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Phytochemical analysis and digestive enzymes inhibition study of *Beta vulgaris*

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Abstract

Beta vulgaris is an annual crop grown for its edible roots and leaves. It is traditionally used for the treatment of diabetes, cancer, obesity, heart problems, kidney problems, and liver diseases. The present work is centered on the phytochemical analysis and assessment of antioxidant, antimicrobial, and antidiabetic activities, and toxicity in the root and leaf extracts and solvent fractions. TPC and TFC were measured using the Folin-Ciocalteu phenol reagent method and $AlCl_3$ colorimetric method respectively. Antioxidant and antidiabetic activity were measured with DPPH assay and α-glucosidase enzyme inhibition assay. Antimicrobial activity was determined with the agar disc diffusion method and brine shrimp assay was performed to measure toxicity. Phytochemical analysis revealed the presence of phenols, flavonoids, glycosides, saponins, tannins, terpenoids, and alkaloids. The ethyl acetate fraction of the root contained the highest amount of phenolics with 84.35 $\pm$ 0.94 mg GAE/g. Total flavonoid content was found highest in the hexane fraction of root at 150.48 $\pm$ 1.10 mg QE/g. The ethyl acetate fraction of the root displayed an IC_{50} of 3.92 $\pm$ 0.06 μg/mL in the DPPH assay. The plant extracts and fractions possessed weak α-glucosidase enzyme inhibition activity. They were inactive against bacterial species of Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, and fungal species of Fusarium solani. Toxicity assay found the plant to be non-toxic against the brine shrimp nauplii with the lowest LC_{50} value being 1166.36 $\pm$ 100.21 μg/mL for the hexane fraction of leaf. The study finds B. vulgaris to be rich in phytochemicals and antioxidant activity with weak α-glucosidase enzyme inhibition activity. It is non-toxic to brine shrimp larvae.

Keywords
Beta vulgaris, α-glucosidase, DPPH, antidiabetic, antimicrobial, toxicity.

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1 Introduction

Humans have been using plants as both food and medicine. Dietary plants contain many important secondary metabolites besides basic nutrients. The secondary metabolites in such food act as functional components that can prevent or treat diseases [1]. Many plants used in traditional medicines are also consumed as spices, vegetables, and fruits. They are known as functional foods and defined as 'Foods or dietary components that may provide a health benefit beyond basic nutrition' [2]. The functional food market has grown due to an increase in the health consciousness among the modern population. Medicinal or functional food plays a supporting role in modern medicine in the treatment of illness. So, it is essential to explore the phytochemicals and biological activities in our fruits and vegetables and assess their medicinal value.

Beta vulgaris subsp. *vulgaris*, commonly known as beetroot is an annual crop plant from the Chenopodiaceae family [3]. Its wild forms are distributed from Europe, and North Africa to Western Asia whereas cultivated beetroots are grown worldwide and commonly consumed in our daily diets [4]. *B. vulgaris* is rich in minerals and vitamins [5]. Proximate analysis by Kale et al., (2018) has revealed the composition of the plant as 1.35 ± 0.2% proteins, 7.59 ± 0.4% carbohydrates, 0.3 ± 0.1% fats, 1.9 ± 0.2% dietary fibers, 1.4 ± 0.2% ash, and 87.4 ± 0.3% moisture [6]. Alkaloids, terpenoids, steroids, flavonoids, tannins, saponins, and glycosides in root extracts and flavonoids, tannins, oxalate, anthocyanins, saponins, phenolics, carotenoids, and phytate in leaf extracts of *B. vulgaris* has been detected in previous studies [7]. The plant is used in traditional medicine for the treatment of diabetes, cancer, obesity, heart, kidney, and liver diseases, and it also improves the immune system and hematopoietic system [8–10]. *B. vulgaris* has also been used for dandruff, diminished libido, constipation, and gastrointestinal and musculoskeletal pain [11]. In addition to traditional uses, the plant has displayed remarkable biological activities in different scientific studies. Rehman et al., (2021) observed significant protein denaturation inhibitory activity, acetylcholinesterase inhibitory activity, and red blood cell stabilizing activity in root and leaf extract of *B. vulgaris* [12]. Mzoughi et al., (2019) have revealed the antioxidant and antidiabetic potential of the plant [13]. The roots of the plant displayed anticancer and antibacterial activities in a study by El-Beltagi et al., (2018) [14]. The plant has also gained significant attraction in scientific research because of its high nitrate (NO_3^-) content that promotes health aids for cardiac ailments through endogenic nitric oxide (NO) synthesis [15]. It is widely consumed and utilized to develop food coloring agents [16]. *B. vulgaris* is a source of a class of bioactive compound, 'betaine' that shows a hepatoprotective effect by increasing the expression of the quinone reductase enzyme [17]. Beetin 27 (BE27) is a protein isolated from *B. vulgaris* that contains antiviral and cytotoxic activities [18]. Additionally, aqueous extract of the plant has been used in the biosynthesis of ZnO nanoparticles that display antimicrobial, anticancer, and antidiabetic activity [19]. Due to its high nutritional and medicinal properties, *B. vulgaris* has attracted many scholars over the years and there have been many studies regarding its nutritional and pharmaceutical activities. The present study adds brine shrimp toxicity assay and comparative phytochemical and biological analysis of the plant using methanol extract and hexane, ethyl acetate, dichloromethane, and aqueous fraction of root and leaf of *B. vulgaris* to the ongoing research on the plant. The findings of the present study may guide future work regarding the isolation, purification, and characterization of active metabolites from *B. vulgaris*.

Diabetes mellitus is a chronic metabolic disorder characterized by high levels of blood sugar and caused by insulin deficiency or insulin resistance [20]. Synthetic antidiabetic drugs are associated with adverse effects including hypoglycemic coma, weight gain, and kidney and liver diseases [21]. So, the World Health Organization (WHO) recommends the treatment of diabetes using medicinal plants that are cheap, effective, and contain fewer side effects [22]. The antidiabetic potential of many fruits and vegetables remains unexplored. So, there is a need to study antidiabetic activity present in our food plants to lower the risk of diabetes with appropriate diets and possible discovery of noble antidiabetic compounds.

Overproduction of highly reactive oxygen and nitrogen species during various metabolic processes in our bodies causes oxidative stress [23]. During oxidative stress, reactive oxygen and nitrogen species oxidize and damage important biomolecules like lipids, proteins, DNA, and RNA [24]. This leads to chronic diseases and disorders including cancer, diabetes mellitus, cataract, cardiovascular disease, neurodegenerative diseases, asthma, and rheumatoid arthritis [23]. Antioxidants are bioactive compounds that are involved in preventing or postponing the oxidation of particles or molecules and protecting our health and well-being [25]. Antioxidants may be enzymatic or non-enzymatic and endogenous or exogenous. The correlation between the intake of antioxidant-rich pomegranate juice and its positive impact on health has been observed in previous studies [26]. Antioxidants in foods assist in preventing oxidative stress-related diabetes, obesity, hypertension, cardiovascular and chronic

inflammation disease, and display neuroprotective and anticancer activity [27].

Foodborne diseases and infections cause about 600 million hospital cases and 420,000 deaths each year [28]. Most of such infections are caused by the growth of pathogenic bacteria and fungi in foods. In addition to health risks, food spoilage is associated with environmental and resource costs [29]. Synthetic preservatives like benzoates, caffeine, saccharin, and sorbic acid are known to produce side effects such as asthma, heart defects, diabetes, and dermatitis [30]. So, there is an urgent need for antimicrobial agents of natural origin that are effective, safe for human consumption and display minimal side effects. Many edible plants display significant antimicrobial activity and they are considered as sources of inhibitory substance against foodborne pathogens [31].

Traditional medicines of natural origin are widely regarded as safer alternatives to modern synthetic drugs. A previous study reported that the fatalities caused by adverse reactions to modern pharmaceuticals were more than 100,000 whereas herbal medicines were responsible for less than 24 deaths each year [32]. Nevertheless, evaluation of toxic and other side effects is incorporated in the assessment of medicinal properties of plants for their potential toxicity and bioactivity, but the safety assessment of edible and cultivated plants is generally ignored as they are considered safe for human consumption. An adequate study of medicinal food requires an assessment of its cytotoxic activity to increase confidence in human consumption and potential pharmaceutical developments.

Figure 1: Photographs of fresh plant sample, herbarium, sample size collection, and drying of plant samples.

2 Materials and Methods

2.1 Chemicals and reagents

The 4-nitrophenyl -D-glucopyranoside (CAS NO: 3767-28-0), α-glucosidase enzyme (CAS NO: 9001-42-7), and quercetin (CAS NO: 117-39-5), were procured from Sigma-Aldrich (Germany). DPPH was purchased from Hi-media (India) and gallic acid was purchased from Molychem (India). Analytical grade (extra pure) organic solvents like methanol, hexane, ethyl acetate, DCM, DMSO, and other chemicals used in the experimentation were purchased from Merck and Fischer Scientific (India).

2.2 Equipment

The equipment used during the study were mortar and pestle, glassware, an electric grinder, a weighing scale (Pioneer, DHAUS), a rotary evaporator (Buchi RE111), a hot air oven (Griffin-Grundy), a microplate reader (Synergy LX, Bio Tek, Instruments, Inc., USA), water-bath (Clifton), pipettes, vials, micropipettes (Erba BIHOT).

2.3 Plant collection and identification

Leaf and root samples of the plant were harvested from the cultivated site in Bhaktapur, Nepal in August 2021. The altitude and coordinates of the collection site are given in Table 1. The plant was identified by the Central Department of Botany, Tribhuvan University, Kathmandu, Nepal as herbarium sample TUCH-210075. Photographs of plant samples are given in Figure 1.

Table 1: Traditional medicinal uses of the plant

Name of the plant	Identification no.	Altitude/plant growing	Plant parts	Medicinal uses and health benefits
Beta vulgaris	TUCH-210075	Altitude 1401 m 85° 25' 48" E, 27° 40' 14" N.	Roots Leaves	Obesity, diabetes, and cardiovascular disease [8, 9] Cancer, heart disease [10] Dandruff, diminished libido, constipation, gastrointestinal, and musculoskeletal pain [11] Hypertension and endothelial function [4] Food coloring [16]

2.4 Extraction and fractionation

Plant samples were cleaned, shade-dried to a constant weight, and ground to powder. The cold percolation method was used for the extraction of phytochemicals. 250 g root powder and 250 g leaf powder were soaked in reagent grade methanol (500 mL) in separate conical flasks at a temperature of 21 ^{0}C for 72 hours. The flasks were shaken vigorously after every 24-hour interval. Then, the contents were filtered with a clean muslin followed by the Whatman-1 filter paper. A rotatory evaporator was used to concentrate the filtrate at reduced pressure and 40 ^{0}C temperature.

Solvent fractions of methanol extract were isolated using hexane, dichloromethane, ethyl acetate, and water. 20 g methanol extract was dissolved in 50 mL of distilled water in a separating funnel and an equal volume of hexane was added. The contents were vigorously shaken and then left undisturbed until clear layers of hexane at the top and water at the bottom were formed. The hexane layer was concentrated in the rotary evaporator at reduced pressure and 40 C temperature. The aqueous layer was subjected to further fractionation with dichloromethane and ethyl acetate.

2.5 Phytochemical analysis

The qualitative and preliminary analysis of the plant extracts was performed according to the standard procedures as described by Savithramma et al., (2011) [33].

2.6 Total phenolic content (TPC)

The Folin-Ciocalteu reagent method as described by Slinkard et al., (1977) was employed to measure the TPC [34]. 20 µL gallic acid solution (10 to 80 µg/mL in methanol) and 20 µL of each extract and fraction (500 µg/mL in 50% DMSO) were loaded in triplicates in a 96-well plate. To each bore, 100 µL of Folin-Ciocalteu reagent and 80 µL of Na_2CO_3 solutions were added and the 96-well plate was placed in the dark for 30 minutes. Then, a microplate reader was used to measure the absorbance at 765 nm. A regression equation obtained from the absorbance versus concentrations of the gallic acid curve was used to calculate TPC. It was expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of extract and fraction.

2.7 Total flavonoid content (TFC)

The $AlCl_3$ colorimetric method as described by Marinova et al., (2005) was used to estimate the total phenolic content [35]. 130 µL standard quercetin solution (10 to 80 µg/mL in methanol) and 20 µL of 500 µg/mL plant extracts and fractions in 50% DMSO were loaded in a 96-well plate. It was followed by adding 110 µL of deionized water to the bores containing plant extracts and fractions. Subsequently, each bore received 60 µL of ethanol, 5 µL of $AlCl_3$, and 5 µL of CH_3COOK. Then, the 96-well plate was placed in the dark. A microplate reader was used to measure the absorbance at 415 nm. A standard absorbance versus concentration of quercetin curve was constructed and TFC was calculated using its regression equation. It was expressed as milligrams of quercetin equivalent per

gram (mg QE/g) of extract and fraction.

2.8 Antioxidant assay

The DPPH assay was used to measure the antioxidant activities [36]. 100 µL of plant extracts and fractions at concentrations 1.5625, 3.125, 6.25, 12.5, 25, and 50 µg/mL in 50% DMSO were loaded to the bores of a 96-well plate in triplicates. To each bore, 100 µL DPPH solution (0.1 mM in methanol) was added and the 96-well plate was placed in the dark. After 30 minutes, a microplate reader was used to measure the absorbance at 517 nm. Quercetin and 50% DMSO were used as the standard and control respectively. The following formula was used to calculate the percentage of radical scavenging

$$\text{Percentage scavenging} = \frac{A_1 - A_2}{A_1} \times 100$$

where A_1=Absorbance of control
A_2=Absorbance of sample
GraphPad Prism 9 software was used to calculate the IC_{50} (half maximal inhibitory concentration) values of extracts and fractions.

2.9 Antidiabetic assay

The α-glucosidase enzyme inhibition assay was used to measure the in-vitro antidiabetic activities [37]. 20 µL each of 0.5 unit/mL α-glucosidase enzyme and 500 µg/mL plant extracts were premixed inside the bores of a 96-well plate. Then, 120 µL of potassium phosphate buffer (pH 6.8) and 40 µL of pNPG substrate were added. After 15 minutes of incubation at 37 °C, the absorbance of the reaction mixture was measured at 405 nm. The reaction mixture with the volume of plant extract replaced by an equal volume of buffer solution was used as a control. The following formula was used to calculate the percentage of enzyme inhibition

$$\text{Percentage inhibition} = \frac{A_1 - A_2}{A_1} \times 100$$

where A_1=Absorbance of control
A_2=Absorbance of sample

2.10 Antibacterial assay

The agar disc diffusion method with slight modifications was used to measure antibacterial activity [38]. The list of pathogenic bacteria, type, and ATCC numbers are given in Table 2. Bacterial broth cultures were prepared in nutrient broth media and incubated overnight. Then, the inoculum (0.5 McFarland standards) was transferred to sterilized Muller-Hinton Agar (MHA) plates using sterile cotton swabs. Discs impregnated with 50 µL of plant extracts and fractions (25 mg/mL) were placed on the surface of MHA plates using sterilized forceps. Once all discs were in place, the plates were covered with lids, inverted, and then incubated at 35 C for 18 hours. Then, zones of inhibition (ZOI) were measured. Ampicillin (1 mg/mL) and 100% DMSO were used as standard and control respectively.

Table 2: Names of the bacteria, types, and ATCC number

Bacteria	Type	ATCC
Klebsiella pneumoniae	Gram-negative	700603
Escherichia coli	Gram-negative	25922
Bacillus subtilis	Gram-positive	35021
Staphylococcus aureus	Gram-positive	25923

2.11 Antifungal assay

The antifungal activities in extracts and fractions were measured using the disc diffusion method [38]. The fungal species *Fusarium solani* (ATCC 11712) was used as a test organism. The broth culture of the test organism was prepared in nutrient broth media and incubated overnight and then the inoculum (0.5 McFarland standards) was spread on potato dextrose agar (PDA) plates. Discs impregnated with 50 µL of 25 mg/mL plant extract and fractions were placed on the surface of the PDA plate. The plate was covered with lids, inverted, and then incubated at 35 ^{0}C for 18 hours. ZOIs were measured after incubation. Cycloheximide (20 mg/mL) and 100% DMSO were used as standard and control respectively.

2.12 Toxicity

The brine shrimp lethality assay was used to measure toxicity [39]. 2 mL of each of the plant extracts and fractions at concentrations 10, 100, and 1000 µg/mL in methanol were added to different test tubes in triplicates. 2 mL of methanol was used as the control. The solvent was evaporated to dryness

using a water bath. After this, artificial seawater (5 mL) was used to redissolve the leftover residue in each test tube. Then, 10 matured brine shrimp larvae were transferred to each test tube. The numbers of surviving brine shrimp nauplii in each test tube were counted after 24 hours. The concentration of plant extract and fraction that kills half of the test organisms (LC_{50}) was calculated from the percentage mortality versus concentration curve.

2.13 Statistical analysis

All the tests were performed in triplicates and the results are expressed as mean ± SE. The results are compared using one-way ANOVA followed by Tukey's test with the help of SPSS version 29 software. The values with $p < 0.05$ were considered statistically different.

3 Results

3.1 Phytochemical Phytochemical analysis

The preliminary phytochemical analysis found phenols, flavonoids, glycosides, saponins, tannins, terpenoids, alkaloids, and carbohydrates in both root and leaf extracts of *B. vulgaris* (Table 3). Protein was detected only in the root extract of *B. vulgaris*.

Table 3: Phytochemical constituents in plant extracts.

Group of Compounds	BRC	BLC
Alkaloids	+	+
Phenols	+	+
Flavonoids	+	+
Glycosides	+	+
Tannins	+	+
Terpenoids	+	+
Saponins	+	+
Reducing Sugars	+	+
Proteins	+	-

BRC: Crude extract of roots, BLC: Crude extract of leaves. '+' present, '-' absent.

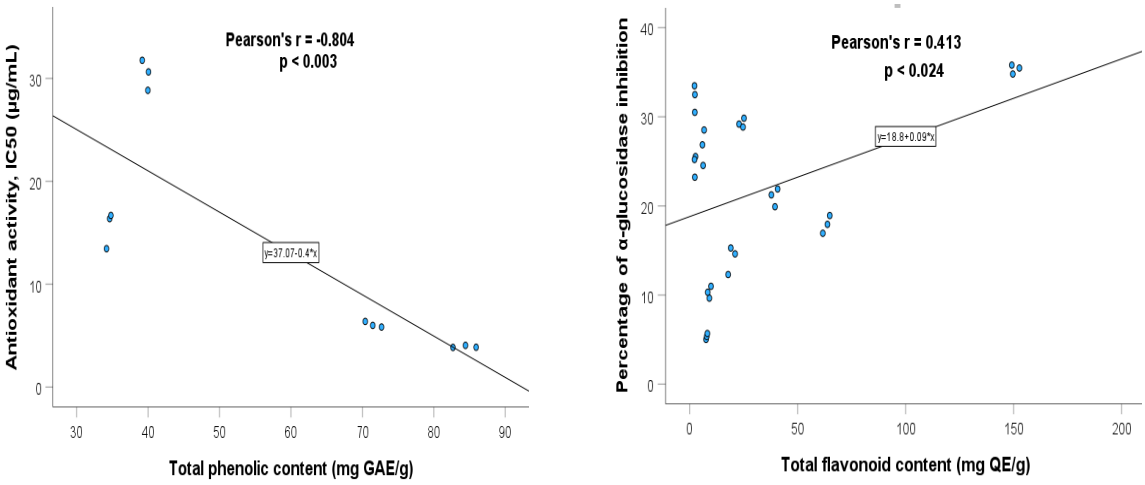
Table 4: TPC, TFC, DPPH free radical scavenging activity (IC_{50}), and α-glucosidase enzyme inhibition activity.

Plant extracts and solvent fractions	TPC (mg GAE/g)	TFC (mg QE/g)	Antioxidant activity IC_{50} (µg/mL)	Antidiabetic activity % enzyme inhibition at 500 µg/mL
BRC	25.75 ± 0.27^{a}	7.94 ± 0.18^{a}	Nd	5.35 ± 0.19
BRH	$34.53 \pm 0.18^{b, c}$	150.48 ± 1.10	15.5 ± 1.02	35.36 ± 0.29^{a}
BRD	42.04 ± 1.14^{d}	19.34 ± 0.93	Nd	14.07 ± 0.90
BRE	84.35 ± 0.94^{e}	24.27 ± 0.70	3.92 ± 0.06^{a}	$29.29 \pm 0.29^{b, c}$
BRA	33.04 ± 1.58^{b}	63.46 ± 0.94	Nd	17.93 ± 0.57^{d}
BLC	83.39 ± 0.35^{e}	9.16 ± 0.44^{a}	Nd	10.31 ± 0.38
BLH	26.51 ± 1.19^{a}	2.49 ± 0.13^{b}	Nd	24.66 ± 0.72^{e}
BLD	71.51 ± 0.66	39.39 ± 0.84	6.07 ± 0.16^{a}	21.01 ± 0.58^{d}
BLE	$39.73 \pm 0.28^{c, d}$	6.31 ± 0.22^{a}	30.41 ± 0.85	$26.64 \pm 1.15^{b, e}$
BLA	15.87 ± 2.28	2.40 ± 0.06^{b}	Nd	$32.16 \pm 0.88^{a, c}$
#Quercetin	Nd	Nd	3.9 ± 0.07^{a}	Nd

BRC: crude extract of root, BRH: hexane fraction of root, BRD: dichloromethane fraction of root, BRE: ethyl acetate fraction of root, BRA: aqueous fraction of root, BLC: crude extract of leaf, BLH: hexane fraction of leaf, BLD: dichloromethane fraction of leaf, BLE: ethyl acetate fraction of leaf, BLA: aqueous fraction of leaf. # positive standard, Nd: values not determined. Values are the mean ± SE (n=3). Values followed by the different letters in the same columns are not significantly different at $p < 0.05$.

3.2 Total phenolic and flavonoid content

B. vulgaris contained a significant amount of phenolics and flavonoids, and the observed results are presented in Table 4. The descending order of phenolic content was 84.35 ± 0.94 (BRE) > 83.39 ± 0.35 (BLC) > 71.51 ± 0.66 (BLD) > 42.04 ± 1.14 (BRD) > 39.73 ± 0.28 (BLE) > 34.53 ± 0.18 (BRH) > 33.04 ± 1.58 (BRA) > 26.51 ± 1.19 (BLH) > 25.75 ± 0.27 (BRC) > 15.87 ± 2.28 mg GAE/g (BLA). Similarly, the descending order of TFC was found as 150.48 ± 1.10 (BRH) > 63.46 ± 0.94 (BRA) > 39.39 ± 0.84 (BLD) > 24.27 ± 0.70 (BRE) > 19.34 ± 0.93 (BRD) > 9.16 ± 0.44 (BLC) > 7.94 ± 0.18 (BRC) > 6.31 ± 0.22 (BLE) > 2.49 ± 0.13 (BLH) > 2.40 ± 0.06 mg QE/g (BLA).



(a) (b)

Figure 2: a) Correlation between total phenolic content and IC_{50} in DPPH assay. b) Correlation between total phenolic content and percentage of α-glucosidase inhibition.

3.3 Antioxidant potential

IC_{50} value of ethyl acetate and dichloromethane fractions of leaves and ethyl acetate and hexane fractions of roots were determined as they displayed the highest radical scavenging activity during screening. These fractions displayed remarkable antioxidant activity. The highest activity was observed in BRE with half maximal inhibitory concentration of 3.92 ± 0.06 µg/mL followed by BLD (6.07 ± 0.16 µg/mL), BRH (15.5 ± 1.02 µg/mL), and BLE (30.41 ± 0.85 µg/mL) (Table 4). The IC_{50} of quercetin was 3.9 ± 0.07 µg/mL.

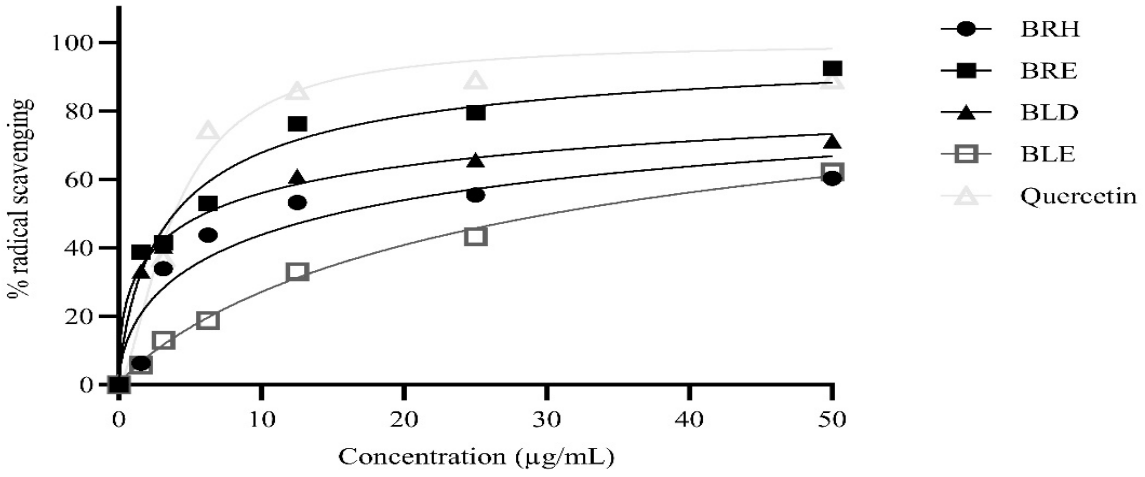


Figure 3: Percentage radical scavenging against concentrations (µg/mL) of plant extracts, solvent fractions, and quercetin.

3.4 Antidiabetic potential

The percentage of α-glucosidase enzyme inhibition for plant extracts and fractions at concentrations of 500 µg/mL is given in Table 4. The descending order of percentage enzyme inhibition is 35.36 ± 0.29 (BRH) > 32.16 ± 0.88 (BLA) > 29.29 ± 0.29 (BRE) > 26.64 ± 1.15 (BLE) > 24.66 ± 0.72 (BLH) > 21.01 ± 0.58 (BLD) > 17.93 ± 0.57 (BRA) > 14.07 ± 0.90 (BRD) > 10.31 ± 0.38 (BLC) > 5.35 ± 0.19 (BRC).

3.5 Antimicrobial activity

Plant extracts and fractions did not display significant ZOI against bacterium and fungal strains used in the study. Photographs of Petri plates are given in Figure 4. Ampicillin used as a standard was found to be most potent against *K. pneumoniae* with ZOI 34 mm, followed by *B. subtilis* (32 mm), *S. aureus* (23 mm), and *E. coli* (23 mm). Cycloheximide used as standard in antifungal assay displayed a ZOI of 15 mm against *F. solani* (Table 5).

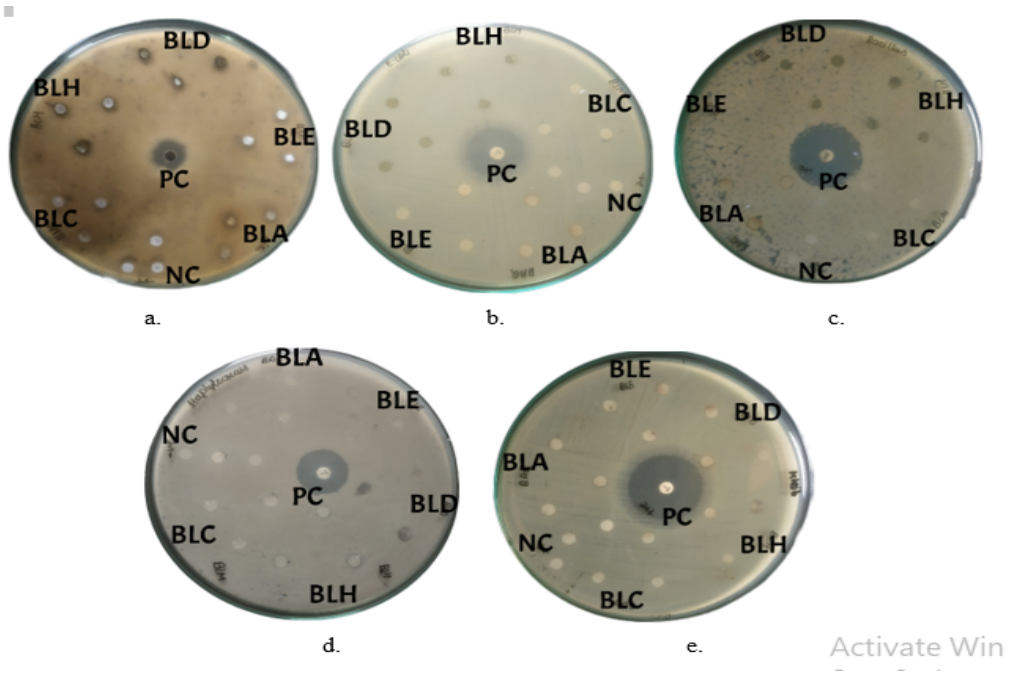


Figure 4: Antimicrobial activities of plant extract and fractions against bacterial and fungal strains (a. *Fusarium solani,* b. *Escherichia coli*, c. *Bacillus subtilis* c. *Staphylococcus aureus*, e. *Klebsiella pneumoniae.*)

Table 5: Antimicrobial activity (ZOI) shown by the extract and solvent fractions.

Plant extracts	***K. pneumoniae***	***S. aureus***	***B. subtilis***	***E. coli***	***F. solani***
#Ampicillin	34	23	32	23	Nd
#Cycloheximide	Nd	Nd	Nd	Nd	15

 # positive standard, Nd: values not determined.

3.6 Toxicity against Brine shrimp nauplii

The toxicities of plant extracts and fractions were measured in terms of their ability to kill brine shrimp nauplii and the results are given in Table 6. The plant extracts and fractions did not display significant toxicities in the assay. All the LC_{50} values were well over 1000 µg/mL. The ascending order of LC_{50} is 1166.36 ± 100.21 (BLH) < 1195.83 ± 115.34 (BLE) < 1573.90 ± 145.26 (BLC) < 1807.78 ± 270.77 (BRC) < 1906.72 ± 285.36 (BRE) < 2337.71 ± 570.09 (BRD) < 2711.11 ± 170.05 (BLD) < 4079.84 ± 809.06 (BRA) < 5287.16 ± 393.51 (BRH) < 5692.20 ± 381.99 g/mL (BLA).

Table 6: Median lethal concentration (LC_{50}) for plant extract and solvent fractions in brine shrimp lethality assay.

Plant extracts	LC50 (μg/mL)
BRC	1807.78 ± 270.77^{a}
BRH	5287.16 ± 393.51^{b}
BRD	$2337.71 \pm 570.09^{a,c}$
BRE	1906.72 ± 285.36^{a}
BRA	$4079.84 \pm 809.06^{b,c}$
BLC	1573.90 ± 145.26^{a}
BLH	1166.36 ± 100.21^{a}
BLD	$2711.11 \pm 170.05^{a,c}$
BLE	1195.83 ± 115.34^{a}
BLA	5692.20 ± 381.99^{b}

Values are the mean ± SE (n=3). Values followed by the different letters are not significantly different at $p < 0.05$.

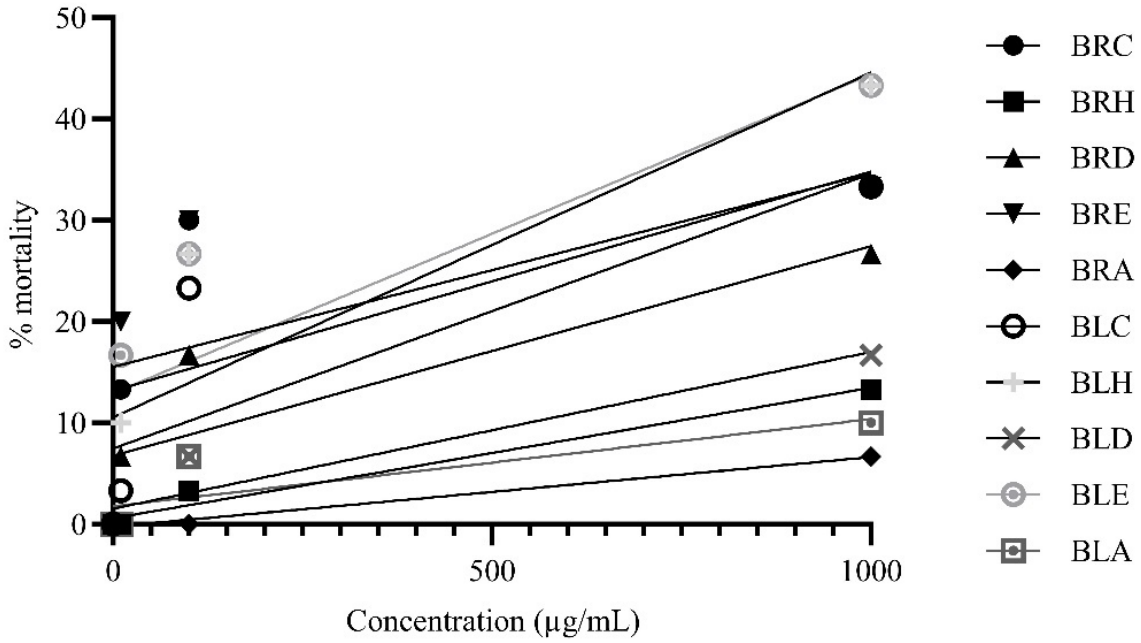


Figure 5: Percentage mortality versus concentrations (µg/mL) of plant extracts and solvent fractions.

4 Discusion

The present study detected the presence of phenolics, flavonoids, glycosides, tannins, alkaloids, and carbohydrates in the methanol extract of the root. All these secondary metabolites are also reported in the literature [40, 41]. However, Ahmad et al., (2013) have reported the absence of proteins and saponins in root extract [40]. Extracts and fractions of *B. vulgaris* displayed a significant amount of phenolics and flavonoids in the present study. The amount of TPC and TFC in different plant extracts were significantly different from each other at $p < 0.05$. The crude extract of the root displayed higher TPC than the crude extract of the leaf, but TFC was found to be higher in the crude extract of the leaf than in the root. The slightly higher concentration of flavonoids in leaf extract is due to the accumulation of flavonoids in leaves [42]. The highest amount of phenolics was recorded for the ethyl acetate fraction of the root whereas the hexane fraction of the root contained the most flavonoid content in the present study. Observed TPC of 25.75 ± 0.27 mg GAE/g for BRC was found intermediatory between previously reported values of 11.23 ± 0.13 mg GAE/g by Ahmad et al., (2013) and 39.75 ± 2.32 mg GAE/g by Edziri et al., (2019) [40, 43]. Similarly, the TFC of 7.94 ± 0.18 mg QE/g was found comparable to 6.41 mg QE/g as reported by Odoh et al., (2012) and lower than 20.73 ± 1.25 mg

QE/g as reported by Edziri et al., (2019) [43, 44]. The number and concentrations of phytochemicals in fruits and vegetables depend upon factors such as cultivar variation, agronomic practices, environmental stress, food processing, and storage methods [45]. Appropriate measures should be taken to preserve the pharmacological activities of fruits and vegetables during the processing, storing, and cooking stages.

Phenolic compounds exert health-promoting effects and therapeutic activities such as antioxidant, antidiabetic, antimicrobial, anticancer, and analgesic functions [46]. These compounds are synthesized from shikimate pathways. The structural feature of phenolics consists of one or more hydroxyl groups with carbon skeletons [47]. They mainly exert their pharmaceutical effect due to their antioxidant activity. The current investigation found a strong negative correlation ($r = -0.804$, $p < 0.003$) between total phenolic content and the IC_{50} value in the DPPH assay. The correlation is graphically presented as a linear regression plot in Figure 2(a). Such correlations are also reported in the literature [48]. Flavonoids are a type of phenolic compound with significant pharmacological importance. They are effective against oxidative stress, bacteria, viruses, malaria, and HIV, and are responsible for natural pigmentation and flavor in many fruits and beverages [49]. The presence of various plant secondary metabolites and the high concentrations of phenolics and flavonoids indicate the high potential of *B. vulgaris* in pharmacological research.

The ethyl acetate, hexane and dichloromethane fractions of roots and leaves of *B. vulgaris* displayed high antioxidant activities. The percentage of free radical scavenging increased with the increase in the concentrations of plant fractions (Figure 3). Antioxidant activities in the ethyl acetate fraction of the root and dichloromethane fraction of the leaf were similar to that of standard quercetin at $p < 0.05$ indicating the possibility of the presence of an active compound. The high antioxidant activity in *B. vulgaris* also makes it an ideal natural food preservative. The antioxidants in fruits and vegetables are mainly due to polyphenols, carotenoids, or vitamins [50]. Further work involving GC-MS and LC-MS analysis could identify specific active principals responsible for bioactivity. Previously, Edziri et al., (2019) have reported IC_{50} values in ABTS and DPPH assay of methanol extract of root as 359.65 and 254.76 µg/mL respectively [43]. The DPPH IC_{50} value of 3.92 ± 0.06 µg/mL for BRE was comparable to the IC_{50} values of 2.32 ± 0.72 µg/mL and 2.20 ± 0.72 µg/mL for root and leaf extracts reported in the literature [12].

α-Glucosidase inhibitors competitively bind with α-glucosidase enzyme and reduce the rate of disaccharide hydrolysis and subsequent glucose absorption in the blood [51]. Thus,α-glucosidase enzyme inhibition assay was used in the present study to measure antidiabetic activity. The plant extracts and fractions displayed weak inhibition activities in the present study. However, a previous study by Mzoughi et al. (2019) has reported the IC_{50} values for ethanolic leaf extract as 0.13 mg/mL and 1.03 mg/mL in α-glucosidase inhibition assay and -amylase inhibition assay respectively [13]. Variations in IC_{50} values may have arisen due to the differences in environmental stress, cultivar variation, or extraction solvent. Mechanism of antidiabetic activity in plants includes blocking of liver gluconeogenesis, stimulation of insulin secretion and sensitivity, inhibition of glucagon secretion, prevention of glucose absorption in the intestine, and reabsorption of glucose in the kidneys [52]. A previous study has found an increase in the number of β-cells of the islet of Langerhans in streptozotocin-induced diabetic rats on oral administration of 2 g/kg *B. vulgaris* extract for 28 days [53]. This indicates antidiabetic activity in the plant may involve stimulation of insulin secretion and inhibition of digestive enzymes. The α-glucosidase inhibition assay displayed a mild positive correlation ($r = 0.413$, $p < 0.024$) with total flavonoid content. The graphical representation of such correlation is given in Figure 2(b).

The leaf extracts and fractions lacked significant zones of inhibition against bacterium and fungal species at a concentration of 25 mg/mL. The findings in this study agreed with the observations made by Rauha et al., (2000) in which they reported the absence of activity against *C. albicans, A. niger, E. coli,* and *S. aureus* for 500 µg of 80% methanol extract of *B. vulgaris* tuber [54]. Another study reported ZOI of 2 mm against *S. aureus* for leaf aqueous extract while methanol extract was found to be inactive [55]. Ethanolic extract of the roots displayed ZOIs of 12.54 ± 0.35, 8.37 ± 0.21, and 0 mm against *S. aureus*, *B. cereus*, and *A. niger* respectively in the study conducted by El-Beltagi et al., (2018) [14]. Thus, there are differences in the antibacterial activity reported in various studies. These differences may be due to variations in the number and concentration of secondary metabolites caused by genetic and environmental factors, the solvent used for extractions, concentrations used, and bacterial susceptibility.

The cytotoxic activity in plant extracts and fractions was measured using a brine shrimp lethality assay. The assay is a reliable method for estimating preliminary cytotoxic activity. Lagarto Parra et al., (2005) have reported a significant correlation between the brine shrimp assay and oral lethality in mice [56]. In the present study, all LC_{50} values were higher than 1000 µg/mL. So, the plant is

relatively safe for human consumption, a fact evident from its worldwide cultivation and inclusion in kitchens. Observed percentage mortality increased with the increase in concentration of plant extracts as shown by Figure 5.

5 Conclusion

The medicinal food *B. vulgaris* contains important phytochemicals such as alkaloids, phenols, flavonoids, glycosides, tannins, terpenoids, and saponins. The extracts and fractions of the plant possess high concentrations of phenolics and flavonoids and display significant antioxidant activities. Thus, the consumption of the plant could reduce the risk of chronic diseases associated with oxidative stress. The plant displayed weak α-glucosidase inhibition activity that was significantly correlated with total flavonoid content. The plant is found to be inactive against *F. solani, K. pneumoniae, E. coli, B. subtilis,* and *S. aureus*. It is not toxic to the brine shrimp nauplii. Thus, the inclusion of *B. vulgaris* in the diet will have potential pharmacological benefits due to the presence of important phytochemicals. Further isolation, purification, and characterization of active metabolites coupled with *in-vivo* studies may lead to increased use of the plant as a medicinal food. Besides the medicinal properties, the plant could be used as a potential source of secondary metabolites that impart high food value from the aspects of nutraceutical properties.

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