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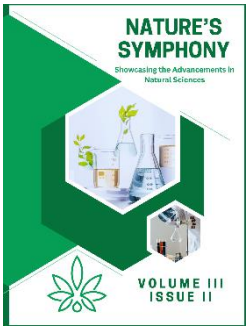

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Research Article

Study of Phytoconstituents, Antioxidant, Antimicrobial, and Antidiabetic Activities of Root of *Berberis Aristata*

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ABSTRACT

Berberis aristata is a traditionally used medicinal herb for managing diabetes, oxidative stress and numerous diseases. This study focuses on the biological activities of its root examining antidiabetic, antibacterial and antioxidant qualities. Soxhlet method was used to collect hexane, methanolic and ethyl acetate extracts from root sample. Extract's antioxidant capacity was evaluated using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), antimicrobial activity was investigated using agar well diffusion method, and antidiabetic activity was analyzed using alpha-amylase inhibition method. Flavonoid compound quantification (TFC) and phenolic compound quantification (TPC) was performed using colorimetric methods.We found that the hexane, ethyl acetate and methanol extracts of the root had various phytoconstituents: glycosides, saponins, alkaloids, quinones, flavonoids, terpenoids, steroids, and tannins. Among three organic solvents, methanolic extract had the highest TPC and TFC values, at 119.06±3.32 mg of gallic acid equivalent per g of extract and 84.11±1.35 mg of quercetin equivalents per g, respectively. The methanolic extract and the ethyl acetate extract's showed IC_{50} value of 51.79±0.73 µg/mL and 54.58±0.96 µg/mL, respectively, comparing to standard quercetin's IC_{50} values of 50.16±0.86 µg/mL, representing significant antioxidant activity of the root extract as compared to other parts of *B. aristata*. In addition, the root extracts also showed antifungal and antibacterial properties. The methanolic root extract from *B. aristata* proved significant against several bacterial strains: *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus epidermidis* (ATCC 1228), and *Bacillus subtilis* (ATCC 6051). The corresponding zones of inhibition (ZOI) were 26.00 mm, 16.10 mm, 28.30 mm, and 28.30 mm, respectively. Efficient Antifungal action was also observed with a ZOI of 31.50 mm against *Candida albicans* (ATCC 2091). The root extract also showed significant antidiabetic activity: the methanolic root extract's IC_{50} value was 117.37 µg/mL and the ethyl acetate extract's was 358.57 µg/mL, as compared to IC_{50} value of reference acarbose (36.84 µg/mL).

Keywords:
Antioxidant, Antidiabetic, Antibacterial, IC_{50}, Phytoconstituent

1. INTRODUCTION

Berberis aristata is a medicinal plant that is also known as "daruharidra." It is most common in Himalayas area. The plant has yellowish-brown woody roots with a thin layer

of bark. As herbal medicine, to cure inflammation, jaundice, diabetes, and wounds, different parts of *B. aristata* have been used since a long time ago. This plant has numerous bioactive substances: berberine, epiberberine, palmatine, oxyacanthine, dehydrocaroline, karachine, jatrorrhizine, pseudopalmatine chloride, pakistanine, 1-O-methyl pakistanine, and pseudoberberine chloride (Potdar *et al.*, 2012). Those bioactive constituents represent several phytochemical activities: antimicrobial, anticancer, hypotensive, immunostimulatory, anti- inflammatory, and antidiabetic properties.

A quaternary isoquinoline alkaloid, Berberine, is the fundamental phytoconstituent, primarily found in the roots, rhizomes, and stem bark, and its concentration is used to assess the quality of the plant. Berberine has antifungal, tuberculostatic, and antihelminthic properties which are key for therapeutic benefits in neurological and cardiovascular disorders (Shakeel *et al.*, 2015). Berberine is also used to treat diarrhea, bacterial intestinal infections and eye infections (Dhiman, 1998).

The diaphoretic, anti-periodic, and antipyretic qualities of *B. aristata* root extract are well known (Rathi *et al.*, 2013). It is found that *B. aristata* methanolic extracts have effective anticancer effects on colon cancer cells, human hepatoma cells and murine leukemia L1210 cells. Significant antioxidant capacity is also shown by plant extracts. Additionally, investigations on animals have demonstrated significant antidysenteric and antidiarrheal benefits from an aqueous extract made from dried *B. aristata* leaves (Joshi *et al.*, 2011). The current investigation aims to assess antimicrobial, antioxidant, and antidiabetic qualities of the root of *B. aristata* in order to investigate the phytoconstituent components (both qualitative and quantitative).

Although several studies have reported the pharmacological potential of different parts of *B. aristata*, a systematic solvent-wise evaluation of phytochemical composition and biological activities of its root remains limited. The present study aims to investigate the phytoconstituent profile, antioxidant, antimicrobial, and antidiabetic activities of hexane, ethyl acetate, and methanolic root extracts of *B. aristata*.

2. EXPERIMENTAL APPROACH AND TECHNIQUES

2.1 Harvesting and Processing of Plant Roots for Extracts

In early April 2019, *B. aristata* roots were gathered from the Mahabharat Mountain Range in Phogatpur, Thakre-8, Dhading District, Bagmati Province, Nepal. Dried and powdered (50 g) roots were extracted using the Soxhlet method with 350 mL of hexane, 300 mL of chloroform, 250 mL of ethyl acetate, and 200 mL of methanol. Following methanol extraction, 500 mL of water was used to reflux the remaining residue for three hr. After being filtered and concentrated using a rotary evaporator (Rota Vapor) to create a semi-solid bulk, it was frozen for more experimental analysis.

2.2 Phytoconstituent Screening

The standard protocol proposed by Ciulei (1982) was used to perform a preliminary phytoconstituent screening of the root extract (Alghamdi, 2021).

2.3 Phytoconstituent Quantification of *B. aristata* Root Samples

2.3.1 *Phenolic Compound Quantification (TPC)*

Using the established protocol, an initial phytoconstituent screening of the root extract was conducted. Folin-Ciocalteu colorimetric technique was used to determine the TPC of root extracts (Okselni *et al.*, 2018). To create standard solutions various concentrations (125,

250, 500, and 1000 µg/ml) of gallic acid were prepared in methanol. To create a final volume of 10 ml one ml of each concentration of gallic acid solution, five ml of 10% Folin-Ciocalteu reagent, four ml of 7% Na_2CO_3 were mixed in a 20 ml test tube. The final blue solution was mixed thoroughly and was incubated at 40 ℃ in a water bath for thirty min. The absorbance was measured at 760 nm after incubation. For reference, a blank solution that contained every reagent but gallic acid was utilized. Each experiment was performed in triplicates for precision. The calibration curve was plotted using the mean absorbance readings at various concentrations. Conventional approach was used to find out the absorbance values for root extracts at different concentrations. TPC of root extracts was expressed using Gallic acid equivalents (GAE) per gram of dry extract (mg/g).

2.3.2 Flavonoid Compound Quantification (TFC)

The TFC was determined using $AlCl_3$ assay where quercetin was the reference standard (Ariffin *et al.*, 2021). At 510 nm, the absorbance for different extract concentrations was determined using the equation (y = 0.0113x0.0837, R^2 = 0.988, and the formula C = cV/m) derived from the calibration curve TFC was determined. Root extract in dry weight (mg of quercetin equivalents per gram) was used to report result (Sharma *et al.*, 2012). The linear correlation coefficient (R^2) value was recorded as the mean ± standard deviation of 3 absorbance values for every concentration, and was computed using Microsoft Office Excel 2007. The equation, y = mx + c, for straight line, where y is the absorbance, m is the calibration curve's slope, x is the concentration, and c is the intercept was used to determine TPC and TFC as well as the concentrations of each extract.

To create standard solutions, quercetin was dissolved at varying concentrations (10, 25, 50, 100, and 125 µg/mL) and 2 mL of methanol and 4 mL of distilled water was added to 1 ml of quercetin solution from each concentration.

2.4 Biological Screening

2.4.1 Free Radical Scavenging Activity

Free radical scavenging activity of root extract was assessed by using DPPH free radical scavenging assay (Mishra *et al.*, 2012; Pokhrel and Neupane, 2021). 0.2 mM DPPH solution was prepared by dissolving 7.8 mg of DPPH in 100mL of methanol. Serial dilution of the corresponding stock solutions was used to create methanolic solutions of the extracts at various concentrations (5, 10, 20, 40, and 80 µg/mL. An aliquot of 0.5 mL extract solution was added to 2.5 mL of 0.2 mM DPPH solution.

A 1:1 mixture of 0.2 mM DPPH and distilled water was used as the control. Mixtures were shaken well and incubated for 30 min at 37 ℃. The absorbance was measured at 517 nm with a Hitachi U2800 UV-Vis- spectrophotometer.

The radical scavenging percentage DPPH inhibition (%) = [1-A1/A0] × 100 (Kim *et al.*, 2017) was used, where A0 = absorbance of the control and A1 = absorbance of the sample. The mean data and standard deviation were calculated from a triplicate set of the data. To determine 50% of the DPPH free radicals, IC_{50} (concentration for 50% inhibition) value was defined as the effective sample concentration required. An inhibition curve was used to calculate IC_{50} by tabulating the measured concentration against the respective scavenging effect (Kumar *et al.*, 2012).

2.4.2 Antidiabetic Assay

Antidiabetic potential was assessed using the % alpha-amylase inhibition assay. The development of a starch-iodine complex, which was identified at 630 nm, indicated the

presence of undigested starch resulting from enzyme inhibition. A starch sample was prepared by dissolving 0.2 g of starch in 25 ml of 0.4 molar NaOH. The pH was then adjusted to 7 with distilled water. Acarbose solution was used as an inhibitor.

200 µL of either acarbose or plant extract at various doses (31.25, 62.50, 125, 250, 500, and 1000 µg/mL) was incubated for 5 min with 400 µL of starch solution at 37 ℃. 200 µL of alpha-amylase enzyme (pH 6.9) was then added and incubated for 15 min at 37 ℃. Thereafter, 800 µL of 0.1 M HCl was added to inhibit the reaction. Following the introduction of 1000 µL of iodine reagent at a concentration of 2.5 mM, the absorbance at 630 nm was measured.

The % of enzyme inhibition was calculated by using the following equation (Timilsina *et al.*, 2020): % inhibition = [1-(Abs2 - Abs1/ Abs4 - Abs3)] ×100. A graph was produced by plotting the concentration on the X-axis and the % inhibition on the Y-axis. This graph was used to calculate each extract's IC_{50} value and compare the outcomes of multiple extracts. The extract with the lowest IC50 is assumed to have the best antidiabetic property.

2.4.3 Antimicrobial Study

2.4.3.1 Sample collection: Ten bacterial species and two fungal species were employed in these tests. Table 1 lists the names and types of microorganisms used.

2.4.3.2 Antibacterial study: Before use, the agar plates were carefully dried to remove any residual moisture. The bacterial culture was then evenly spread over the solid nutrient agar layer with the help of a sterile swab. Each plate was labeled with the test disc code and the name of the bacterial strain to be tested. The antibacterial potential of the extracts was determined using the agar well diffusion method, and the effectiveness was evaluated by measuring the zone of inhibition (ZOI) formed against bacterial growth (Erhonyota *et al.*, 2023).

Four wells were carefully punched into each petri plate, and their diameters were recorded. Plant extracts were dispensed into the designated wells using a micropipette, while chloramphenicol was used as the positive control. The plates were then covered and left undisturbed for 30 min. After this diffusion period, the plates were incubated for 24 hr.

Following incubation, the appearance of clean zones around the wells indicated bacterial inhibition, and the diameter of each ZOI was measured using a mm ruler (Thakur *et al.*, 2020; Rahman *et al.*, 2022).

2.4.3.3 Antifungal study: The antifungal activity was tested using the disc diffusion method. Blood agar plates were inoculated with each fungus culture. Different amounts of plant extracts were deposited onto filter paper discs (6 mm in diameter). Before being applied to test organism-seeded plates, the extract was completely evaporated after being dissolved in DMSO. Clotrimazole (10µg per disc) served as the positive control, while a blank disk impregnated with solvent and dried off served as the negative control. Following an incubation period of one week at 28±2℃, the activity was assessed. Diameter of ZOI was measured in mm.

Table 1. Name and type of microorganisms used in this study

S.No.	Microorganisms	Reference Cultures	Strains
	Bacteria		
1.	*Bacillus subtilis*	ATCC -6051	Gram (+)
2.	*Enterococcus faecalis*	ATCC -29212	Gram (+)
3.	*Escherichia coli*	ATCC -8739	Gram (-)
4.	*Klebsiella pneumonia*	ATCC -700603	Gram (-)
5.	*Proteus vulgaris*	ATCC -6380	Gram (-)
6.	*Pseudomonas aeruginosa*	ATCC -9027	Gram (-)
7.	*Salmonella enteric*	ATCC -29630	Gram (-)
8.	*Shigella dysenteriae*	ATCC -13313	Gram (-)
9.	*Staphylococcus aureus*	ATCC -6538P	Gram (+)
10.	*Staphylococcus epidermidis*	ATCC -1228	Gram (+)
	Fungi		
1.	*Candida albicans*	ATCC -2091	
2.	*Saccharomyces cerevisiae*	ATCC -18824	

Table 2. Phytochemical screening of extracts in different solvents

S.No.	Parameter	Technique used	Hexane Extract	Ethyl acetate extract	Methanol Extract	percolated extract
1.	Alkaloids	Meyers Test	_	_	+	_
2.	Flavonoids	Shinoda test	+	+	+	_
3.	Glycosides	Molish's Test	+	+	+	_
4.	Quinones	Shinodas Test	+	+	+	+
5.	Tannins	Braemer's test	_	_	_	_
6.	Saponins	Froth flotation method	_	_	+	_
7	Steroids	Libermann- Buchard Test	_	_	_	_
8.	Carbohydrate	Fehling test	_	_	+	_
9.	Phenolic Group	Ferric chloride test	+	+	+	_
10.	Terpenoids test	Salkowski test	+	+	+	+

(+) indicate the presence and (-) indicate absence

3. RESULTS

3.1 Screening of Phytoconstituents

Chemical contents found in the plant extracts were determined using phytoconstituent screening. *B. aristata* extracts were screened for Phytoconstituents in hexane, ethyl acetate, and methanol. Table 2 shows the results of phytoconstituent screening. Phytoconstituent analysis of *B. aristata* ethyl acetate extracts showed that alkaloids, steroids, tannins, carbohydrates, and saponins were absent, while flavonoids, quinones, phenolic groups, terpenoids, and glycosides were present. Hexane extracts of *B. aristata* contained flavonoids, quinones, phenolic groups, terpenoids, and glycosides, but not alkaloids, steroids, tannins, carbohydrates, or saponins.

Alkaloids, flavonoids, phenolic groups, glycosides, and quinones were found in methanolic extracts of *B. aristata* in the above table, but tannins and steroids were not found. Quinones and terpenoids were present in the percolated *B. aristata* sample, but the remaining phytoconstituents were not.

3.2 Quantification Study

3.2.1 Phenolic Compound Quantification (TPC)

Total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent (FCR), and the results were expressed in terms of gallic acid equivalents. A standard calibration curve was generated by recording the absorbance of different concentrations of gallic acid at 760 nm. The TPC values for *B. aristata* root extracts obtained using methanol and ethyl acetate are presented in Table 3. The TPC of the ethyl acetate extract was found to be 60.57 ± 3.382 mg gallic acid equivalent per g of extract, while the methanolic extract showed a higher TPC value of 119.06 ± 3.317 mg gallic acid equivalent per g of extract.

Table 3. Determination of TPC in *B. aristata* Extracts Using Ethyl Acetate and Methanol

S.No.	Concentration (µg/ml)	Ethyl acetate extract			Methanol Extract		
		Absorbance (760nm)	TPC	Mean TPC±SD	Absorbance (760nm)	TPC	Mean TPC±SD
1.	125	0.161	65.13		0.257	117.83	
2.	250	0.262	60.27	60.57± 3.382	0.497	123.34	119.06± 3.317
3.	500	0.485	60.27		0.896	115.47	
4.	1000	0.879	56.76		1.806	119.61	

Table 4. Determination of Total Flavonoid Content in *B. aristata* Extracts Using Ethyl Acetate and Methanol

S.No.	Concentration (µg/ml)	Ethyl acetate extract			Methanol Extract		
		Absorbance (510nm)	TFC	Mean TFC±SD	Absorbance (510nm)	TFC	Mean TFC±SD
1.	125	0.027	64.00		0.035	84.64	
2.	250	0.058	72.00	66.69± 3.195	0.067	83.61	84.11± 1.354
3.	500	0.102	64.39		0.135	85.67	
4.	1000	0.208	66.38		0.258	82.52	

3.2.2 Flavonoid Compound Quantification (TFC)

To quantify flavonoids in the methanolic and ethyl acetate extracts of *B. aristata*, a method involving aluminum chloride complex formation was employed. Table 4 presents the data for both solvent extracts. This approach depends on the interaction between $AlCl_3$ and specific functional groups in flavone and flavonol structures — particularly the carbonyl group at position C-4 and -OH groups at either C-3 or C-5 — forming stable acidic complexes. For this assay, catechin served as the calibration standard. The concentration of flavonoids was determined by measuring the color change produced during complex formation using a spectrophotometric technique (Pękal and Pyrzynska, 2014). *B. aristata* methanolic extracts showed a higher TFC value of 84.11±1.354 mg quercetin equivalents per g, while ethyl acetate extract has a lower TFC value of 66.69±3.195 mg of quercetin equivalents per g.

3.3 Biological Screening

3.3.1 Evaluation of Antioxidant Potential through DPPH Radical Scavenging

Methanolic and ethyl acetate extracts were tested, and their capability to neutralize free radicals was compared. According to the data in Table 5, the methanolic extract demonstrated an IC_{50} value of 51.79 ± 0.73 µg/mL, while the ethyl acetate extract showed a slightly higher IC_{50} of 54.58 ± 0.96 µg/mL, suggesting comparable antioxidant effectiveness. Quercetin, a commonly used standard in antioxidant studies, was also tested and exhibited an IC_{50} of 50.16 ± 0.89 µg/mL, serving as the benchmark for comparing extract performance.

3.3.2 Assay for Antidiabetic Activity

Acarbose was used as a reference to test the methanol and ethyl acetate extract of *B. aristata* for its antidiabetic properties. Using the starch-iodine method, the % alpha-amylase inhibition assay was conducted to evaluate the antidiabetic activity of root extract (Giri *et al.*, 2023). Table 6 displays the percentage of % alpha- amylase inhibition of amylase at different acarbose and plant extract dosages.

Table 5. IC_{50} values in methanol and ethyl acetate extract of *B. aristata* in DPPH assay

S. No.	Concentration (µg/ml)	Ethyl acetate extract			Methanol Extract		
		Absorbance (517nm)	Scavenging activity (%)	IC_{50} value (µg/ml)	Absorbance (517nm)	Scavenging activity (%)	IC_{50} Value (µg/ml)
01	5	0.351	4.61	54.58	0.391	6.68	51.79
02	10	0.328	10.86		0.363	13.36	
03	20	0.289	21.46		0.322	23.15	
04	40	0.223	39.4		0.247	41.05	
05	80	0.107	70.92		0.109	73.98	

From the data in Table 6, % alpha-amylase inhibition versus concentration curve for acarbose and methanolic extract, and acarbose and ethyl acetate extract of *B. aristata* are presented as shown in Figures 1 and 2 respectively. The ability of methanolic and ethyl acetate extracts of *B. aristata* to suppress alpha - amylase activity was studied in this study. It was found that the IC_{50} of the methanolic extract was 117.37 µg/mL, whereas the IC_{50} of standard acarbose was 36.84 µg/mL. The ethyl acetate extract was found to have an IC_{50} of 358.57 µg/mL.

Table 6. % alpha-amylase inhibition of plant extracts and acarbose for antidiabetic assay

S.N	Concentration (ppm)		% of % alpha-amylase inhibition	
		Acarbose	Ethyl acetate extract	Methanolic extract
1.	0.00	0.00	0.00	0.00
2.	31.25	38.50	11.49	27.27
3.	62.5	46.79	18.71	33.42
4.	125	59.62	22.99	45.18
5.	250	72.45	35.56	51.34
6.	500	83.68	43.04	64.70
7.	1000	99.10	51.87	72.19

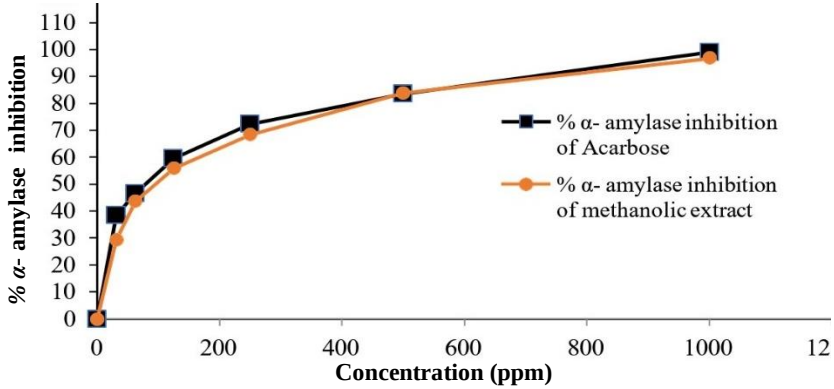

Figure 1. Comparison of % alpha-amylase inhibitory action of acarbose and methanolic extracts of *B. aristata*

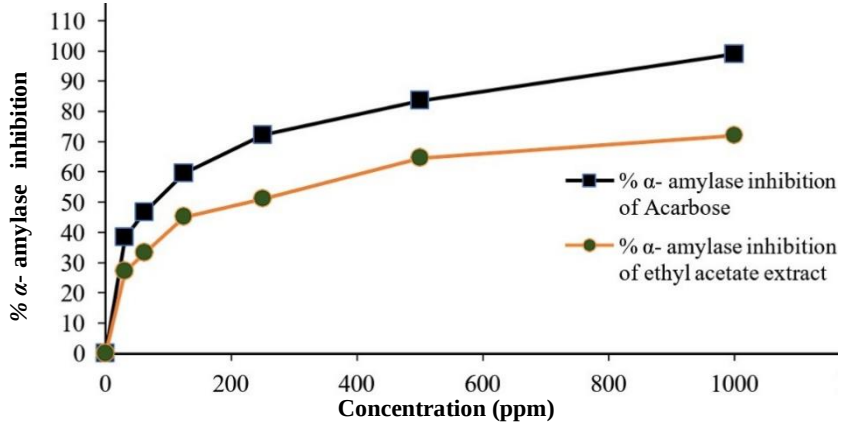

Figure 2. Comparison of alpha-amylase inhibitory action of acarbose and ethyl acetate extracts of *B. aristata*

3.3.3. Antimicrobial Study

The antimicrobial potential of the methanolic *B. aristata* root extract was assessed using the agar well diffusion technique against various bacterial and fungal strains. The outcomes of this assay are summarized in Table 7. The ZOI value in an agar well diffusion assay for plant extracts' antimicrobial activity indicates the well's diameter of incubation against a number of bacteria, including *Escherichia coli* (ATCC-8739), *Pseudomonas aeruginosa* (ATCC-9027), *Bacillus subtilis* (ATCC- 6051), *Enterococcus faecalis* (ATCC-29212), and the fungal strain *Candida albicans* (ATCC-29212). For *B. subtilis*, the methanol extract of *B. aristata* showed 16.10 mm ZOI, whereas *E. faecalis*, *Staphylococcus epidermidis*, and *P. aeruginosa* showed 28.30 mm, 26.00 mm, 28.30 mm ZOI respectively. Due to the inability of *the B. aristata* root extract to

Table 7. Antimicrobial action of methanol extracts of *Berberis aristata*

S.N.	Microorganism	Reference Culture	Test Extract	Positive control	
			ZOI (mm)	Name	ZOI (mm)
	Bacteria				
1.	*Bacillus subtilis*	ATCC 6051	16.10	Chloramphenicol	26.58
2.	*Enterococcus faecalis*	ATCC 29212	28.30	Chloramphenicol	20.86
3.	*Escherichia coli*	ATCC 8739	0.00	Chloramphenicol	22.75
4.	*Klebsiella pneumonia*	ATCC 700603	0.00	Chloramphenicol	12.28
5.	*Proteus vulgaris*	ATCC 6380	0.00	Chloramphenicol	0.00
6.	*Pseudomonas aeruginosa*	ATCC 9027	28.30	Chloramphenicol	0.00
7.	*Salmonella enterica*	ATCC 29630	0.00	Chloramphenicol	27.44
8.	*Shigella dysenteriae*	ATCC 13313	0.00	Chloramphenicol	28.99
9.	*Staphylococcus aureus*	ATCC 6538P	0.00	Chloramphenicol	28.40
10.	*Staphylococcus epidermidis*	ATCC 1228	26.00	Chloramphenicol	31.47
	Fungi				
1.	*Candida albicans*	ATCC 2091	31.50	Clotrimazole	32.33
2.	*Saccharomyces cerevisiae*	ATCC 18824	0.00	Clotrimazole	24.35

inhibit them, the remaining bacteria—*Staphylococcus aureus*, *Salmonella enterica*, *Proteus vulgaris*, *Shigella dysenteriae*, *E. coli*, and *Klebsiella pneumoniae*—did not exhibit ZOI. *Saccharomyces cerevisiae* did not exhibit 31.50 mm ZOI between two fungi, while *C. albicans* did.

4. DISCUSSION

The findings of this study were compared with previously published literature to better understand the biological potential of *B. aristata* root extracts. Overall, the results align with earlier reports while also revealing notable differences that help contextualize the extract's phytochemical and pharmacological behavior. All experiments were performed in triplicate, and the results are expressed as the mean ± standard deviation. Calibration curves showed good linearity, confirming the reliability and reproducibility of the quantitative analysis. Solvents with increasing polarity were selected in order to extract a broad range of phytochemicals, ranging from non-polar to highly polar compounds. This approach enables effective isolation of diverse secondary metabolites such as terpenoids, alkaloids, phenolics, and flavonoids.

Thakur *et al.* (2020) found bioactive chemicals such as flavonoids, saponins, alkaloids, carbohydrates, and phenolics in a methanolic extract of *B. aristata*. This comparison supports the consistency of flavonoid and phenolic presence across different studies, even though variations in other constituents may arise from methodological or environmental factors. The methanol-based extraction yielded a greater concentration of phenolic compounds compared to ethyl acetate. However, Bhatt *et al.* (2018) found that the TPC value of *B. aristata* bark extract

was 11.04±2.20 mg of gallic acid equivalent per g of extract. *B. aristata* leaf and stem extracts showed TPC values of 50.44±8.10 mg of gallic acid equivalent per g of extract and 17.70±2.97 mg of gallic acid equivalent per gram of extract, respectively. Sharmila *et al.* (2020) reported that the TPC value of the extract of *B. aristata* was 11.04±2.20 mg of gallic acid equivalent per g of extract. This explains why the root of *B. aristata* has a greater TPC value than the leaf, stem, or bark. The concentration and composition of polyphenols in different plants are affected by a variety of parameters such as growth condition, climate, processing, degree of ripeness, harvesting time, environmental factors, storage, and analytical procedures (Heimler *et al.*, 2017). These variables likely contribute to the observed differences in phenolic content across plant parts and studies.

In terms of antioxidant activity, the methanolic and ethyl acetate extracts showed IC_{50} values close to that of quercetin, suggesting comparable antioxidant effectiveness. Supporting studies by Bhatt *et al.* (2018) reported DPPH scavenging for *B. aristata* bark extract at various concentrations. IC_{50} values for bark ranged from 59.10 ± 0.85 µg/mL, 71.02 ± 3 µg/mL, to 96.92 ± 0.71 µg/mL when tested at 0.625 mg/g, 1.25 mg/g, and 10 mg/g, respectively. These results reinforce the extract's consistent ability to scavenge free radicals across different dosages. According to Lamichhane *et al.* (2014), *B. aristata* exhibited DPPH radical scavenging activity with an IC_{50} of 53.31 µg/mL, whereas the commonly used antioxidant L-ascorbic acid showed a much lower IC_{50} of 9.6 µg/mL. At a test concentration of 100 µg/mL, the *B. aristata* extract achieved 81.8% scavenging of hydrogen peroxide radicals, which closely resembled the inhibition rate of L-ascorbic acid, measured at 86.7%. The antioxidant activity of the extracts may be attributed to the presence of phenolic and flavonoid compounds, which are capable of donating hydrogen atoms or electrons to neutralize free radicals. Lower IC_{50} values therefore indicate stronger free-radical scavenging ability.

Another study reported an IC_{50} of 54.23 µg/mL for L-ascorbic acid, while *B. aristata* displayed a slightly higher IC_{50} of 60.06 µg/mL. Research by Sharmila *et al.* (2020) indicated that the aqueous extract of the aerial parts of *B. aristata* showed the highest DPPH radical inhibition, with 99.29 ± 0.95% effectiveness at 300 µg/mL. However, when compared with the standard antioxidant (ascorbic acid, IC_{50} = 1.98 µg/mL), the IC_{50} for *B. aristata* was considerably higher at 491.40 µg/mL. This suggests that the root extract of *B. aristata* may offer stronger antioxidant potential compared to other plant parts, as reflected by its relatively lower IC_{50} value.For antidiabetic activity, Chakrabarti *et al.* (2014) reported that the methanolic extract of *B. aristata* (bark), however, inhibited alpha- amylase with an IC50 of 177.9 µg/ml. According to Khan (2023) reported that the ethanolic and methanolic extracts derived from the stem and bark of *B. aristata* significantly lowered elevated blood glucose levels in alloxan-induced diabetic rats. Similarly, 50% aqueous ethanol extracts from the roots of *B. aristata* demonstrated both antioxidant and anti-hyperglycemic properties in diabetic animal models. These root extracts were not only effective but also safe, as they significantly decreased blood glucose levels without inducing hypoglycemia in non-diabetic (control) rats. The α-amylase inhibitory activity of the extracts may be associated with the interaction of polyphenolic and flavonoid compounds with the enzyme's active site through hydrogen bonding and other non-covalent interactions, resulting in reduced starch hydrolysis.

Additionally, the extract of *B. aristata* root has been found to play a vital role in maintaining glucose balance by inhibiting gluconeogenesis. This suggests that bioactive constituents in the root may act through stimulating insulin secretion or exhibit insulin-mimicking effects, contributing to their strong antidiabetic potential when administered orally.

Differences in IC_{50} values among various plant extracts can be credited to features such as plant maturity at the time of harvest, environmental influences, post-harvest treatment, and storage conditions—all of which affect the concentration of polyphenols. Therefore, even if plant extracts show a higher IC_{50} compared to acarbose (a standard antidiabetic agent with IC_{50} = 36.84 µg/mL), this is not unexpected. Unlike acarbose, which is a pure compound, plant extracts are complex mixtures of numerous bioactive constituents. These factors collectively explain the variability in antidiabetic potency across studies and extraction methods.

According to Rizwan *et al.* (2017), extracts from *B. aristata* significantly restricted the growth of the microbiological isolates that were employed. The leaf extract of *B. aristata* did not exhibit any inhibitory activity against *Paecilomyces lilacinus*, *Aspergillus niger*, *Rhizopus arrhizus*, or *Curvularia lunata*. Nonetheless, significant antifungal effects were recorded against *Curvularia* (30 ± 0.57 mm) and *Trichoderma* (36 ± 0.57 mm), with the root and stem extracts demonstrating the most notable suppression. Among these, the leaf extract showed the greatest antifungal potential against Trichoderma, with a ZOI of 19 ± 0.57 mm.

Regarding antibacterial activity, both leaf and stem extracts showed inhibitory effects against *S. aureus* (29 ± 0.57 mm) and *P. vulgaris* (22 ± 0.00 mm). The root extract, however, demonstrated the highest level of inhibition against *Vibrio cholerae* and *Shigella*, each with a ZOI of 29 ± 0.57 mm. The presence of alkaloids, flavonoids, phenolic compounds, and glycosides in the root extracts suggests their potential contribution to the observed biological activities. These phytochemical classes are well known for their antioxidant, antimicrobial, and enzyme-inhibitory properties. The observed antimicrobial activity may be linked to the presence of alkaloids and phenolic compounds, which are known to disrupt microbial cell membranes, interfere with enzyme systems, and inhibit microbial growth.

Lamichhane *et al.* (2014) looked at experimental evidence showing the extract was sensitive to *P. aeruginosa, C. albicans, E. coli, and S. typhii*, but not to *K. pneumoniae or S. aureus*. Thus, it appears that *B. subtilis, E. coli*, *P. aeruginosa, E. faecalis*, and other bacteria, as well as fungi such as *C. albicans* and *Saccharomyces cerevisiae*, have extremely strong inhibitory activity in the root extract of *B. aristata*. These observations collectively support the broad-spectrum antimicrobial potential of the root extract, particularly against Gram-positive bacteria and certain fungal strains.

4. CONCLUSION

Overall, the combined evidence from phytochemical, antioxidant, antidiabetic, and antimicrobial analyses underscores the therapeutic relevance of *B. aristata* root extract and highlights its superiority over other plant parts in several biological activities.

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Conflict of interest
The authors declare no conflict of interest.

Author Contributions
BH and SK performed the experiments. BH drafted and edited the manuscript. SP designed and supervised the research.

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