	18
P. aeruginosa	-	-	9	6.4	-	22
S. aureus	20.2	19	17.4	15.9	17.9	21
Salmonella typhi	9	12.4	11.8	13.3	11	17
E. coli	-	-	-	-	-	20

Antifungal Activity

The findings from the evaluation of the antifungal activity of methanolic extract and essential oils of different samples against various fungal strains are succinctly presented in Tables 8 and 9. The antifungal activity of the methanolic extract of gingers showed intermediate results as compared to essential oils. In the case of the methanolic extract of ginger samples, for *Fusarium oxysporum*, none of the samples exhibited any Zone of Inhibition (ZOI). Moving to *Aspergillus flavus*, G5 showed a ZOI of 9.4 mm, followed by G1 with 8.2 mm, G2 with 7 mm, G4 with 6.7 mm, and G3 with 6.6 mm. *Candida albicans* demonstrated ZOIs of 13 mm for G1, 12 mm for G2 and G5, 7 mm for G3, and 8.3 mm for G4. Similarly, *Candida parapsilosis* exhibited ZOIs of 14 mm by G1, 8 mm by G2 and G5, 7 mm by G3, and 11 mm by G4. These data indicate methanolic extract of ginger from different districts can act as an antifungal agents. Likewise, essential oils from the gingers of different districts exhibited excellent antifungal activity. Regarding *Fusarium oxysporum*, G5 exhibited a modest ZOI of 9.4 mm. Similarly, shifting focus to *Aspergillus flavus*, G2 demonstrated the highest ZOI at 17.5 mm, followed by G3 and G4, both displayed ZOIs of 15.3 mm. G1 exhibited a ZOI of 14.7 mm, while G5 displayed a comparatively lower ZOI of 11.9 mm. The investigation into *Candida albicans* showcased the highest inhibition by G4 with 19.6 mm of ZOI and the lowest ZOI by G1 with ZOI of 14.3 mm. Samples of essential oils for *Candida parapsilosis* displayed consistent ZOIs, ranging from 16.5 mm for G1 and G2 to 19.3 mm for G5. Comparatively, essential oils exhibited strong antifungal activity against different fungi compared to methanolic extract of ginger. This result is found comparable with the result reported by Rawal et, al., andJantan et. al., these results presented the zone of inhibition for *C. ablicans* was 12.3 ±2.5 mm and antifungal activity increased with increasing concentration [23, 24].

Table 7. Antifungal activity of ginger methanolic extracts

Fungi	G1 ZOI (mm)	G2 ZOI (mm)	G3 ZOI (mm)	G4 ZOI (mm)	G5 ZOI (mm)	PC
Fusarium oxysporum	-	-	-	-	-	20
Aspergillus flavus	8.2	7	6.6	6.7	9.4	20
Candida ablicans	13	12	7	8.3	12	19
Candida parapsilosis	14	8	7	11	8	16

Table 8. Antifungal activity of essential oils of ginger samples

Fungi	G1 ZOI (mm)	G2 ZOI (mm)	G3 ZOI (mm)	G4 ZOI (mm)	G5 ZOI (mm)	PC
Fusarium oxysporum	-	-	-	-	9.4	25
Aspergillus flavus	14.7	17.5	15.3	15.3	11.9	23
Candida ablicans	14.3	17.8	17.7	19.6	17.6	24
Candida parapsilosis	16.5	16.5	18.2	18.4	19.3	21

Brine Shrimp Lethality Test

The biological activities of the distinct ginger extracts were assessed to determine their toxicity towards newly hatched brine shrimp. The study involved determining the lethal concentration at which 50% of the exposed *A. salina* population is killed (LC_{50} values) in µg/mL which is calculated with the help of the brine shrimp lethality assay. This approach aimed to gauge the potentially harmful effects of ginger extracts on the brine shrimp population, providing valuable insights into their toxicity levels. Among the tested ginger samples, G2 exhibited the most notable cytotoxic activity, with an LC_{50} value of 3×10^3. This was followed by G4 with an LC_{50} of 3.5×10^4, G3 with 4.6×10^4, and G5 with 6.7×10^4, while G1 displayed the highest LC_{50} value of 1×10^5. These results provide insights into the varying cytotoxic potential of different ginger samples. Chemical species with LC_{50} values below 1000 µg/mL are generally considered to possess pharmacological activity. In the context of this study, the determined LC_{50} values were found to exceed 1000 µg/mL, indicating that the ginger extracts from all districts exhibit pharmacological inactivity and non-toxicity. Consequently, ginger can be safely consumed as a spice without posing any harmful effects.

Table 9. Brine shrimp toxicity of the ginger extracts

Ginger samples	**LC_{50} (µg/mL)**
G1	1×10^5
G2	3×10^3
G3	4.6×10^4
G4	3.5×10^4
G5	6.7×10^4

Conclusions

The methanolic extract of ginger was obtained through cold percolation, and the essential oil was collected by hydrodistillation using a Clevenger apparatus. Among the ginger extract samples, G4 and G5 displayed the highest total phenolic content (TPC) and total flavonoid content respectively. The DPPH assay was employed to measure radical scavenging capacity. Ginger sample G4 and the essential oil of ginger sample G5 exhibited remarkable scavenging ability compared to other samples. Antibacterial activity was assessed using the agar well diffusion method. The results of the antibacterial activity assessment of the methanolic extract and essential oil of ginger samples against various bacterial strains indicated that almost all samples are effective against *Staphylococcus aureus*, *Klebsiella pneumonia,* and *Salmonella typhi*. For *Candida albicans*, *Candida parapsilosis*, and *Aspergillus flavus*, all ginger methanolic extract and essential oil demonstrated a zone of inhibition.

Toxicity was assessed using the brine shrimp lethality assay. Sample G1 exhibited the highest LC_{50} value, recorded at 1×10^5µg/mL. These findings suggest the non-toxic nature of the methanolic extracts of ginger, as evidenced by the accompanying LC_{50} values. In conclusion, the outcomes of this study have shed light on the antioxidative, antibacterial, antifungal, and toxicity

properties of the *Zingiber officinale* collected from the different regions of Nepal. Furthermore, *Zingiber officinale* shows a potential natural source of antioxidants, antibacterial as well as antifungal agents-demonstrating non-toxic characteristics. This underscores its utility as a culinary spice. Exploring further the isolation and extraction of bioactive compounds from these plant extracts could pave the way for the development of innovative pharmaceutical formulations.

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