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Phytochemical analysis, antioxidant and antibacterial activities of methanolic rhizome extract of *Bergenia pacumbis*: A valuable medicinal herb

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ABSTRACT

Bergenia pacumbis, a perennial herb of the Saxifragaceae family, usually referred to as 'Pashanbheda' is geographically distributed within the temperate Himalaya including Nepal. The purpose of this study was to evaluate the phytochemical analysis as well as *in vitro* antioxidant and antibacterial activities of methanolic rhizome extract of *B. pacumbis*. The rhizome of *B. pacumbis* was extracted in methanol using the Soxhlet apparatus and then the extract was concentrated. The rhizome extract was tested for phytochemicals using the standard protocol. The *in vitro* antioxidant activity of the extract was evaluated using the 2, 2- diphenyl-1-picrylhydrazyl free radical scavenging assay. The 50 % inhibitory concentration (IC₅₀) values of ascorbic acid and *B. pacumbis* were found to be 11.91 ± 0.03 $\mu\text{g/mL}$ and 11.99 ± 0.05 $\mu\text{g/mL}$ respectively that indicate *B. pacumbis* is a good source of antioxidant agents. The extract was further tested for antibacterial activity through the agar well diffusion method and found to be effective against *Staphylococcus aureus* (16 mm) and *Klebsiella pneumoniae* (12 mm). The present study validates the traditional use of *B. pacumbis*.

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1. Introduction

Plant metabolites such as proteins, lipids, fatty acids, carbohydrates, and so on play a direct role in plant growth, development, and reproduction whereas secondary plant metabolites are used for defense against predators, parasites, and diseases, etc. [1]. Plants contain a wide range of phytochemicals, such as polyphenols, alkaloids, flavonoids, tannins, carotenoids, terpenoids, quinine, and berberine, which are responsible for curing a variety of diseases and have properties including *in vitro* antioxidants, antimicrobial, antiviral, anti-mutagenic, anti-inflammatory, and other properties to a greater or lesser extent [2–4]. Because of their adequacy, accessibility, compatibility, and feasibility, medicinal plants are an important part of primary health care [5]. In developing countries like Nepal, medicinal plants play a vital supplementary role in the limited modern health care availability. World Health Organization (WHO) estimates that approximately 80 % of people in developing countries rely on medicinal plants for their basic healthcare needs [6, 7]. In industrialized countries, nearly 25 % of prescribed organic drugs and approximately 95 % of modern drugs have been derived directly or indirectly from plant sources [8, 9]. In the last few decades, the unprecedented rise in antibacterial resistant pathogenic microorganisms has increased the demand for novel/ alternative antibacterial agents [10–13].

Antioxidants are a class of chemical compounds thought to protect enzymes and genetic material from structural and functional damage caused by an excess of free radicals [14]. The production of free radicals in the body that exceeds the body's antioxidant capacity and the resulting damage is called oxidative stress [15]. Oxidative stress is responsible for the pathogenesis of cardiovascular and neurodegenerative diseases, such as Alzheimer's, aging, cancer, diabetes, and others [16]. Destroying free radicals may help with good health [17]. Phytochemicals

such as polyphenols, flavonoids, ascorbic acid, tocopherols, carotenoids, etc. can absorb and neutralize free radicals and have significant antioxidant capacities [18, 19]. Over the years, the demand for safe dietary natural antioxidants in pharmaceuticals, nutraceuticals, cosmetics, and the food industry has grown significantly [20].

The *Bergenia* species, commonly known as 'Pashanbheda' (Paashan = rockstone & bheda = piercing) [21], are perennial herbs native to central Asia that grow widely in the temperate Himalaya and are the most promising medicinal plants of the Saxifragaceae family [22]. The different species of *Bergenia* flourish well in rocky areas and on the cliffs are used for the dissolution of kidney and gall bladder stones. The root and rhizome of the *Bergenia* species are used to cure fractured bones, fresh cuts, boils, wounds, fever, cough, vomiting, pulmonary infections, diarrhea, and ophthalmia by local peoples since ancient times in Nepal, India, Bhutan, and China. In addition, *Bergenia* species have been reported with pharmacological activities such as analgesic, astringent, antiviral, antioxidant, antipyretic, antiulcer, antidiabetic, hepatoprotective, celebro-protective, gastroprotective, adaptogenic, hemorrhoidal, antilithiatic, anticancer, antihypertensive, antitussive, anti-inflammatory, antimalarial, antimicrobial, anti-obesity, insecticidal, diuretic, and skin whitening properties [23–25]. The virtues of *Bergenia* species are largely attributed to their secondary metabolites such as arbutin, bergenin, catechin, gallic acid, and tannins, which are therapeutic and lead to variation in medical activities [26–28].

Bergenia pacumbis (Buch.-Ham. Ex D. Don) C. Y. Wu & J. T. Pan is traditionally used in the treatment of various human diseases in different parts of Nepal. Its leaves are used for the remedy of wounds, stomach ulcers, intestinal complaints, and heavy sneezing, while roots and rhizomes are used for acidity, dysentery, cough, fever, diarrhea, headache,

kidney stone, internal injuries, and intestinal complaints [21, 29]. To treat diseases, a cup of boiled leaves in water is taken twice a day, while roots or rhizomes are crushed and boiled in water, then taken twice a day [29]. As reported by Pandey et al. (2020), methanolic extract of *B. pacumbis* contains flavonoids and phenolic compounds such as afzelechin, arbutin, astilbin, bergenin, catechin, diosmetin, gallic acid, hyperoside, kaempferol-7-O-glucoside, methyl gallate, morin, paashaanolactone, phloretin, protocatechuic acid, quercetin, and syringic acid. The scientific data related to the biological and chemical properties of *B. pacumbis* is scanty, hence, this study reports phytochemical analysis and antioxidant and antibacterial activities of methanolic extract of *B. pacumbis*.

2. Methodology

2.1 Chemicals and equipment

The analytical grade chemicals/reagents used in the study were purchased from Fisher Scientific in Germany. Instruments such as Soxhlet apparatus (Borosilicate Genuine, JSW), rotary evaporator (IKA RV 10 digital V with IKA HB 10 digital), and UV-visible spectrophotometer (Shimadzu UV-Vis spectrophotometer-1800) were also used in this research.

2.2 Collection of plant materials

The plant materials (plant & rhizomes) (Figure 1) were collected in August 2018 from the Salyan district (Tharkot hill, altitude: 2,400 m above sea level), Karnali province, Nepal. The herbarium of the plant was recognized and verified as *Bergenia pacumbis* (Buch.-Ham. Ex D. Don) C. Y. Wu & J. T. Pan by Taxonomists in National Herbarium and Plant Laboratories, Lalitpur, Nepal. The rhizomes were washed with tap water, followed by sterilized distilled water, to remove adhered dust, dirt, and other foreign materials, and then dried in shade at room temperature. The dried rhizomes were powdered and then stored in an airtight polyethylene bag in a cool, dry location for ongoing studies.



(a)

(b)

Fig. 1. (a) Aerial part and (b) Rhizomes of *Bergenia pacumbis*

2.3 Extraction of plant materials

The plant powder (60 g) was placed in the Soxhlet apparatus and refluxed for 8 h with 360 mL methanol over a heating mantle at 63 °C. The solvent from plant extract was removed by evaporating with the help of a rotary evaporator under reduced pressure at 40 °C. The resulting extract (semisolid) was poured into a Petri dish and left at room temperature to evaporate the excess solvent. The dried extract was weighed, and the percentage yield was calculated using equation (1). The dry sample was refrigerated at 4 °C until required for use.

Percentage yield

$$= \frac{\text{Mass of crude extract}}{\text{The total mass of sample used}} \times 100 \% \quad (1)$$

2.4 Preliminary phytochemical screening

The crude methanolic extract was subjected to qualitative tests for glycosides, flavonoids, alkaloids, phenolic compounds, terpenoids, steroids, carbohydrates, saponins, tannins, fixed oils and fats, quinones, reducing compounds, proteins, and coumarins according to the methods discussed in the literature. For the phytochemical tests, the stock solution was made by dissolving one gram of crude extract in 40 mL methanol. The related tests were mentioned below: **(1) Test for glycosides (Keller-Killiani test):** With distilled water (5 mL), the crude extract (0.5 g) was shaken. To this, glacial acetic acid (2 mL) with one drop of ferric chloride solution was added, followed by sulphuric acid (1 mL) along the side of the test tube. A brown ring at the interface indicated the presence of cardiac glycosides (a violet ring may appear below the brown ring, while a greenish ring may form in the acetic acid layer) [30, 31]; **(2) Test for flavonoids: a) Shinoda test:** To 2-3 mL of crude extract, a piece of magnesium ribbon and 1 mL of concentrated hydrochloric acid were added. The presence of flavonoids was indicated by the formation of

red or pink-red coloration of the solution [2, 32], and **b) Lead acetate test:** 1 mL of crude extract was mixed with 1 mL of 10% lead acetate solution. The presence of flavonoids was indicated by the formation of a yellow precipitate [33]; **(3) Test for alkaloids: a) Mayer's test:** The crude extract (0.5 g) was mixed with 5 mL of 1 % dilute hydrochloric acid and then filtered. A few drops of Mayer's reagent (Potassium mercuric iodide solution) were added to the filtrate. The presence of alkaloids was determined by the formation of a white creamy precipitate [30, 34], and **b) Dragendorff's test:** The crude extract (0.5 g) was mixed with 5 mL of 1 % dilute HCl before being filtered. The filtrate was treated with a few drops of Dragendorff's reagent (Potassium bismuth iodide solution). The presence of alkaloids was indicated by the formation of an orange-red precipitate [2, 30]; **(4) Test for phenolic compounds (ferric chloride test):** In 5 mL of distilled water, the crude extract (0.2 g) was dissolved. A few drops of neutral 5 % ferric chloride solution were added to this. The presence of phenolic compounds was indicated by the formation of green, blue, or violet colored solution [32–34]; **(5) Test for terpenoids (Salkowski test):** Five mL of extract solution was mixed with 2 mL of chloroform, and 3 mL of concentrated sulphuric acid was added along the side of the tube. The presence of terpenoids was indicated by the reddish-brown color at the interface [30, 31]; **(6) Test for steroids (Salkowski test):** With 2 mL of chloroform, the crude extract (0.2 g) was mixed and then 2 mL of concentrated sulphuric acid was added along the side of the test tube. The presence of steroids was indicated by the development of a reddish-brown color by the chloroform layer and green color by the acid layer [12]; **(7) Test for carbohydrates: a) Fehling's test:** Fehling A and Fehling B reagents were mixed with equal parts, and 2 mL of it was added to the crude extract solution and gently boiled in a water bath. The appearance of a brick-red

precipitate at the bottom of the test tube indicated the presence of carbohydrates (reducing sugars) [31, 32], and **b) Molisch's test:** 2 drops of Molisch's reagent were added to 2 mL of the crude extract and thoroughly shaken. Along the side of the test tube, 2 mL of concentrated sulphuric acid was added. The presence of carbohydrates was determined by the formation of a reddish-violet ring at the junction of the two layers [32, 33]; **(8) Test for saponins (foam test):** In a graduated cylinder, the extract (0.5 g) was shaken for 15 minutes with distilled water (20 mL). The presence of saponins was indicated by the formation of stable foam [12, 30]; **(9) Test for tannins (ferric chloride test):** The extract (0.5 g) was mixed with distilled water (10 mL) before being filtered. A few drops of 5% ferric chloride solution were then added. The formation of a black or blue-green coloration or precipitate is the indication of the presence of tannins [12, 30]; **(10) Test for fixed oils and fats (spot test):** In between two filter papers, a drop of the concentrated extract was pressed and then kept undisturbed. An oil stain on the paper indicated the presence of oils and fats [35, 36]; **(11) Test for quinones (alcoholic potassium hydroxide test):** One mL of crude extract was treated with a few mL of alcoholic potassium hydroxide solution. The presence of quinones was indicated by the color change from red to blue [33, 36]; **(12) Test for reducing compounds (Fehling's test):** The water (1 mL) was added to the extract (1 mL). To this solution, Fehling's reagent (5-8 drops) was added and then the mixture was warmed over a water bath. The presence of reducing compounds was indicated by brick-red precipitation [31, 32]; **(13) Test for proteins (Xanthoproteic test):** A few drops of concentrated nitric acid were added to the extract (1 mL). The presence of proteins was indicated by the formation of a yellow-colored solution [37]; and **(14) Test for coumarins (sodium hydroxide test):** To about 1 mL of extract, 1.5 mL of 10 % NaOH was

added. The formation of yellow color is the indication of the presence of coumarins [37].

2.5 Antioxidant activity

The antioxidant activity of the plant extract and ascorbic acid (standard) was assessed *in vitro* based on free radical scavenging ability using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, which was slightly modified from the method described previously [38]. Various concentrations (5, 12.5, 25, 50, 100 & 200 µg/mL) of plant extract and ascorbic acid were prepared in methanol on the clean and dry test tubes. The sample (plant extract or ascorbic acid) was taken 2 mL in each test tube. To this sample, 2 mL of the 0.2 mM methanolic DPPH solutions was added [6]. The tubes were shaken vigorously for uniform mixing. These tubes were incubated for half an hour in darkness at room temperature. The control was made in the same way but without the plant extract or ascorbic acid, i.e. DPPH solution in methanol. Methanol was taken to collect the baseline on the spectrophotometer i.e. methanol as a blank [31]. The absorbance was measured at the wavelength (λ) = 517 nm using a UV-visible spectrophotometer (UV-1800, Shimadzu). The percentage of DPPH free radical scavenging activity (% inhibition) was calculated by using the formula given as equation (2).

$$\% \text{ Inhibition} =$$

$$\frac{A_c - A_t}{A_c} \times 100 \%$$

(2)

Where A_c denotes control absorbance and A_t denotes test sample (extract/standard) absorbance. Then, the concentration corresponding to 50 % inhibition (IC_{50} value) was calculated by using a four-parameter logistic regression model in GraphPad Prism. The lower IC_{50} value is an indication of a more potent antioxidant activity [14, 39].

2.6 Antibacterial activity

B. pacumbis extract was tested for the *in vitro* antibacterial activity against Gram-negative

bacteria *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, and *Salmonella typhimurium* ATCC 14028, and a positive bacterium *Staphylococcus aureus* ATCC 25923. The antibacterial activity of plant extract was assessed using the modified Agar Well Diffusion Method [40–44] as reported by Kardong et al. (2013) and Dwivedi et al. (2020). All glassware, media, and reagents were sterilized by autoclaving at 121 °C. The standard cultures of bacteria under study were collected. After obtaining the culture, the test organisms were streaked on nutrient agar plates [10] and incubated. From the isolated colony, Gram staining was performed. The organism thus obtained were tested for their purity. The standard culture inoculum was prepared from primary culture plates. The isolated colony was sub-cultured on nutrient agar plates with the inoculating loop aseptically. It was then transferred to a tube containing sterile nutrient broth and incubated for 24 h at 37 °C.

The bacterial suspension (inoculum) was swabbed uniformly onto the agar surface of Mueller-Hinton Agar (MHA) plates. The four wells of 6 mm diameter were made in the incubated media (MHA) plates with the assistance of a sterile cork-borer at four equidistant points at about 1.5 cm away from the dish-wall. Then 40 µL of the working solution (50 mg/mL) of plant extract was loaded into the respective well of each Petri dish. At the same time, the solvent (DMSO) was tested for activity as a negative control in a separate well. The cultured agar plates were loaded with 40 µL neomycin (1 mg/mL) as a positive control. One of the wells was also loaded with 20 µL neomycin plus 20 µL extract solution to study synergistic or antagonistic effect. The zone of inhibition (ZOI) was measured in a millimeter unit after a 24 h of incubation period at 37 °C to determine the antibacterial efficacy.

2.7 Statistical analysis

All experiments were carried out in triplicate, and data were presented as the average of three replicates. For the statistical analysis, a one-way analysis of variance (ANOVA) ($p < 0.05$) was used. MS Excel 2013 and GraphPad Prism 9.2.0 (332) were used to conduct the statistical analysis and create the graph.

3. Results and Discussion

3.1 Percentage yield of extraction

The percentage yield of rhizome extract of *B. pacumbis* in methanol was calculated and it was found to be 28.17 %. The percentage yield of plant extraction is ascertained by the ability of solvent that depends upon the phytochemical composition, extraction procedure and conditions, and polarity of the extracting solvent [3, 33, 45].

3.2 Phytochemical screening

As shown in **Table 1**, the phytochemical screening of a crude methanolic extract of *B. pacumbis* rhizome revealed the presence of various phytochemicals such as cardiac glycosides, flavonoids, alkaloids, terpenoids, steroids, phenolic compounds, carbohydrates, saponins, fixed oils and fats, quinones, reducing compounds, and proteins, whereas tannins and coumarins were absent. However, the phytochemical composition may differ in different environmental conditions or stress conditions, extraction method, and type of solvent used [12, 39, 45].

It is revealed that the phytochemicals are known to have medicinal properties [46], antimicrobial [11, 47], and antioxidant activity [14, 17, 48]. For example, alkaloids show biological properties such as antimicrobial, antimalarial, anti-inflammatory, antispasmodic, cytotoxicity, and pharmacological effects. Similarly, steroids have antibacterial and insecticidal properties, along with cardiogenic effects. According to research, cardiac glycosides are being used to treat cardiac arrhythmia and congestive heart failure [30]. Other phytochemicals called terpenoids show

medicinal properties such as antimicrobial, anticarcinogenic, antimalarial, hepaticidal, anti-ulcer, and anticancer activities [33]. According to several reports, flavonoid groups exhibited biological activities such as antioxidant, anti-viral, antimicrobial, anti-inflammatory, anti-cancer, anti-angiogenic, and anti-allergic reactions [8, 38]. Flavonoids are essential for plant growth and development as well as a plant defense against pests and microorganisms [49]. Some flavonoids such as quercetin [22] and rutin [49] are known for their antioxidant, antiviral, anti-inflammatory, and antihistaminic activities. Saponins and glycosides are important antidiarrheal agents [33]. Saponins are antimicrobial, anti-inflammatory, anti-malarial, anti-diabetic, anti-allergic, and insecticidal [9]. Saponins are also involved in plant defense systems due to their antimicrobial activity [38]. The antibacterial properties of steroids are known [40]. Several studies have also shown that phenolic compounds have good antioxidant activity and a wide range of pharmacological activities such as anti-apoptosis, anti-carcinogenicity, anti-aging, anti-inflammation, anti-atherosclerosis, inhibition of angiogenesis, cardiovascular protection, and endothelial function improvement [10, 39]. Scientific reports support the fact that flavonoids or polyphenols lower the risk of cardiovascular disease, cancer, and diabetes [49]. The medicinal and nutritional values of food are mainly based on total phenolic and flavonoid content [9].

The presence of biologically important phytochemicals in the rhizome extract of *B. pacumbis*, as tested in this study, may contribute to its medicinal value and a potential source for useful drugs. Pandey et al. (2020) used high-resolution mass spectrometry (HRMS) to detect flavonoids and phenolic compounds in a crude methanol extract of *B. pacumbis*. But, according to the present study, phytochemical screening indicates the presence of other phytochemicals like cardiac glycosides, alkaloids, terpenoids, steroids,

carbohydrates, saponins, fixed oils and fats, quinones, reducing compounds and proteins along with flavonoids and phenolic compounds. Hence, to find the more chemical compounds present in *B. pacumbis*, gas chromatography-mass spectrometry (GC-MS), ¹H NMR (nuclear magnetic resonance), and ¹³C NMR spectroscopy methods are required. According to several reports, different species of *Bergenia* possess many phytochemicals, for example, 58 chemical compounds were found to be present in *B. ciliata* [24], 103 compounds were found in *B. crassifolia* [23], and 31 compounds were isolated from *B. ligulata* [21]. Therefore, the phytochemicals present in *B. pacumbis* are responsible for the use of the plant as ethnomedicine in different parts of Nepal.

Table 1. Phytochemical screening of a crude extract of *Bergenia pacumbis* rhizome

S. No.	Phytochemicals screened	Standard test	Methanol extract of <i>B. pacumbis</i>
1	Glycosides	Keller-Killiani test	+
2	Flavonoids	Shinoda test	+
		Lead acetate test	+
3	Alkaloids	Mayer's test	+
		Dragendorff's test	+
4	Phenolic compounds	Ferric chloride test	+
5	Terpenoids	Salkowski test	+

6	Steroids	Salkowski test	+
7	Carbohydrates	Fehling's test	+
		Molisch's test	+
8	Saponins	Foam test	+
9	Tannins	Ferric chloride test	-
10	Fixed oils and fats	Spot test	+
11	Quinones	Alcoholic potassium hydroxide test	+
12	Reducing compounds	Fehling's test	+
13	Proteins	Xanthoproteic test	+
14	Coumarins	Sodium hydroxide test	-

Note: '+' denotes the presence and '-' denotes the absence of phytochemicals.

3.3 Antioxidant activity

The DPPH free radical scavenging method was used to investigate the *in vitro* antioxidant activity of the methanolic rhizome extract of *B. pacumbis* because this experiment is reliable, quick, and requires a small amount of sample [20]. DPPH is a very stable nitrogen-centered organic free radical with a purple color and a strong absorption at 517 nm that has been successfully used to evaluate free radical scavenging activity [3, 30]. When free radical scavengers are added, DPPH is reduced to DPPH-H, and its color changes to yellow due to the efficacy of antioxidants [18, 50]. The ability of a test sample to scavenge DPPH free radicals determines its free radical scavenging capacity or antioxidant potential, which

demonstrates its effectiveness, prevention, interception, and repair mechanism against injury in a biological system [49, 51]. The percentage of DPPH radical scavenging ability (% inhibition) of plant extract and ascorbic acid were plotted against respective various concentrations (5 – 200 $\mu\text{g/mL}$) as displayed in Figure 2. The percentage inhibition was found to increase with increasing extract concentration [49]. The percentage inhibition was found to be 87.36 % and 94.01 % at 200 $\mu\text{g/mL}$ for rhizome extract and ascorbic acid (standard) respectively. The antioxidant activity of a substance is directly proportional to its DPPH free radical scavenging power [52]. The concentration required for 50% DPPH inhibition (IC_{50}) values of ascorbic acid and *B. pacumbis* were found to be 11.91 ± 0.03 and 11.99 ± 0.05 $\mu\text{g/mL}$ respectively that indicating the IC_{50} value of *B. pacumbis* was close to ascorbic acid. The lower IC_{50} value showed the rhizome extract of *B. pacumbis* is the potential of high antioxidant activity. Pandey et al. (2020) reported that the IC_{50} value of the crude methanol extract of *B. pacumbis* rhizome was 40.87 ± 0.32 $\mu\text{g/mL}$. Moreover, a variety of factors influence the antioxidant activity of plant extract, such as the test method, extraction procedure, and phytoconstituents in the extract [30, 39].

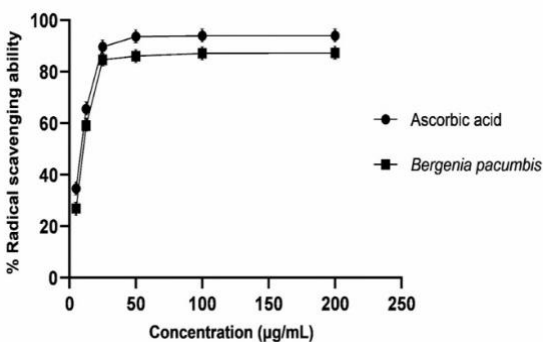


Fig. 2. DPPH free radical scavenging ability of *B. pacumbis* and ascorbic acid at various concentrations (5-200 $\mu\text{g/mL}$).

The antioxidant activity of phytochemicals varies from slight to extremely high depending upon their reactive groups. Due to the lower bond dissociation energy phenolic compounds having the O–H group are more active than compounds having the N–H group [17]. Further, activity also depends on the structure, molecular weight, and substitution pattern of functional groups [50]. According to a previous report by Pandey et al. (2020), the major compounds identified in the extract of *B. pacumbis* were afzelechin, arbutin, astilbin, bergenin, catechin, diosmetin, gallic acid, hyperoside, kaempferol-7-O-glucoside, methyl gallate, morin, paashaanolactone, phloretin, protocatechuic acid, quercetin, and syringic acid. Out of these various flavonoids and phenolic compounds, according to reports, bergenin, catechin, arbutin, morin, afzelechin, and quercetin show significant antioxidant activity [22]. Because of their hydrogen donating ability, these compounds were able to discolor DPPH solution [38, 45]. Moreover, phytochemical screening of *B. pacumbis* performed in this study showed the presence of polyphenols, flavonoids, alkaloids, steroids, terpenoids, saponins, and reducing sugars, which may responsible for good antioxidant activity of the plant.

The antioxidant activity of a plant can be figured out by phenolic and flavonoid contents [48, 53]. Phenolic compounds are the key contributors in plant constituents that bestow the highest scavenging ability on free radicals [4, 19]. On the other hand, flavonoids which are subgroups of phenolic compounds [19] suppress reactive oxygen species (ROS) formation [54], chelate trace elements involved in the free-radical generation, scavenge reactive species, inhibit oxidative enzymes and up-regulate or protect antioxidant defenses [16, 53, 55].

Therefore, *B. pacumbis* could be a good source of natural antioxidant alternative to synthetic

antioxidants. Further investigation regarding *in vivo* antioxidant activity and its mechanism could be considered.

3.4 Antibacterial activity

Table 2 displays the *in vitro* antibacterial activity of methanolic rhizome extract of *B. pacumbis* in terms of zone of inhibition (ZOI).

Table 2. Antibacterial activity of the rhizome extract of *Bergenia pacumbis*

Plant extract ^a	Pathogens ^b	Zone of inhibition (ZOI) in mm			
		Neomycin (1 mg/mL) 40 µL (1) ^c	Extract solution (50 mg/mL) 40 µL (2) ^d	Neomycin + extract solution (20 µL + 20 µL) (3) ^e	DMSO 40 µL (4) ^f
<i>B. pacumbis</i>	<i>S. aureus</i>	24	16	20	0
	<i>E. coli</i>	18	0	16	0
	<i>K. pneumoniae</i>	22	12	21	0
	<i>S. typhimurium</i>	19	0	0	0

Notes: ^a source of plant extract, ^b bacterial species, ^c antibiotic as a positive control, ^d plant extract dissolved in methanol for working solution, ^e antibiotic + solution of plant extract dissolved in methanol, to determine the synergistic or antagonistic effect, ^f DMSO as a negative control

The antibacterial activity was evaluated in comparison to neomycin (positive control) and DMSO (negative control). Figure 3 shows the antibacterial activity of the crude extract against bacteria tested by the agar well diffusion method.

The antibacterial activity in terms of ZOI was found to vary significantly with test microbes. The result revealed that the extract of *B. pacumbis* showed significant ZOI against *S. aureus* (16 mm) and *K. pneumoniae* (12 mm) out of four bacteria viz. *S. aureus*, *E. coli*, *K. pneumoniae*, and *S. typhimurium* and ZOI is statistically comparable with neomycin. The extract did not display any inhibition against *E. coli* and *S. typhimurium*. The extract portrayed the highest ZOI against Gram-positive bacterium – *S. aureus* followed by Gram-negative bacterium – *K. pneumoniae*. The positive control exhibited the greatest inhibition, while the negative control did not exhibit inhibitory action against either of the microbes. The antibiotic plus plant extract had shown the antagonistic effect [56, 57] in all cases because the combined effect was less than the effect of either individual agent [58]. The plant extract shows antibacterial activity in a concentration-dependent manner [3, 38]. The extraction method and the solvent used also affect the antibacterial activity of the plant extract [9, 11].

The antibacterial activity was more pronounced on the Gram-positive bacterium than the Gram-negative bacterium due to the difference in morphological constitutions. The Gram-positive bacterium contains only an outer peptidoglycan layer, which is an ineffective permeability barrier to antibacterial compounds [53]. On the other hand, the Gram-negative bacterium has an outer membrane that contains lipopolysaccharide (LPS) in the outer leaflet and phospholipids in the inner leaflet, as well as an inner peptidoglycan layer, making the cell wall impermeable to antibacterial compounds. The cell wall of Gram-negative bacterium is more complex in a layout that acts

as a diffusion barrier and makes it less susceptible to antibacterial agents than the Gram-positive bacterium [33, 37]. The presence of porins at the outer membrane and the active efflux processes involved in Gram-negative bacterium is responsible for higher resistance [4]. Therefore, the phytochemicals present in the extract are more active against the Gram-positive bacterium as being better permeable into the cell structure. The broad-spectrum antibacterial activity of the rhizome extract of *B. pacumbis*

might be because of the presence of phytochemicals like alkaloids, phenolics, flavonoids, steroids, saponins, and terpenoids and these compounds could be soluble in an organic polar solvent. Alkaloids and flavonoids have been known to have bactericidal effects [59, 60]. Specific phenolic compounds possess potential antibacterial activity [61, 62]. It is reported that the antibacterial activity of most polyphenols is related to interactions between polyphenols and bacterial cell surfaces [63]. Steroids, the important compound for sex hormones, have the strongest antibacterial properties. Saponins have been reported to possess antibacterial activity [40, 43]. Terpenoids are also for inhibition of bacterial growth [64]. The bacterial growth inhibition occurs through several mechanisms: (i) destruction of bacterial cell membrane and function, (ii) interruption of nucleic acid synthesis and function, (iii) inhibition of a metabolic pathway, and (iv) coagulation of cytoplasmic constituents [59, 60, 65]

4. Conclusion

The medicinal plant contains phytochemicals, especially secondary metabolites, that can be used to cure a variety of human ailments. The phytochemical screening tests of methanolic rhizome extract of *B. pacumbis* revealed the presence of a variety of phytochemicals, such as cardiac glycosides, flavonoids, alkaloids, terpenoids, steroids, phenolic compounds, carbohydrates, saponins, fixed oils and fats, quinones, reducing compounds, and proteins.

The study showed that the rhizome extract possesses an astonishing potential on *in vitro* antioxidant activity was investigated by DPPH free radical scavenging method and the good broad-spectrum *in vitro* antibacterial activity using agar well diffusion method. The present study found that *B. pacumbis* extract contains phytochemicals with antioxidant and antibacterial properties, making it a potential natural source alternative to antimicrobials and antioxidants. The results suggest that *B. pacumbis* is therapeutically important to cure various diseases. The study validates the traditional use of *B. pacumbis* and it is the foundation for further research. A more detailed study is required to investigate the in-depth medicinal properties and the mechanism of action by the phytochemicals.

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