Comparative investigation on antihyperglycemic and antioxidant activity of *Zingiber officinale* growing in different regions of Nepal

Khaga Raj Sharma[*] and Nav Raj Khanal[*]

[*]Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Abstract: The aim of this research is to collect *Zingiber officinale* from the different altitudes of Nepal for the study of antioxidant activity, quantification of phenolic and flavonoid content, α-amylase enzyme inhibition and the toxicity test. The phenolic and flavonoid content in methanol extracts of *Z. officinale* was estimated by Folin-Ciocalteu colorimetric method and aluminium chloride colorimetric assay. The antioxidant potential was determined by DPPH free radical scavenging assay and the toxicity of the extracts was performed by brine shrimp lethality assay. Similarly, the antidiabetic activity of extracts was evaluated by the α-amylase enzyme inhibition assay. The antioxidant potential of *Zingiber officinale* was evaluated as inhibitory concentration IC_{50} values which were found to be in the range from 44.43 µg/mL to 65.71 µg/mL in the sample collected from the different regions of Nepal. The total phenolic content in *Z. officinale* collected from different regions was found to be in a range from 118.19±3.32 mg GAE/g to 222.04±24.93 mg GAE/g. The total flavonoid content was found to be lower than that of total phenolic content in *Z. officinale* collected from different altitudes of Nepal. The total flavonoid was found to be in a range from 42.10 ± 4.48 mg QE/g to 62.95 ± 0.70 mg QE/g. The brine shrimp lethality LC_{50} in *Z. officinale* collected from the different regions was found to be higher than 1000 µg/mL. The α-amylase enzyme inhibition IC_{50} of *Z. officinale* growing in different regions of Nepal was found to be in a range from 161.25 µg/mL to 216.59 µg/mL. This study is the first attempt for comparing the antioxidant and α-amylase enzyme inhibition activity of *Z. officinale* growing at different altitudes of Nepal.

Keywords: *Zingiber officinale;* DPPH; Antioxidant; Antidiabetic; Antibacterial.

Introduction

Nepal is rich in biodiversity due to the wide range of climatic conditions existing in Nepal. Nepal is rich in natural resources in which medicinal herbs are the second most bioresources after water resources[1]. In Nepal wide variety of medicinal herbs have been distributed throughout the country and people have better practices for using such medicinal herbs for the care of simple to life-threatening diseases. But there is a lack of systematic study, exploration and preservation of such plant resources located in different ecology of the country. Medicinal herbs are used by the people in different communities of Nepal as well as in the world for many years to prevent and care for several diseases as a source of drugs or their precursors[2]. In the traditional medicinal practices, it is found that people have been using the plant secondary metabolites as the medicine and the source of medicine for the treatment of different diseases[3]. The earliest record of using medicinal plants for the treatment of different diseases are mentioned in

Author for correspondence: Khaga Raj Sharma, Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal.
Email: khagaraj_sharma33@yahoo.com

Received: 22 Feb 2022; First Review: 03 Mar 2022; Second Review: 10 Mar 2022; Accepted: 13 Mar 2022.
Doi: https://doi.org/10.3126/sw.v15i15.45885

Rigveda. Similarly, in Ayurveda the importance of medicinal herbs has been reported. The Charak or Caraka Samhita and Susruta Samhita mention the art of surgery, therapeutic uses of such medicinal herbs[4]. Nearly 80% of the world's population still relies on traditional medicines for primary health care most of which involves the use of plant extracts. Total 60% of the population in developing countries rely on traditional medicines mostly in medicinal herbs for their primary health care needs[5].

However, the study of active chemical constituents of plants by chemical analysis was started after the isolation of morphine from *Papaver somniferum*. Morphine and Codeine were obtained from the opium poppy. Various other active components from natural resources have been identified and used in modern synthetic medicines. Atropine obtained from *Atropa belladonna* is used for the treatment of asthma and to decrease saliva production during surgery, taxol is obtained from the bark of the pacific yew tree which has been used as a potent anti-cancer drug, artemisinin from *Artemisia alba* has been used for the treatment of malaria[6,7,8].

Medicinal herbs are a good source of plant secondary metabolites that can be used for the treatment of infectious diseases, diabetes and several other simple to life-threatening diseases. These herbs are the sources of natural antioxidant, antidiabetic, antibacterial and are rich in phenolic and flavonoid compounds. Globally, Nepal is the third largest ginger producer country after China and India. A large quantity of *Zingiber* is being produced in various geographical locations of Nepal[9].

Ginger has been used as an important ingredient in Chinese, Ayurvedic and Tibetan-Unani herbal medicine for the treatment of arthritis, rheumatological conditions and muscular discomfort, treatment of conditions like atherosclerosis, migraine headaches, rheumatoid arthritis, high cholesterol, ulcers, depression and impotence[10]. Ginger has been used in both fresh and dried forms for the treatment of cough, common cold, flu-like symptoms rheumatism, asthma, stroke, diabetes and even menstrual periods[11]. Similarly, the powdered rhizome has been used as a traditional remedy for gastrointestinal complaints including peptic ulcer treatment & generally regarded as an excellent carminative and intestinal spasmolytic and reduce motion sickness[12]. People have been using ginger to kill or inhibit the growth of pathogenic bacteria and to control or inhibit the multiplication of digestive bacteria[13]. The few bioactive chemical compounds reported from the rhizome of *Z. officinale* are displayed in Figure 1.

This research aimed at the collection of Z. officinale rhizomes from a different altitude of Nepal for the comparative study of biological activity along with the quantification of phenolic and flavonoid compounds. This is the first attempt in which we perform a comparative study of the antidiabetic, antioxidant activity, estimation of total phenolic and flavonoid content and toxicity test on the Z. officinale growing in different altitudes of Nepal.

Materials and methods

Chemicals and equipment

The chemicals and solvents used in this research were of analytical and laboratory grade. Methanol Fischer Scientific), acetone (Fischer Scientific), hexane (Merck), gallic acid, quercetin, ascorbic acid, iodine trichloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH), $NaNO_2$, $AlCl_3$, NaOH, Folin-Ciocalteu reagent (FCR), α-amylase enzyme, acarbose and DMSO (Merck) were purchased from the local market.

The equipment used in this research is an electric grinder, mortar and pestle, digital weighing balance (GT 210), hot air oven (Griffin-Grundy), rotatory evaporator (Buchi RE 111) with a water bath (Buchi 461), spectrophotometer (WPA, supplied by Philip Harris Shenstone, England), iodine chamber, cuvettes, burettes, pipettes and micropipettes (Erba BIHOT).

Collection of samples

The fresh rhizomes of Z. officinale was collected from the various geographical regions of Nepal. The regions where ginger cultivation is massive were selected for the

collection of the samples. The samples and the regions of the collection are presented in Table 1.

Sample preparation

The contaminants in the samples of Z. officinale rhizomes were removed by washing with tap water. The rhizomes were chopped, shade dried, ground into powder form and stored at 4°C in the clean plastic bag until required for the estimation of phytochemicals and investigation of biological activity.

Extraction of secondary metabolites

The secondary metabolites of plant samples were extracted by cold percolation methods using methanol as a solvent. The filtrates were concentrated in the rotatory evaporator and were kept in a beaker wrapping with aluminium foil with small pores for the complete evaporation of the solvent. The yield percentage of the extracts were calculated from the dry extracts.

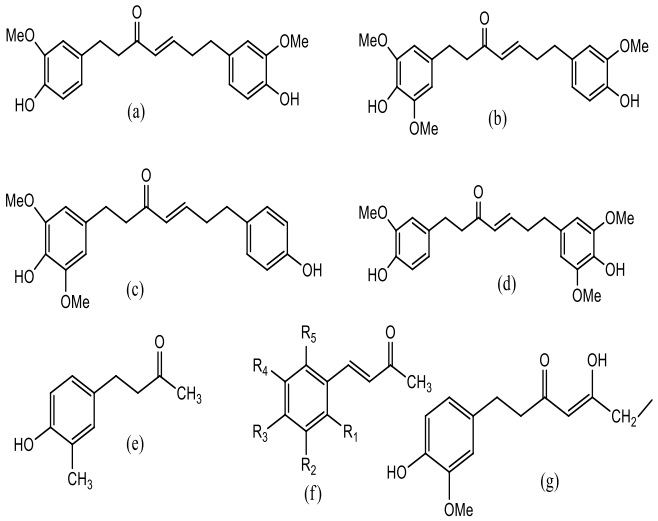

Figure 1: Structure of compounds reported from the Z.officinale (a) Gingerenone A (b) Isogingerenone B (c) Gingerenone C (d) Gingerenone B (e) Zingerone f. dehydrozingerone (g) Shogaol

Table 1. List of samples Z. officinale collected from different altitudes

Sample code	District	Place of collection	Altitude (meter)
NK1	Salyan	Malneta	1380
NK2	Syangja	Chilaunebas	1275
NK3	Dang	Kabhre	1250
NK4	Palpa	Barangdi	1250
NK5	Tanahau	Rishing	1260
NK6	Dhading	Kebalpur	1260
NK7	Nuwakot	Haldekalika	1150
NK8	Morang	Letang	800

Estimation of total phenolic content

The phenolic content in extracts was estimated by Folin-Ciocalteu colorimetric method adopting the standard procedure based on oxidation-reduction reaction[14].

The calibration curve was constructed by using the gallic acid solution of different concentrations as standard. Gallic acid solution (1 mL) from each concentration was poured into test tubes. Then, 5 mL of 10 % Folin- Ciocalteu reagent (FCR) and 4 mL of 7 % sodium carbonate solution (Na_2CO_3) were added to these test tubes to make a total volume of 10 mL. The blue coloured mixture was shaken well and incubated for 30 minutes at 40 ºC in a water bath. Finally, the absorbance of the solution was noted using a spectrophotometer at 760 nm against a blank solution

having all the reagents except gallic acid. Then the various concentration of the extracts (20, 40, 80, 160, 320 and 640 µg/mL) was prepared by serial dilution and their absorbance were noted in the spectrophotometer.

The total phenolic content was calculated as:

$$C = \frac{cV}{m} \quad \text{..... (1)}$$

Where, C = Total phenolic content, (mg GAE/g), c = Concentration of gallic acid established from the calibration curve (mg/mL) and V = Volume of extract (mL)

Statistical analysis

The data were recorded as a mean of three determinations (n=3) of absorbance for each concentration, from which linear correlation coefficient (R^2) was calculated.

$y = mx + c$..... (2)

Where, y = Absorbance of the extract, m = Slope, x = Concentration of the extract, c = Intercept.

The concentration of the extracts was calculated with the help of a regression equation. Similarly, the phenolic content was quantified from the calculated value of the concentration of the extract.

Estimation of total flavonoid content

The aluminium chloride colorimetric assay was used to estimate the total flavonoid content in the *Z. officinale* samples using quercetin as standard to construct the calibration curve[15].

The stock solution was prepared by dissolving 20 mg of extract in 2 mL of methanol and the working solutions of different concentrations were prepared as 20, 40, 80 and 160 and 320 µg/mL by serial dilution. Then the absorbance of each concentration was noted following the same procedure as adopted for the standard quercetin.

The flavonoid content in the samples was calculated by using the equation as,

$$c = \frac{cV}{m} \quad \text{..... (3)}$$

Where, C = Total flavonoid content (mg QE/g), c = Concentration of quercetin established from calibration curve (mg/mL), V = Volume of the extract (mL), m = Weight of the plant extract (g)

Statistical analysis

The data were recorded as a mean of three determinations (n=3) of absorbance for each concentration to calculate the linear correlation coefficient.

$$y = mx + c \quad \text{..... (4)}$$

Where, y = Absorbance of the extract, m = Slope, x = Concentration of the extract, c = Intercept

The concentration of the extracts was calculated with the help of a regression equation. The flavonoid content in the extracts was estimated from the calculated value of the concentrations of the extracts.

Evaluation of antioxidant activity

The *Z. officinale* extracts collected from different regions were evaluated for the antioxidant potential following DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay with some modifications[16,17]. The working solutions of extracts were prepared by serial dilution of stock solution. The ascorbic acid was used as positive control The free radical scavenging activity of the extracts was calculated as,

$$\%\ \text{radical scavenging} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}}\ X\ 100$$

IC_{50} was calculated graphically by plotting extract concentrations against the percentage of radical scavenging.

α-Amylase inhibition

The α-amylase enzyme inhibition was performed by using the procedure with slight modification[18]. The undigested starch due to enzyme inhibition was detected spectrophotometrically at 630 nm due to the formation of a blue starch iodine complex. The iodine solution, phosphate buffer, starch solution, α-amylase, hydrochloric acid and extracts were prepared to adopt the protocol[19]. The enzyme solution was prepared in a phosphate buffer of pH 6.9. The substrate 400 µL was pre-incubated in 200 µL of extracts and acarbose separately at 37 °C for 5 min. After this, the

α-amylase solution (200 µL) was mixed with each of them and incubated at 37 °C for 15 min. The enzymatic reaction was quenched by 800 µL of HCl (0.1 M). Then, 1000 µL of iodine solution was added to the content and the absorbance was noted spectrophotometrically at 630 nm.

The Percentage of enzyme inhibition was calculated as,

$$\% \text{ Inhibition} = 1 - [A_2 - A_1 / A_4 - A_3] \times 100$$

Where, A_1=Absorbance of the mixture plant extract, starch, and amylase, A_2=Absorbance of the mixture of plant extract and starch, A_3=Absorbance of the mixture of starch and α-amylase, A_4=Absorbance of the starch. The IC_{50} for each sample was calculated graphically.

Toxicity test

The toxicity test was performed by adopting the standard procedure[19]. The artificial seawater was prepared by dissolving 15 mg of sodium chloride, 0.45 g of potassium chloride, 0.55 g of calcium chloride and 1.76 g of magnesium sulphate in distilled water to make a 500 ml solution. The brine shrimp nauplii hatching tank was filled with water and the eggs were sprinkled into the covered part of the tank. This method shows the toxicity of extracts towards the nauplii by determining the LC_{50} (µg/mL). LC_{50} value is the lethal concentration required to kill 50 percent of the brine shrimp nauplii. The extracts showing the LC_{50} less than 1000 ppm are considered toxic.

$$\alpha = \frac{1}{n}[\Sigma y - \beta \Sigma x] \quad \text{..... (5)}$$

$$\beta = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} \quad \text{..... (6)}$$

Where 'n' is the number of replicates (here three), 'x' is the log of constituents in mg/mL (log10, log100, and log1000 for three dose levels respectively). 'Y' is prohibited for the average survivor of all replicates.

From probit regression,

$$Y = \alpha + \beta X \quad \text{..... (7)}$$

$$X = \frac{(Y - \alpha)}{\beta} \quad \text{..... (8)}$$

Where Y is constant having value 5 calculating LC_{50} values Thus,

$$LC_{50} = \text{antilog } X \quad \text{..... (9)}$$

$$\text{Percentage mortality} = \frac{\text{Number of dead nauplii}}{\text{Total number of nauplii}} \text{ x } 100$$

Results

The yield percentage of each extract of *Z. officinale* collected from the different altitudes are presented in table 2. The quantitative estimation of the *Z. officinale* was studied in methanol solvent. The yield percentage of extracts was found in the range from 6.05% to 9.5% collected from the different districts of Nepal respectively.

Antioxidant activity

The radical scavenging activity of the Z. officinale extracts was evaluated by DPPH assay. The percentage of radical scavenging against the different concentration of the sample extracts are displayed in Tables 3 and 4.

Table 2. The percentage yield of extracts of Z. *officinale*

Sample	Quantity of sample taken (g)	Quantity of extract obtained (g)	% Yield
NK1	200	14.29	7.14
NK2	200	18.10	9.5
NK3	200	13.50	6.75
NK4	200	14.05	7.03
NK5	200	17.70	8.85
NK6	200	13.72	6.86
NK7	200	14.64	7.32
NK8	200	12.10	6.05

Table 3. The DPPH radical scavenging activity of Z. *officinale* against different concentrations.

Concentration (µg/mL)	Percentage of radical scavenging				
	NK1	NK2	NK3	NK4	NK5
20	37.29±7.85	33.82±5.40	21.37±4.91	19.00±5.73	11.26±2.96
40	46.63±3.15	50.61±1.87	49.63±2.66	42.93±1.75	25.68±8.22
60	60.78±5.30	73.55±2.11	60.66±1.89	59.37±5.12	49.38±6.65
80	72.86±1.17	82.18±0.31	72.95±2.21	65.47±6.26	61.77±1.75
100	75.73±3.51	83.35±1.03	77.98±3.45	76.78±6.33	75.06±1.57
120	83.11±1.49	86.18±0.51	78.66±2.30	79.94±2.73	85.06±1.56

Table 4. The DPPH radical scavenging activity of *Z. officinale* against different concentrations

Concentration (µg/mL)	Percentage of radical scavenging			
	Ascorbic acid	NK6	NK7	NK8
20	39.60±1.86	39.60±1.86	26.10±15.74	26.10±15.74
40	54.20±1.97	54.20±1.97	48.64±1.37	48.64±1.37
60	69.33±0.78	69.33±0.78	66.63±4.34	66.63±4.34
80	81.86±3.24	81.86±3.24	72.28±1.64	72.28±1.64
100	91.10±1.08	91.10±1.08	77.94±0.70	77.94±0.70
120	94.44±0.30	94.44±0.30	80.42±1.69	80.42±1.69

The reducing power of the extracts was measured in inhibitory concentration IC_{50}, which is the amount of the extracts required to scavenge 50% DPPH free radicals. The inhibitory concentration was calculated graphically by plotting the concentration of sample extracts against the percentage of radical scavenging. The IC50 of the plant extract and the standard ascorbic acid is presented in Table 5. The IC50 for the various extracts of Z. officinale is in ranges from 44.43 µg/mL to 65.71 µg/mL. The sample of Z. officinale collected from Chilaunebas of Syangja district had shown the lowest IC50 having high antioxidant potential as compared to the standard ascorbic acid 48.74 µg/mL. The sample having low IC50 reflects the greater radical scavenging activity.

Table 5. The antioxidant potential and inhibitory concentration IC50 of the different extracts of Z. *officinale*

Extracts of *Z. officinale* and ascorbic acid	Inhibitory concentration (IC_{50}) µg/mL
NK1	53.64
NK2	44.43
NK3	57.58
NK4	61.37
NK5	65.71
NK6	53.33
NK7	55.21
NK8	59.65
Ascorbic acid	48.74

Total phenolic content

The amount of phenolic content in the different extracts of Z. officinale are presented in Table 6. The total phenolic content in the extracts of Z. *officinale* collected from Letang of Morang district is found in a ramge from 118.19±3.32 mg GAE/g to 222.04 ± 24.93 mg GAE/g collected from Chilaunebas of Syangja district of Nepal . The rest of the samples of Z. *officinale* showed a moderate amount of total phenolic content.

Table 6. The spectrophotometric estimation of phenolic content in different samples of *Z. officinale* (n=3)

Extracts of *Z. officinale*	Absorbance			Phenolic content (mg GAE/g)			Mean±SD (mg GAE/g)
	A_1	A_2	A_3	P_1	P_2	P_3	
NK1	1.446	1.559	1.395	145.70	157.55	117.80	140.35 ± 20.41
NK2	1.905	2.356	2.261	193.83	241.12	231.16	222.04 ± 24.93
NK3	1.391	1.40	2.389	139.93	140.87	244.56	175.13 ±6 0.15
NK4	1.334	1.497	1.510	133.95	151.05	152.41	145.81 ± 10.28
NK5	1.875	1.772	1.754	190.68	179.88	177.99	182.86 ± 6.84
NK6	1.514	1.576	1.078	152.83	159.33	107.11	139.76 ± 28.46
NK7	1.251	1.314	1.183	125.25	131.86	118.12	125.07 ± 6.87
NK8	1.219	1.158	1.174	121.90	115.49	117.18	118.19 ± 3.32

Total flavonoid content

The amount of flavonoid content in the extracts of Z. officinale is presented in table 7. The total flavonoid content was found to be lower than that of total phenolic content in the extracts of Z. officinale collected from different regions of Nepal. The total flavonoid content in the sample collected from the Letang of Morang district was found to be in a range from 42.10 ± 4.48 mg QE/g to 62.95 ± 0.70 mg QE/g in the sample collected from Haldekalika of Nuwakot district of Nepal. The rest of the Z. officinale samples collected from the different regions showed a moderate amount of flavonoid content.

α- Amylase enzyme inhibition activity

The comparison of α-amylase enzyme inhibition activity of the extracts of Z. officinale to the standard acarbose of different concentrations is displayed in Figure 2. The plots showed the variation of enzyme inhibition activity against the different concentrations of the extracts and the standard acarbose. The inhibition activity of the standard acarbose was found almost the same as the inhibition shown by the extracts of Z. officinale.

Table 7. Estimation of total flavonoid content in different samples of Z. officinale (n=3)

Extracts of *Z. officinale*	Absorbance			Total flavonoid content (mg QE /g)			Mean ± SD (mg QE/g)
	A_1	A_2	A_3	P_1	P_2	P_3	
NK1	1.564	1.401	1.361	57.22	51.01	49.48	52.57±4.10
NK2	1.576	1.579	1.576	57.67	57.78	57.56	57.67±0.10
NK3	1.459	1.452	1.446	53.22	52.95	52.72	52.96±0.24
NK4	1.301	1.313	1.288	47.19	47.65	45.28	46.71±1.25
NK5	1.257	1.319	1.267	45.52	47.88	45.89	46.43±1.27
NK6	1.474	1.553	1.557	53.79	56.80	56.95	55.84±1.78
NK7	1.74	1.70	1.70	63.77	62.51	62.59	62.95±0.70
NK8	1.161	1.053	1.288	41.85	37.74	46.69	42.10±4.48

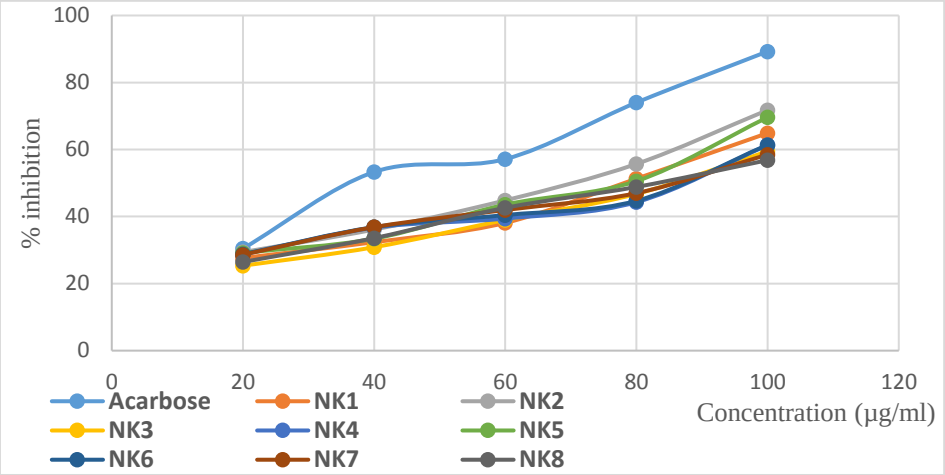

Figure 2. Percentage inhibition of α-amylase against the concentration of the extracts and acarbose

The percentage inhibition of α-amylase was observed to be proportional to the increase in the concentration of extracts. The standard acarbose showed the highest percentage inhibition as compared to the extracts of *Z. officinale* collected from different altitudes. The IC_{50} of standard acarbose and *Z. officinale* extracts was calculated graphically. The IC_{50} of standard acarbose was found to be 100.23 µg/mL whereas, the methanol extracts of Z. officinale growing in different altitudes showed IC50 ranging from 161.25 µg/mL to 216.59 µg/mL. Most of the extracts showed moderate antidiabetic activity as compared to the standard acarbose. The IC_{50} for the different extracts of *Z. officinale* is presented in Table 8.

Table 8. The IC50 for α-amylase inhibition of the extracts of Z. officinale

Z. officinale /acarbose	IC_{50} (µg/mL)
NK1	191.37
NK2	161.25
NK3	215.83
NK4	209.57
NK5	174.94
NK6	207.63
NK7	212.19
NK8	216.59
Acarbose	100.23

Toxicity test

The toxicity of Z. officinale was evaluated by brine shrimp lethality bioassay. The lethal concentration required to kill 50% of the brine shrimp larva (LC50) is shown in Table 9.

Table 9. The LC50 of Z. officinale showing the toxicity effect

Extracts of *Z. officinale*	LC_{50} (µg/mL)
NK1	3630780548
NK2	67608297.54
NK3	2152781735
NK4	45708.81
NK5	15505.16
NK6	6807693.58
NK7	46773.51
NK8	457088.18

The degree of lethality was found to be directly proportional to the concentration of the extracts that are maximum mortalities of the brine shrimp larvae took place at the concentration of 1000 µg/mL and the least mortalities were at 10 µg/mL. The extracts having LC50 values less than 1000 µg/mL are found to be toxic. The LC50 values for Z. officinale collected from the different altitudes were found

greater than 1000 µg/mL which shows Z. officinale are found nontoxic.

Discussion

The yield percentage of the extracts of Z. officinale showed the sample growing in Rishing of Tanahau district has the highest yield percentage indicating the rich source of secondary metabolites. The Z. officinale collected from the Letang of Morang district was found poor source of secondary metabolites with the least yield percentage. The rest of the sample showed a moderate amount of secondary metabolites. The antioxidant potential of Z. officinale extracts was calculated in terms of inhibitory concentration. The sample with low IC50 is found to be a potential antioxidants. The sample collected from the Chilaunebas of Syangja district showed the highest antioxidant potential whereas the sample collected from Rishing of Tanauhau district showed the least antioxidant potential. This variation in the antioxidant potential of Z. officinale is due to the altitudinal variations and the other environmental conditions. The presence of phenolic compounds such as eugenol, shogaols, zingerone, gingerdiols, gingerols etc. in ginger oil and oleoresin may be responsible for their antioxidant properties20. The difference in the antioxidant activity may be attributed to the difference in essential oil and oleoresins composition of Z. officinale growing in different regions may be altered due to the different environmental conditions like altitude, different solar exposition, different soil types and also the genetic differences21. The half-maximal inhibitory concentration (IC50) of radical scavenging activity (RSA) reported in aqueous and methanol fraction of Z. officinale was 23.30 ±1.04 µg/mL and 9.78 ± 0.33 µg/mL, respectively and it was found comparable to the results of the present study[22]. The antioxidant activity shown by Z. officinale growing in different altitudes of Nepal is found nearly the same as the methanol extracts of different parts of Z. officinale observed by Ghasemzadeh et al. (2010) 23. The excessive formation of free radicals in the body might cause various cell damage and incite different issues. However, the natural antioxidants from the natural origin like plant and plant-based products can repair and neutralize these free radicals that stop the oxidative damage of the cells and other cell components.

The total phenolic and flavonoid content in the extracts of Z. officinale growing in the different altitudes of Nepal were determined spectrophotometrically and results are presented in tables 6 and 7. The total phenolic content was found maximum (222.04±24.93 mg GAE/g) in Z. officinale collected from Chilaunebas of Syangjs district. The lowest phenolic content (118.19±3.32 mg GAE/g) was found in the sample of Z. officinale collected from Letang of Morang district. These values were found higher than those reported by EI-ghorab et al. (2010) 95.2±6.2 mg GAE/g and 87.5±2.3 mg GAE/g in the methanol and hexane extracts of fresh ginger rhizomes24. Buchana and Jones (2000) and Dias et al. (2016) has reported that phenolic compounds are usually generated as products of defense mechanism against the pathogen attack or in response to a stressful environment of the plants. The results showed that the samples of Z. officinale collected from the different altitudes of Nepal are found the rich sources of phenolic compounds and can be used as the source of these supplements that could be used for the treatment of simple to life threatening diseases 25,26. The highest flavonoid content (62.95±0.70 mg QE/g) was estimated in Z.officinale growing in the Haldekalika of Nuwakot district while the extract of Z. officinale growing in the Rishing of Tanahaun district showed the least amount (46.43±1.27 mg QE/g). The rest of the extracts showed a moderate amount of flavonoid content and vary with the altitude and the environmental conditions for plants growing. The flavonoid content in Z. officinale extracts collected from different altitudes of Nepal was found higher as compared to the previously reported values (40.25±0.21 mg QE/g) and (6.55±0.10 mg QE/g)27. The antioxidant capacity of the plant extract showed a positive correlation to the amount of total phenolic and total flavonoid content. Higher amounts of polyphenols and flavonoids are responsible to impart the higher antioxidant activity of the plant extracts although quantitative determination of phenolic compounds in plant extracts are hampered by their complex structure, diversity,

type of analytical assay method, selection of standard and presence of interfering substances. Phenolic and flavonoid compounds are reported to act as a reducing agent which can be hydrogen donors or oxygen quenchers as well as free radical inhibitors. The antidiabetic potential of Z. officinale extracts was evaluated by the inhibition of the enzyme α-amylase. The extracts of Z. officinale collected from Chilaunebas of Syangja district displayed the highest α-amylase enzyme inhibition (161.25 µg/mL) while the extract of Z. officinale collected from Letang of Morang district showed the minimum (216.59 µg/mL) enzyme inhibition activity. Lower the value of inhibitory concentration (IC50) higher will be the enzyme inhibition activity.

The present research showed Z. officinale growing in the different altitudes of Nepal have high values of inhibitory concentration (IC50) indicating the poor α-amylase inhibition as compared to the previously reported value (3.14 µg/mL) for red ginger28. The Z. officinale from the different altitudes of Nepal were found nontoxic against the brine shrimp lethality test. All the samples showed an LC50 greater than 1000 µg/mL indicating the nontoxic effect.

Conclusion

This study showed Z. officinale are a rich source of plant secondary metabolites that could be used to cure different diseases in human beings. The results of the present study suggested that the Z. officinale growing in the different altitudes of Nepal are a rich source of phenolic and flavonoid compounds. The findings of this study showed that ginger samples are found to be the sources of natural antioxidants and could be used as the source of natural radical scavengers. The Z. officinale growing in the Chilaunebas of Syangja district was found to be a good antioxidant as compared to the standard ascorbic acid. It can be concluded that Z. officinale are rich in the total phenolic and flavonoid content that exhibit antioxidant activity for pharmacological, cosmetic and agronomic industries. It also concluded that Z. officinale growing at different altitudes is nontoxic and is considered to be a safe herbal medicine.

Acknowledgements

The authors are grateful to the Central Department of Chemistry, Tribhuvan University for providing the laboratory facility and some chemicals to carry out this research.

References

1. Plant Resources: A Scientific Publication, *Bulletin of Department of Plant Resources*, 2016. 38
2. Thomas, M. and Singh, S. 2013. Review article on antimicrobial resistance. *Indian Journal of Research Pharmacy and Biotechnology*. **62(1)**: 2320-3471.
3. Bourgaud, F., Gravot, A., Milesi, S. and Gontier, E. 2001. Production of plant secondary metabolites: a historical perspective. *Plant science*. **159**: 839-851.
4. Kunwar, R. M., Nepal, B. K., Kshhetri H. B., Rai, S. K. and Bussmann, R. W. 2006. Ethnomedicine in himalaya: a case study from Dolpa, Humla, Jumla and Manang district of Nepal. *Journal of Ethnobiology and Ethnomedicine*. **2(27)**: 1-6.
5. Savithramma, N., Rao, M. L. and Suhrulatha, D. 2011. Screening of medicinal plants for secondary metabolites. Middle-East. *Journal of Scientific Research*. **8(3)**: 579-584.
6. Vesna, D., Nikolic, I., Ivan, M., Savic, 1., Ivana, M., Savic,1., Ljubisa, B., Nikolic, 1., Mihajlo, Z., Stankovic, 1., Valentina, D. and Marinkovic. 2011. Paclitaxel as an anticancer agent: isolation, activity, synthesis and stability. *Central European Journal of Medicine*. **6(5)**: 527-536.
7. Flemming, K. and MSc. R. N. 2010. The use of morphine to treat cancer-related pain: a synthesis of quantitative and qualitative research. *Journal of Pain and Symptom Management*. **39 (1)**: 139-154.
8. Jian, Li. and Zhou, B. 2010. Biological actions of artemisinin: insights from medicinal chemistry studies. *Molecules*. **15**: 1378-1397.
9. Gautam, J. and Mainali, R. P. 2016. Management of ginger rhizome fly (calobata sp.) and associated rhizome rot (pythium sp.). *World Journal of Agricultural Research*. **4(4)**: 128-131.
10. Al-Yahya, M. A., Rafatullah, S., Mossa, J. S., Ageel, A. M., Parmar, N. S. and Tariq, M. 1989. Gastroprotective activity of ginger Zingiber Officinale roscoe in albino rats. *American Journal of Chinese Medicine*. **17(1)**: 51-56.
11. Feng, T., Su, J., Ding, Z. H., Zheng, Y. T., Li, Y., Leng, Y. and Liu, J. K. 2011. chemical constituents and their bioactivities of "tongling white ginger" (Zingiber officinale). *Journal of Agricultural and Food Chemistry*. **59**: 11690-11695.
12. Phillips, S. 1993. Zingiber officinale (Ginger)-an antiemetic for day case surgery, anaesthesia. **48**: 715-717.
13. Gewali, M. B. and Awale, S. 2008. Aspects of traditional medicines in Nepal. Institue of the natural medicine university of Toyama.

14. Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *The American Journal of Enology and Viticulture*. **16**: 144–158.
15. Miliauskas, G., Venskutonis, P. R. and Van Beek, T. A. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*. **85**: 231.
16. Khadayat, K., Marasini, B. P., Gautam, H., Ghaju, S. and Parajuli, N. 2020. Evaluation of the alpha-amylase inhibitory activity of Nepalese medicinal plants used in the treatment of diabetes mellitus. *Clinical Phytoscience*. **6(34)**: 1–8.
17. Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*. **28(1)**: 25-30.
18. Bernfeld, P. 1955. Amylases alpha and beta. in: methods in enzymology. 1. New York: Academic Press, 149-5.
19. Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. J. and McLaughlin, J. L. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*. **45(05)**: 31-4.
20. Singh, G., Kapoor, I. P. S., Singh, P., Heluani, C. S., Lampasona, M. P. and Catalan, C. A. N. 2008. Chemistry, antioxidant and antimicrobial investigation of essential oil and oleoresins of Zingiber officinale. *Food and Chemical Toxicology*. **46**: 3295-3302.
21. Figueiredo, C., José, A., Barroso, G., Luis, G. and Johannes, J. C. 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils and factors affecting volatile and essential oil production in plants. *Flavour Fragrance journal*. **23**: 213–226.
22. Bekkouch, O., Harnafi, M., Touiss, I., Khatib, S., Harnafi, H., Alem, C. and Amrani, S. 2019. In-vitro antioxidant and in vivo lipid-lowering properties of zingiber officinale crude aqueous extract and methanolic fraction: A follow-up study. Evidence-based complementary and alternative medicine. 1-13.
23. Ghasemzadeh, A., Jaafar, H. Z. E. and Rahmat, A. 2010. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). *Molecules*. **15(6)**: 4324–4333.
24. EL-ghorab, A. H., Nauman, M., Anjum, F. M., Hussain, S. and Nadeem, M. A. 2010. Comparative study on chemical composition and antioxidant activity of ginger (Zingiber officinale) and cumin (Cuminum cyminum). *Journal of Agriculture and Food Chemistry*. **58**: 8231.
25. Buchanan, B. B. and Jones, R. L. and Rockville, M. D. 2000. *American Society of Plant Physiologists. Biochemistry and molecular biology of plants*. 40.
26. Dias, M. I., Sousa, M. J., Alves, R. C. and Ferreira, ICFR. 2016. Exploring plant tissue culture to improve the production of phenolic compounds: A review. *Industrial Crops and Products*. **82**: 9–12.
27. Ahmed Alia, A. M., Mohamed El-Noura, M. E. and Yagib, S. M. 2018. Total phenolic and flavonoid contents and antioxidant activity of ginger (Zingiber officinale Rosc.) rhizome, callus and callus treated with some elicitors. *Journal of Genetic Engineering and Biotechnology*. **16**: 677–682.
28. Oboh, G., Akinyemi, A. J., Ademiluyi, A. O. and Adefegha, S. A. 2010. Inhibitory effects of aqueous extracts of two varieties of ginger on some key enzymes linked to type-2 diabetes in vitro. *Journal of Food and Nutrition Research*. **49(1)**: 14–20.