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Phytochemical analysis and biological activities on solvent extracts of two traditionally used medicinal plants

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Abstract

The plants Buddleja asiatica and Buddleja paniculata are two closely related species of which the former has widespread uses in traditional medicine while the latter remains relatively unexplored. The present study focused on the analysis of phytoconstituents and the estimation of different biological activities of extracts and semi-purified fractions. The ethyl acetate stem fraction and methanolic extract of the leaf of B. asiatica displayed the highest phenolic contents of 390.98 ± 5.32 and 383.72 ± 7.31 mg GAE/g. TFC was measured as 207.33 ± 0.34 and 138.58 ± 0.53 mg QE/g for ethyl acetate leaf and stem fraction of B. asiatica. The highest antioxidant activity was displayed by crude stem extract of B. paniculata with IC_{50} 35.65±0.61 µg/mL in DPPH assay followed by ethyl acetate stem fraction of B. asiatica with IC_{50} 36.17±0.92 µg/mL. The DCM leaf fraction and stem fraction of B. asiatica were found active against gram-negative and gram-positive bacteria. The median lethal concentration (LC_{50}) in brine shrimp assay ranged from 346.96 ± 25.39 in hexane fraction of leaf to 2719.32 ± 706.5 µg/mL in crude methanol extract of B. paniculata. The present study showed these medicinal plants are rich in an important class of organic compounds that play a significant role in the cure of bacterial infection which ultimately supports pharmaceutical research.

Keywords
Buddleja asiatica, Buddleja paniculata, antimicrobial, antioxidant, phytochemical, toxicity.

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1 Introduction

Plants have a huge importance in both traditional medicine and the modern health care system. The biological activities and therapeutic properties of medicinal plants are the results of specific or synergistic interactions of their secondary metabolites. These compounds are not required for central processes of growth and development in plants, but they are important for defense, signaling, symbiosis, metal transport, and competition [1]. They are synthesized through various biosynthesis pathways in plants [2]. About 200,000 plant secondary metabolites have been isolated and characterized with many more expected in the future [3]. Specific sec-

ondary metabolites may be exclusive to a particular species or group of species. The qualitative and quantitative composition of metabolites may vary within or between species and influenced by environmental stresses like nutrition, drought, temperature, and light [4].

Genus *Buddleja* belonging to the family *Scrophulariaceae* includes more than 100 species that grow in temperate and drier regions of Asia, Africa, and America [5, 6]. These plants are mostly shrubs or small trees that can grow up to 5 m. *Buddleja* species are used to treat bronchial complaints, liver diseases, and wounds and they are known to possess antimicrobial, antihyperglycemic, antioxidant activities, sedative functions, and, analgesic potential [6]. Although *Buddleja* has been used as a diuretic and topical antiseptic in traditional medicine, its phytochemical investigation has been somewhat neglected [7]. *Buddleja asiatica* (Bhimsenpati) and *Buddleja paniculata* (Narayanpati) are two species of the genus *Buddleja* found in the Kathmandu Valley, Nepal. *B. asiatica* is reported to have antihepatotoxic, anti-inflammatory, analgesic, antipyretic, hypotensive, hypoglycemic, antimicrobial, neuroprotective, and anticataract properties [8]. It possesses chemical compounds like monoterpenoids, diterpenes, triterpenes, flavonoids, phenylpropanoids, and steroids [6]. A novel compound buddlin has been isolated from the plant [9]. *B. asiatica* is used as an additive in the preparation of fermentation starter culture in Arunachal Pradesh, India [10]. The leaf juice is used for treatment of gastrointestinal issues, skin conditions [11], abortifacient, cure for loss of weight [12]' and, beverage fermentation [13]. Root paste with rice water is used as a toner in Burma [14]. In Laos, the same procedure is applied to make a base for a medicine taken during childbirth, and leaves are used to treat headaches [15]. Ullah et al., (2014) evaluated the cytotoxic and phytotoxic activities of *B. asiatica* which showed that the plant is safe for human consumption [16]. The leaves of *B. paniculata* are used by the indigenous Chepang community of Nepal in fermentation [17]. While many scholars have evaluated the biological activities and medicinal properties of *B. asiatica*, closely related species *B. paniculata* remains unexplored. The present work is a comparative study of qualitative and quantitative phytochemical analysis, antioxidant and antimicrobial, and cytotoxicity in methanol extracts and fractions of *B. asiatica* and *B. paniculata*.

Reactive oxygen species (ROS) of both free radical and non-radical nature are produced inside the cell during mitochondrial oxidative phosphorylation or interaction with exogenous sources such as xenobiotic compounds [18]. They are involved in many signaling pathways and influence cell proliferation, necrosis, apoptosis, and gene expression in animal and host defense by programmed cell death that prevents the spread of invading pathogens in plants [19]. The steadily forming prooxidants (ROS) are consumed by antioxidant molecules of enzymatic and non-enzymatic nature and a prooxidant-antioxidant equilibrium is maintained [20]. The imbalance in this equilibrium causes oxidative stress during which the highly reactive free radical can attack and cause structural damage to important biomolecules such as DNA, proteins, carbohydrates, lipids, and enzymes [21]. Oxidative stress promotes and assists development of various health issues including neurodegenerative diseases, diabetes, cancer, and cardiovascular diseases [22]. Plant extracts and metabolites with rich antioxidant activity can prevent or delay the oxidation of substrate biomolecules and exert various health-promoting and therapeutic effects. Although the exact mechanisms by which a given herbal medicine exerts its pharmacological effects are not established, most such medicinal plants are known to contain significant antioxidant activity [23].

Adverse or side effects of synthetic drugs kill approximately 100,000 people in the USA each year and they are also responsible for about 8% of total hospital admission cases in the country [24]. Although plant-based traditional medicines and secondary metabolites have far fewer side effects or toxicity than modern synthetic drugs due to their natural origin, they can potentially be toxic [23].

2 Materials and Methods

2.1 Plant Collection and Identification

Leaf and stem of *B. asiatica* and *B. paniculata* were collected from Dakshinkali 1, Champadevi, Lalitpur, and Bhaktapur 1, Dudhapati, Bhaktapur, Nepal respectively in September 2021. The herbariums of the plant specimens were prepared and were identified at the Central Department of Botany, Tribhuvan University, Kathmandu, Nepal, having voucher no. TUCH-210070 and TUCH-210071 of *B. asiatica* and *B. paniculata* respectively. The photographs of fresh plants and herbarium are given in Figure 1 and traditional uses of plants are listed in Table 1.

2.2 Extraction and Fractionation

he collected plant samples were cleaned and shade-dried up to a constant weight that was not attained. Then, the plant samples were ground to powder using a mechanical grinder. 100 g of the powder was soaked with 300 mL of reagent-grade methanol solvent inside a conical flask. After 72 hours, the content in the flask was filtered using muslin cloth followed by Whatman no 1 filter

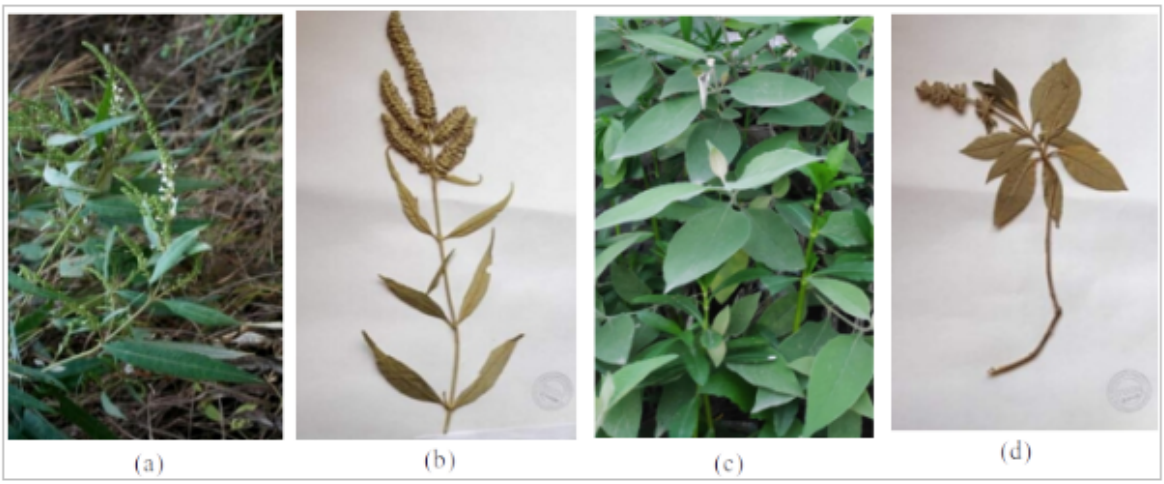

Figure 1: (a) Fresh plant sample *B. asiatica* (b) herbarium of plant sample *B. asiatica* (c) Fresh plant sample of *B. paniculata* (d) herbarium of plant sample *B. paniculata.*

paper. The filtrate was concentrated at a controlled temperature (40 C) and reduced pressure in a rotatory evaporator. The process was repeated after intervals of 48 hours and then 24 hours for maximum and complete extraction of phytochemicals.

Table 1: The plant samples used in the study, voucher number, and traditional medical uses

S.N.	Scientific name	Identification no.	Altitude/plant growing	Plant parts	Traditional Uses
1	Buddleja asiatica	TUCH-210070	Altitude-2180m Coordinates-27°38' 57" N 85°17' 58" E	Leaves and stems	Fermentation [10, 13] Gastrointestinal issues, skin condition [11] Abortifacient, cure for weight loss [12] Toner [14] Childbirth, headache [15]
2	Buddleja paniculata	TUCH-210071	Altitude-1996m Coordinates-27°40' 08" N 85°26' 32" E	Leaves and stems	Fermentation [17]

2.3 Separation of Semi-purified Solvent Fractions

A series of reagent-grade solvents with increasing polarity (hexane, DCM, ethyl acetate, water) were used to separate the different solvent fractions of methanol extract. Differences in the polarities of these solvents will result in the separation of plant secondary metabolites into different solvent fractions. Thus, it will help isolate the active compound in future works. 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 shaken vigorously at first and then left undisturbed until a clear upper layer of hexane and lower layer of water formed and then both layers were collected in separate beakers. The hexane layer was concentrated in the rotary evaporator to obtain a hexane soluble fraction. The aqueous layer was subjected to further fractionation with DCM and ethyl acetate for the separation of DCM and ethyl

acetate soluble fractions.

2.4 Phytochemical Analysis

The phytochemical analysis of extracts and fractions was performed by following the procedures described by Harborne (1998) and Mallikharjuna et al. (2007) [25, 26]. The preliminary qualitative analysis for the presence and absence of secondary metabolites was carried out by the color differentiation method adopting separate protocols.

2.5 Estimation of Total Phenolic Content (TPC)

Phenolic content was measured using the Folin-Ciocalteu reagent method with slight modifications [27]. 20 µL each of different concentrations of standard (10 µg/mL to 100 µg/mL gallic acid solutions in methanol) and plant extracts (500 µg/mL in 50% DMSO) were loaded in separate bores of a 96 well plate in triplicates. Then, 100 µL Folin-Ciocaleu phenol reagent and 80 µL of Na_2CO_3 were added to each bore, and the well plate was placed in the dark. After 30 minutes, absorbance was taken at 765 nm using a microplate reader (Synergy LX, BioTek, Instruments, Inc., USA) with Gene 5 software. TPC was calculated using the regression equation obtained from the gallic acid calibration curve and expressed as mg of gallic acid equivalent per gram dry extract or fraction (mg GAE/g).

2.6 Estimation of Total Flavonoid Content (TFC)

$AlCl_3$ colorimetric method as described by Zhishen et al., (1999) was used to determine total phenolic content [28]. 130 µL of different concentrations of the standard (15.4 µg/mL to 154 µg/mL quercetin in methanol) and 20 µL of plant extracts (500 µg/mL in 50% DMSO) were loaded in the bores of a 96-well plate in triplicates. 110 µL of distilled water was added to each bore containing plant extract. Then, 60 µL of ethanol, 5 µL of $AlCl_3$, and 5 µL of CH_3COOK were added to each bore, and the well plate was placed in the dark. After 30 minutes, absorbance was taken at 415 nm using the microplate reader. TFC was calculated using the regression equation from the quercetin calibration curve and expressed in terms of milligrams of quercetin equivalent per gram dry extract or fraction (mg QE/g).

2.7 Antioxidant Activity

DPPH assay was employed to determine the antioxidant activity of plant extracts and fractions by following standard protocol [29]. 100 µL each of different concentrations of plant extracts and fractions (15.625 µg/mL to 500 µg/mL in 50% DMSO) were loaded in triplicates to the bores of a 96-well plate. 100 µL of 0.1 mM DPPH solution was added to each bore. The reaction mixture was placed in the dark for 25 minutes and absorbance was taken at 517 nm using the microplate reader. Quercetin was used as standard and 50% DMSO was used as control. The relationship given below was used to calculate the percentage of radical scavenging.

GraphPad Prism 9 software was used to calculate the concentration of plant extract that scavenges 50% of the available free radical (IC_{50}).

2.8 Antibacterial Assay

The agar well diffusion method was used to measure the antibacterial activity in extracts and fractions [30]. The types of bacteria and their ATCC numbers are given in Table 2.

Overnight incubated broth cultures of test organisms were prepared in nutrient broth media. The concentration of bacteria was diluted and maintained at 0.5 McFarland standard ($10^{6\text{-}8}$ CFU/mL). 100 µL of inoculum was spread on Muller Hinton Agar (MHA) plates and wells having 7 mm of diameter were bored on the plates. 20 µL of 25 mg/mL (in DMSO) plant extracts were added to the bores in triplicates. Then, the plates were incubated at 37 C, and the zone of inhibition (ZOI) was measured after 24 hours. 100% DMSO was used as control and 1 mg/mL Ampicillin was used as standard.

Table 2: Bacterial strain, type, and ATCC number of test organisms

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

2.9 Antifungal Activity

The agar well diffusion method was used to measure the antifungal activity in plant extracts [30]. Overnight incubated broth culture of test organisms *Fusarium solani* (ATCC 11712) was prepared in nutrient broth media and concentration

was maintained at 0.5 McFarland standard. 100 µL of inoculum was spread on potato dextrose agar (PDA) plates and wells having 7 mm of diameter were bored. 20 µL of 25 mg/mL plant fraction solutions were added in triplicates to those bores. The plates were incubated at 37 C and ZOI was measured after 48 hours. 100% DMSO was used as control and 20 mg/mL cycloheximide was used as standard.

2.10 Toxicity

The toxicity of plant fractions was measured by using the brine shrimp assay [31]. 2 mL of each of the different concentrations of plant fractions (10, 100, 1000 µg/mL in methanol) was added in triplicates to test tubes. The solvent was evaporated using a water bath. The leftover residue in the test tube was dissolved using 5 mL of artificial seawater followed by the addition of 10 brine shrimp nauplii. The number of live nauplii was counted after 24 hours. The concentration of plant extracts lethal to 50% of the organism (LC_{50}) was calculated from the percentage mortality versus concentration curve. 100% methanol was used as a control.

2.11 Statistical Analysis

All the experiments were performed in triplicates. Values were presented as mean ± standard error (SE). Comparisons were made using one-way ANOVA followed by Tukey's test performed with SPSS version 29 software. Values with $p < 0.05$ were considered statistically different.

3 Results

3.1 Phytochemical Analysis

The results of qualitative phytochemical analysis of the crude extracts are given in Table 3.

ALC, *B. asiatica* crude leaf extract; ASC, *B. asiatica* crude stem extract; PLC, *B. paniculata* crude leaf extract; PSC, *B. paniculata* crude stem extract; '+', present; '-', absent.

Important phytochemicals such as polyphenols, flavonoids, and quinones are observed in the leaf and stem extracts of both plants. Alkaloids were present only in leaf extracts and terpenoids were exclusive to the stem. The absence of coumarin in PLC and glycoside in PSC was recorded.

Table 3: Qualitative phytochemical analysis of crude extracts

Group of compounds	ALC	ASC	PLC	PSC
Polyphenols	+	+	+	+
Alkaloids	+	-	+	-
Coumarins	+	+	-	+
Glycosides	+	+	+	-
Quinones	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	-	+	-	+

ALC, B. asiatica crude leaf extract; ASC, B. asiatica crude stem extract; PLC, B. paniculata crude leaf extract; PSC, B. paniculata crude stem extract; '+', present; '-', absent.

3.2 Phytochemical Analysis

The total phenolic content and flavonoid content were measured using the Folin-Ciocalteu phenol reagent method and aluminum chloride colorimetric method respectively and the results are provided in Table 4. The observed phenolic content ranged from 39.5 ± 1.18 in PLC to 390.98 ± 5.32 mg GAE/g in ASE. The descending order of TPC in crude extracts and solvent fractions is 390.98 ± 5.32 (ASE) > 383.72 ± 7.31 (ALC) > 374.32 ± 5.72 (ASD) > 295.57 ± 0.54 (ASC) > 271.58 ± 5.83 (PSC) > 177.05 ± 7.91 (PLD) > 131.34 ± 3.82 (ALE) > 93.6 ± 3.97 (ALD) > 60.39 ± 3.48 (PLH) > 39.5 ± 1.18 mg GAE/g (PLC). The measured amount of flavonoid content ranged from 13.44 ± 0.18 in PLD to 207.33 ± 0.34 mg QE/g in ALE. The descending order of TFC in crude extracts and fractions is 207.33 ± 0.34 (ALE) > 138.58 ± 0.53 (ASE) > 43.33 ± 0.14 (ALC) > 42.48 ± 1.09 (ASC) > 40.58 ± 0.58 (PLC) > 37.22 ± 0.19 (PSC) > 30.96 ± 0.18 (PLH) > 15.41 ± 0.44 (ALD) > 13.89 ± 0.32 (ASD) > 13.44 ± 0.18 mg QE/g (PLD). TPC and TFC values are graphically presented in Figure 2 and Figure 3.

3.3 Antioxidant Properties

The antioxidant activity of the plant extract was measured using DPPH assay. Concentration-dependent increments in the percentage of free radical scavenging are observed (Figure 4). The concentration of plant extract or fraction that scavenges 50% of available free radical (IC_{50})

Table 4: TPC, TFC, and half maximal inhibitory concentration (IC50) in DPPH assay of plant extracts and fractions

Plant extracts and fractions	TPC (mg GAE/g)	TFC (mg QE/g)	IC50 (/)
ALC	$383.72 \pm 7.31^{a,b}$	43.33 ± 0.14^{a}	51.91 ± 1.09
ALE	131.34 ± 3.82	207.33 ± 0.34	139.70 ± 2.30
ALD	93.6 ± 3.97	$15.41 \pm 0.44^{b,c}$	119.91 ± 0.41
ASC	295.57 ± 0.54^{c}	$42.48 \pm 1.09^{a,d}$	71.86 ± 1.67
ASE	$390.98 \pm 5.32^{a,d}$	138.58 ± 0.53	36.17 ± 0.92^{a}
ASD	$374.32 \pm 5.72^{b,d}$	$13.89 \pm 0.32^{b,e}$	83.59 ± 1.57
PLC	39.5 ± 1.18^{e}	40.58 ± 0.58^{d}	374.7 ± 0.55
PLD	177.05 ± 7.91	$13.44 \pm 0.18^{c,e}$	177 ± 0.72
PLH	60.39 ± 3.48^{e}	30.96 ± 0.18	315.3 ± 0.45
PSC	271.58 ± 5.83^{c}	37.22 ± 0.19	35.65 ± 0.61^{a}
Quercetin	#	#	3.28 ± 0.89

ALE, B. asiatica ethyl acetate leaf fraction; ALD, B. asiatica dichloromethane leaf fraction; ASE, B. asiatica ethyl acetate stem fraction; ASD, B. asiatica dichloromethane stem fraction; PLD, B. paniculata dichloromethane leaf fraction; PLH, B. paniculata hexane stem fraction; * positive control; #, value not measured; Values are the mean ± SE (n=3); values marked by the same letter within the same column are not significantly different at $p < 0.05$.

ranged from 35.65±0.61 in PSC to 315.30±0.45 µg/mL in PLC (Table 4). The decreasing order of antioxidant activity is 35.65±0.61 (PSC) > 36.17 ± 0.92 (ASE) > 51.91 ± 1.09 (ALC) > 71.86 ± 1.67 (ASC) > 83.59 ± 1.57 (ASD) > 119.91 ± 0.41 (ALD) > 139.70 ± 2.30 (ALE) > 177 ± 0.72 (PLD) > 315.3 ± 0.45 (PLH) > 315.30±0.45 µg/mL (PLC). The IC_{50} values for extract and fractions were higher than 3.28 ± 0.89 µg/mL recorded for quercetin.

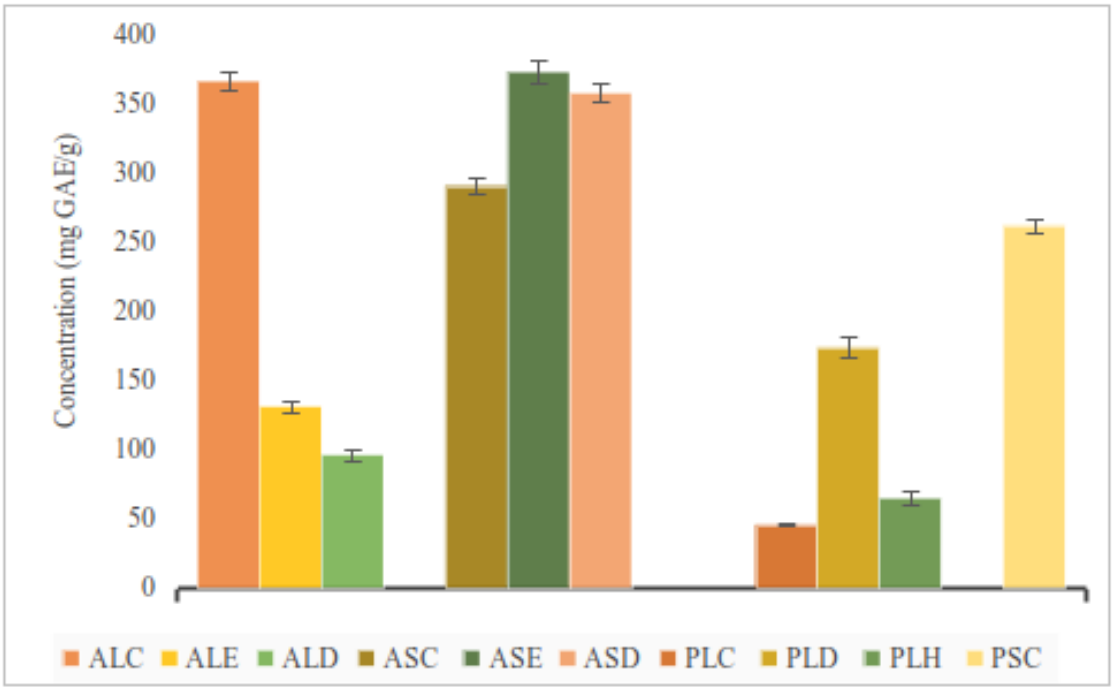

Figure 2: Total phenolic content (mg GAE/g) of plant extracts and solvent fractions.

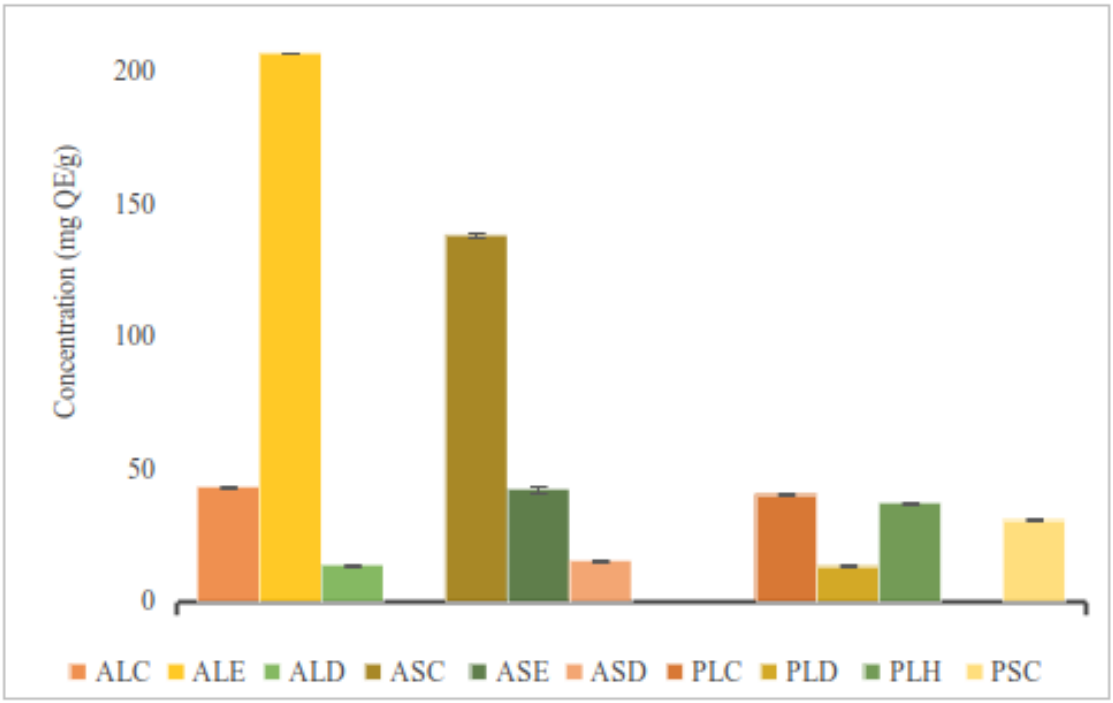

Figure 3: Total flavonoid content (mg QE/g) of plant extracts and solvent fractions.

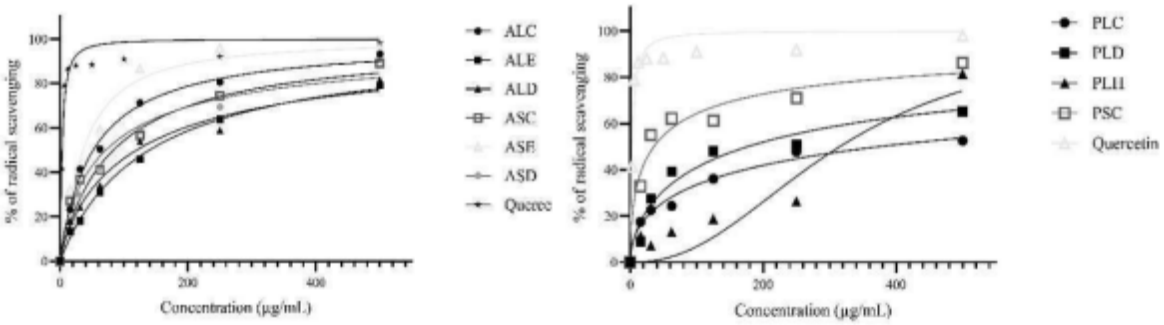

Figure 4: Percentage radical scavenging versus concentration (µg/mL) of plant extracts and solvent fractions.

3.4 Antimicrobial Properties

The antibacterial and antifungal potential exhibited by the crude extracts and the solvent fractions is shown in Table 5. The antibacterial activities of plant extracts were tested against an American-type culture collection of gram-positive and gram-negative bacteria. The dichloromethane fraction of the stem of *B. asiatica* displayed ZOIs of 8.33 ± 0.67 mm against *S. aureus* and 8.33 ± 0.33 mm against *K. pneumoniae*. Similarly, the dichloromethane fraction of the leaf of the plant displayed a ZOI of 8.67 ± 0.33 mm against *S. aureus*. Other extracts and solvent fractions did not display significant ZOI. ZOI of 27.5 ± 0.25 mm, 13 mm, 16.5 ± 1.25 mm, and 22 ± 0.5 mm were recorded against *S. aureus, B. subtilis, E. coli,* and *K. pneumoniae* for Ampicillin used as positive standard. Plant extracts and solvent fractions were found to be inactive against fungal species *F. solani* but the cyclohexamide used as a standard displayed a ZOI of 17.5 mm. The photographs of Petri plates from the antimicrobial assay are given in Figure 5.

ALC = *B.asiatica* crude extract, ASC = *B. Asiatica* crude stem extract, PLC = *B. Paniculata* crude leaf extract, PSC = *B. paniculata* crude stem extract, ALE = *B. asiatica*

Table 5: Zone of inhibition (ZOI) shown by the plant extracts and solvent fractions against bacterial and fungal strains

Plant extracts and fractions	ZOI (mm) of plant extracts and fractions				
	S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*	*F. solani*
ALC	-	#	-	-	-
ALE	-	-	-	-	#
ALD	8.33 ± 0.33^a	-	-	-	-
ASC	-	#	-	-	#
ASE	-	-	-	-	-
ASD	8.33 ± 0.67^a	-	-	8.67 ± 0.33^a	#
PLC	-	#	-	-	#
PLD	-	-	-	-	#
PLH	-	#	-	-	-
PSC	-	#	-	-	-
Ampicillin*	27.5 ± 0.25	13	16.5 ± 1.25	22 ± 0.5	#
Cycloheximide*	#	#	#	#	17.5

Values are the mean ± SE (n=3); * positive control; -, no significant ZOI; #, value not measured; *a* p < 0.05 versus ampicillin.

ethyl acetate leaf fraction, ALD = *B. asiatica* dichloromethane leaf fraction, ASE = *B. asiatica* ethyl acetate stem fraction, PLD = *B. paniculata* dichloromethane leaf fraction, PLH = *B. paniculata* hexane stem fraction;

3.5 Toxicity

The results of the toxicity analysis of the crude extracts and the solvent fractions of the plants are displayed in Table 6. The assay revealed the non-toxic to mildly toxic nature of plant extracts and the solvent fractions.

Values are the mean ± SE (n=3); [a] $p < 0.05$ versus PSC.

The LC_{50} value ranged from 346.96 ± 25.39 in PLH to 2719.32 ± 706.5 µg/mL in PSC. The descending order of toxicity can be presented as 346.96 ± 25.39 (PLH) > 353.68 ± 83.62 (ASE) > 472.95 ± 51.36 (PLD) > 540.46 ± 39.87 (ASD) > 824.48 ± 103.54 (ALE) > 971.34 ± 253.48 (ALC) > 1074.83 ± 115.76 (ASC) > 1362.93 ± 393.21 (ALD) > 1366.7 ± 258.69 (PLC) > 2719.32 ± 706.5 µg/mL (PSC).

Table 6: Half maximal lethal concentration (LC50) shown by plant extracts and solvent fractions against brine shrimp nauplii

Plant extracts and fractions	LC50 ($\mu g/mL$)
ALC	971.34 ± 253.48^a
ALE	824.48 ± 103.54^a
ALD	1362.93 ± 393.21
ASC	1074.83 ± 115.76^a
ASE	353.68 ± 83.62^a
ASD	540.46 ± 39.87^a
PLC	1366.7 ± 258.69
PLD	472.95 ± 51.36^a
PLH	346.96 ± 25.39^a
PSC	2719.32 ± 706.5

Values are the mean ± SE (n=3); *a* $p < 0.05$ versus PSC.

4 Discussion

The observed results were supported by previous studies as the presence of phenols, flavonoids, glycosides, and terpenoids in the leaf of *B. asiatica* was reported by Sai et al., (2019) and Nafees et al., (2022) [13, 32]. Among different plant secondary metabolites, phenolics are a large group of phytochemicals that includes a diverse family of compounds such as phenolic acids, flavonoids, phenylpropanoids, quinones, tannins, lignins, and hydroxycinammic acids [33]. Among

them, flavonoids are involved in pigmentation, signaling, plant growth and development, and plant defense mechanisms [34]. The significance of phenolics in the modern drug discovery process is illustrated by the fact that 17 out of 29 small molecules approved by the Federal Drug Administration, USA in 2020 contained phenol moieties [35].

The crude methanol extracts and solvent fractions of *B. asiatica* and *B. paniculata* are found rich in phenolic and flavonoid contents but significant variations were observed between the two plant species and their crude extracts and fractions. Crude extracts of *B. asiatica* possessed higher phenolic content than crude extracts of *B. paniculata*. Stem extracts and fractions contained more phenolics than their corresponding leaf extract. The concentration of phenolics was higher in more polar solvent ethyl acetate 131.34 ± 3.82 (ALE), 390.98 ± 5.32 (ASE) than in less polar solvent dimethyl chloride 93.6 ± 3.97 (ALD), 374.32 ± 5.72 mg GAE/g (ASD). Similarly, more polar fraction PLD contained higher phenolic compounds than PLH. The ability of a more polar solvent to extract a higher amount of phenolic content is also mentioned by Khanal et al., (2022) in bark extracts of *Beilschmiedia roxburghiana* [36]. Sai et. al., (2019) reported a TPC of 127.48±1.58 mg GAE/g for 80% ethanol extracts of *B. asiatica* leaves [13]. The biological activities of phenolics, flavonoids, alkaloids, and terpenoids in the plant should be responsible for the traditional use of *B. asiatica* in gastrointestinal issues, childbirth, headache, skin conditions, weight loss, cancer, and diabetes [8, 11, 12, 15].

Similar to phenolic contents, the flavonoid content was found to be higher in crude extracts of *B. asiatica* than in *B. paniculata*. Also, the concentrations of flavonoids were higher in crude extracts and fractions of leaves than in stems. This is expected as high concentrations of flavonoids are reported in UV-exposed mesophyll cells that seem to act as protection for chloroplast from photo-oxidative damage [37]. More polar ethyl acetate contained significantly higher TFC 207.33 ± 0.34 (ALE), 138.58 ± 0.53 (ASE) than less polar dimethyl chloride 15.41 ± 0.44 (ALD), 13.89 ± 0.32 mg QE/g (ASD). Sai et al., (2019) reported a TFC of 648.42±2.88 µg/mL for 80% ethanol extract of the leaf of *B. asiatica* [13, 33].

Extracts and fractions with higher phenolic and flavonoid content displayed more antioxidant activity. Phenolic compounds donate hydrogen atoms from their hydroxyl group and form stable, unreactive antioxidant radicals [38]. The antioxidant activity of such compounds depends upon the number and position of the hydroxyl group [39]. In addition to phenolics, El-Sayed et al., (2008) isolated non-phenolic antioxidant compounds from the methanol extract of the leaf of *B. asiatica* [40]. Previous scholars have reported IC_{50} values of 3.04±0.04 µg/mL for 80% ethanol extract and 16.28 µg/mL for methanol extract of the leaf of *B. asiatica* [13, 40]. The present and past studies confirm the presence of high antioxidant activity in *B. asiatica* and *B. paniculata*. Antioxidant compounds present in medicinal plants prevent oxidation, which is the initial step of many neurodegenerative diseases and cancers [41]. They also reduce the risk of diabetes and cardiovascular diseases [22]. High antioxidant activity in *B. asiatica* and *B. paniculata* could be responsible for the traditional uses of the plants as anticancer, antidiabetic, and neuroprotective medicine [42, 43]. The plant is also used for the treatment of rheumatism in China [16]. The significant antioxidant activity in *B. asiatica* and *B. paniculata* makes them valuable medicinal plants.

Only the dichloromethane fractions of the leaf and stem of *B. asiatica* displayed significant ZOI. Both plants do not possess significant activity against *B. subtilis*, *E. coli*, and *F. solani* in the present study. The lack of ZOI against *K. pneumoniae* for methanol extract of *B. asiatica* in the present study is in agreement with the study conducted by Ali et al., (2011) in which no ZOI was observed against *K. pneumoniae* for methanolic extract, hexane, and ethyl acetate fractions of whole plant, but the ZOIs of 15 and 5 mm observed against *E. coli* and *B. subtilis* was different from the present study [12]. The essential oil of the whole plant was reported to be active against bacterial species *E. coli*, *B. subtilis,* and *S. aureus* and, fungal species *F. solani* [44]. The accumulation and concentration of plant secondary metabolites depend upon plant growth and development stage, genetics, and environmental stress such as radiation, drought, temperature, and salinity [45]. The antimicrobial activity of plant extract and fractions is subject to change with the presence or absence of specific metabolites, their concentration, and synergistic interactions.

Crude extracts of *B. asiatica* were more toxic than that of *B. paniculata*. The LC_{50} value of 971.34 ± 253.48 µg/mL for ALC was higher than the reported value of 469.63 µg/mL for ethanol extract of *B. asiatica* leaves [16]. The same study also reported higher toxicity in leaf than bark extracts in agreement with higher toxicity in ALC than ASC observed in the present study. A good correlation between acute toxicity and the results of the Brine shrimp lethality assay has been established in previous studies [46]. Thus, the absence of toxicity means it is potentially safe for humans.

5 Conclusion

Stem and leaf of *B. asiatica* and *B. paniculata* displayed the presence of secondary metabolites such as polyphenols, flavonoids, quinones, alkaloids, coumarins, glycosides, and terpenoids. Plant extracts and solvent fractions of both plants are rich in phenolics and flavonoids and they also

display significant antioxidant activity. The concentration of phenolics and flavonoids was found higher in polar solvents than in less polar ones. There is a weak correlation between phenolic and flavonoid concentration against antioxidant activity. The dichloromethane fraction of the leaf and stem of *B. asiatica* was found active against both gram-positive and gram-negative bacterial strains. Both plants were found inactive against the fungal species *F. solani*. The plant extracts and solvent fractions displayed low toxicities against the brine shrimp nauplii. Thus, the plants are safe for use as a source for isolating natural antioxidants rich in secondary metabolites. The crude extracts of *B. asiatica* displayed higher TPC and TFC along with the potential antibacterial activity and toxicity than crude extracts of *B. paniculata*, however, both plants are found rich in phytochemicals and biological activity. Further work on isolation, purification, and characterization of bioactive compounds from both plants may lead to the discovery of novel therapeutic agents. In this way, the findings of the present study support the use of these two medicinal plants as traditional medicine against infectious diseases and diabetes for many years by the people of Nepal.

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