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Research Article

Evaluation of Antioxidant, Antimicrobial, and Cytotoxic Activities and Correlation with Phytoconstituents in Some Medicinal Plants of Nepal

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Traditional herbal medicines have been consumed in Nepal and other parts of the eastern hemisphere since ancient times. Many of these plants reportedly have been effective against ailments as well. This study aims to analyze the phytochemical constituents from the extracts of ten such plants and evaluate their antimicrobial, cytotoxicity, and antioxidant properties. In addition, the study aims to study the correlation of cytotoxicity and antioxidant activities with the total phenolic, flavonoid, and tannin contents. The plants investigated were *Oroxylum indicum*, *Kalanchoe pinnata*, *Phragmites vallatoria*, *Ehretia acuminata*, *Cirsium wallichii*, *Ampelocissus tomentosa*, *Dichrocephala integrifolia*, *Boenninghausenia albiflora*, *Cynoglossum zeylanicum*, and *Clerodendrum serratum*. Phytochemical analyses were performed to evaluate secondary metabolites, such as glycosides, flavonoids, terpenoids, saponins, alkaloids, and fats. The total phenolic contents of the extracts ranged from 14.94 to 229.89 mg GAE/g, the total flavonoid contents varied from 66.67 to 900 mg QE/g, and the total tannin contents were 42 to 168 mg GAE/g. The results of the antioxidant studies showed that the highest antioxidant activity was exhibited by the extract of *A. tomentosa* (IC50 = 7.89 μg/mL) followed by *E. acuminata* (IC50 = 24.82 μg/mL) and *C. serratum* (IC50 = 32.91 μg/mL). The extracts from *P. vallatoria* and *A. tomentosa* exhibited substantial antimicrobial activity. The extracts of *A. tomentosa* and *B. albiflora* showed lethality against brine shrimp with LC_{50} values of 33.11 μg/mL.

1. Introduction

Despite modern medical advances, people in the eastern hemisphere have traditional medicines ingrained in their culture. Traditional medicines are of choice in certain societies because of their ease of access, cost-effectiveness, and in some cases, lack of awareness of modern health facilities. It is generally agreed that traditional medicines carry fewer adverse effects in such parts of the world [1, 2]. Nepal is a Himalayan nation with great biodiversity owing to its topographical, geographical, and climatic variations in a small land area coverage [3]. More than 700 plant species in Nepal have been reported to possess therapeutic potential. These plants are found to be distributed among ethnic groups based on geography. Such communities have been using these medicinal plants to treat human maladies and some of such practices are part of Ayurveda [4]. Medicinal plants have been shown to contain antioxidant, anticancer, antibacterial, antiviral, and anti-inflammatory agents. Flavonoids and other phenolic compounds found in the leaves, fruits, barks, stems,

and roots of medicinal plants are natural phytochemicals that have led to their therapeutic applications in the treatment of many ailments [5]. Numerous degenerative diseases, including atherosclerosis, cancer, and gastric ulcers, are caused mainly by oxidative stress, which is carried on by oxygen-free radicals. Many antioxidants that actively scavenge oxygen can be found in medicinal plants [6]. Disease prevention and treatment are made possible by the phytochemicals, such as phenolics, flavonoids, anthocyanins, terpenoids, and tannins found in medicinal plants [7]. Numerous medicinal plants have been identified as valuable sources of natural antimicrobial agents as potential alternatives to traditional treatment for bacterial infections [8].

Oroxylum indicum (family Bignoniaceae) is native to the Indian subcontinent, and the major chemical constituents of this species are chrysin, baicalein, scutellarein, aloe emodin, and tetuin [9]. *Kalanchoe pinnata* (family Crassulaceae) is widely distributed in tropical and subtropical regions. It is used for the treatment of periodontal diseases, cheilitis, ear infection, and dysentery [10]. *Phragmites vallatoria* is a grass plant that belongs to the family Poaceae and is primarily used for treating wound healing, diabetes, arthritis, and rheumatism [11]. *Ehretia acuminata* (family Boraginaceae) is traditionally used to treat dysentery [12]. Likewise, *Clerodendrum serratum* (family Lamiaceae) is native to tropical Africa and Southern Asia, and this plant has been used as a traditional medicine to treat asthma, infectious disorders, and other inflammatory diseases [13]. Studies on the roots of Ampelocissus sp. have revealed constituents that possess inhibitory activities against cancer cells [14]. Similarly, *Cynoglossum zeylanicum* is distributed widely throughout Nepal at 900–3500 m, common in open places and on uncultivated land, and it is found to contain alkaloids such as echinatine, isoechinatine, neocorromandaline, cynaustraline, lactodine, viridinatine [15, 16].

Hence, the main aim of the study was to analyze phytochemicals and the biological activities of ten medicinal plants collected from various locations in Nepal. In addition, the study is focused on correlating the composition of phytochemicals with antioxidant and cytotoxic activities using principal component analysis (PCA).

2. Materials and Methods

2.1. Chemicals. Most of the chemicals and solvents were of the analytical grade. Gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Molychem and Hi-Media (India), respectively. Quercetin, dimethyl sulfoxide (DMSO), methanol, acetone, and other solvents were purchased from Fisher Scientific (India), E. Merck, and Qualigens.

2.2. Collection and Identification of the Plants. Medicinal plants were collected from different parts of Nepal. *Oroxylum indicum* (Linn) Vent, *Kalanchoe pinnata* (Lam.) Pers and *Ehretia acuminata* were collected from Bardiya, Nepal, while *Clerodendrum serratum* (L.) Moon was collected from Banke National Park, Nepal, and *Phragmites vallatoria* (L.) Poit from Kirtipur, Kathmandu. Likewise, *Cirsium wallichii, Ampelocissus tomentosa, Dichrocephala integrifolia, Boenninghausenia albiflora*, and *Cynoglossum zeylanicum* were collected from Parbat, Nepal. The local names, scientific names, parts of medicinal plants used, and their ethnomedicinal usage are shown in Table 1. The taxonomic identification of the collected plants was conducted by the National Herbarium, Godavari, and the Central Department of Botany, Tribhuvan University, Nepal. Figure 1 shows the chemical structure of some secondary metabolites of the plants under study obtained through the literature survey.

2.3. Preparation of Extract. The plants were properly cleaned with water, dried in the shade at room temperature avoiding direct sunshine, and then crushed. Using the Soxhlet extractor and polar solvents (ethanol and methanol), the powder of different plants was extracted, and then, the extracts were concentrated under reduced pressure in a rotatory evaporator until a solid mass was obtained. The extracted plant material was kept in a sealed vial at 4°C until further analysis. The percentage yield of different plant extracts was calculated using the following formula:

$$\%\ \text{Yield} = \frac{\text{Dry weight of extract}}{\text{Dry weight of sample}} \times 100\%. \quad (1)$$

2.4. Phytochemical Screening. Phytochemical analyses of plant extracts were performed based on the procedure adopted from Sasidharan et al. [27], Pal et al. [28], and Yadav and Agrawala [29]. Mainly those tests were conducted to determine glycosides, flavonoids, alkaloids, phenolic compounds, terpenoids, steroids, carbohydrates, saponins, tannins, fixed oils, and fats in the extracts.

2.5. Determination of Total Phenolic Contents. The total phenolic contents (TPCs) in the plant extracts were determined by using the Folin-Ciocalteu colorimetric method, which is based on an oxidation-reduction reaction as described by Sengul et al. [30]. At first, 1 mL of plant extract (10 mg/mL in methanol) was added with 5 mL of 1 : 10 Folin-Ciocalteu Reagent (FCR) and 4 mL of 7% aqueous Na_2CO_3 in a 10 mL test tube. Then, the obtained blue mixture was thoroughly agitated and incubated in the dark at room temperature for 30 minutes. Following that, an untreated blank containing all reagents except gallic acid was used to measure the absorbance at 760 nm. Gallic acid was used for the standard calibration curve and TPCs were expressed in milligrams of gallic acid equivalent per Gram of dry weight of the extract (mg GAE/g).

2.6. Determination of Total Flavonoid Contents. The total flavonoid contents (TFCs) of plant extracts were determined using aluminum chloride colorimetric assay, a method developed by Hassan et al. [31]. Quercetin was used for the standard calibration curve, and TFCs were expressed in milligrams of quercetin equivalent per Gram of dry weight of the extract (mg QE/g).

Table 1: Description of the selected medicinal plants.

Scientific name	Local name	Family	Part used	Traditional usage	References
O. indicum	*Tatelo*	Bignoniaceae	Bark	Used to treat diarrhea, dysentery, diaphoretic, asthma, and rheumatism	[17]
K. pinnata	*Patharchur*	Crassulaceae	Leaves	Used to treat urinary bladder stones, and headaches	[18]
P. vallatoria	*Nalkot*	Poaceae	Roots	Used for antidiabetic, wound healing, and rheumatoid arthritis	[19]
E. acuminata	*Arjun*	Boraginaceae	Leaves	Used as an antidiabetic, anti-inflammatory agent, and diarrhea treatment	[20]
C. serratum	*Vawarment*	Lamiaceae	Roots	Used for the treatment of rheumatism, asthma, and other inflammatory diseases	[21]
C. wallichii	*Thakailo*	Compositae	Roots	Used for the treatment of gastric troubles	[22]
A. tomentosa	*Purene*	Vitaceae	Vines	Used externally for cuts and wounds	[23]
D. integrifolia	*Hachhyun jhar*	Asteraceae	Leaves	Used for treating sinusitis and migraine	[24]
B. albiflora	*Uruse jhar*	Rutaceae	Leaves and small twigs	Used as a pain killer and against fever	[25]
C. zeylanicum	*Kanike kuro*	Boraginaceae	Leaves	Used for treating indigestion	[26]

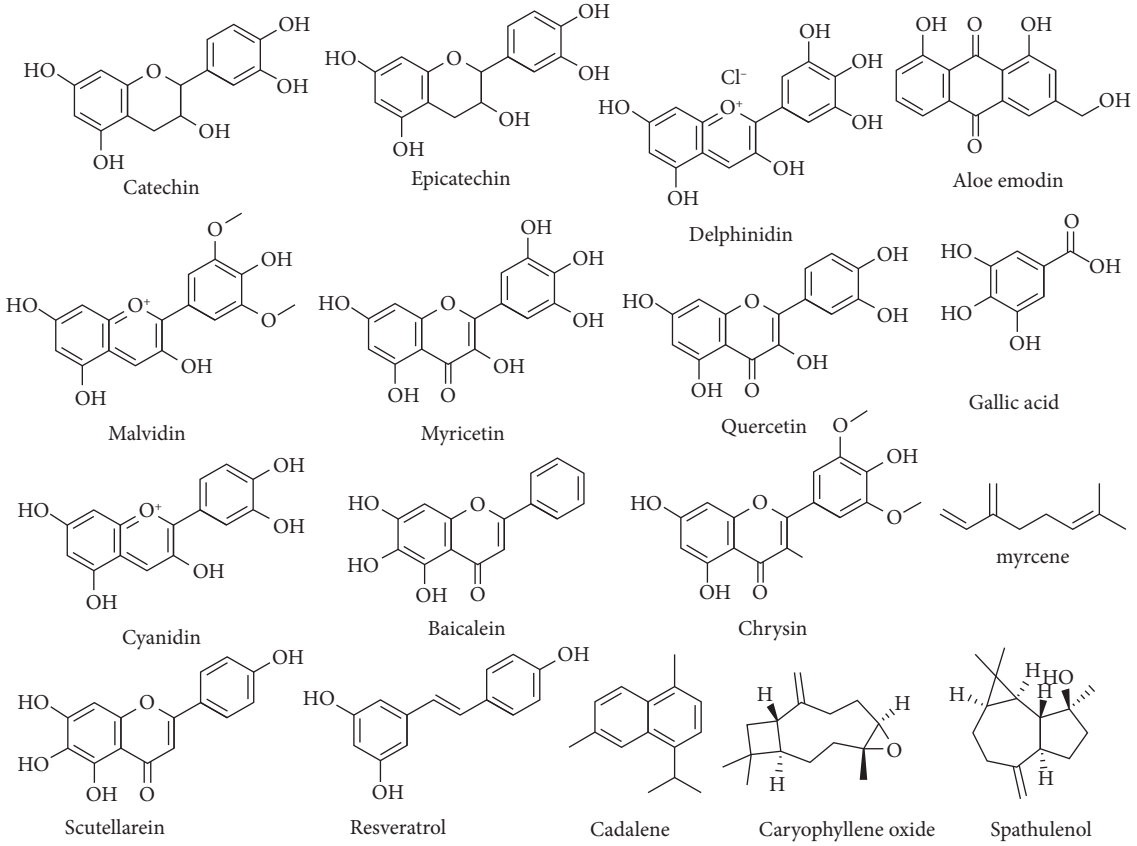

Figure 1: Chemical structure of some secondary metabolites present in the collected plants.

2.7. Determination of Total Tannin Contents. The total tannin contents (TTCs) were determined using the Folin and Ciocalteu methods, which are procedures developed by Tamilselvi et al. [32]. Before adding 0.5 mL of 10% FCR, 0.1 mL of the sample extract solution (10 mg/mL in methanol) was mixed with 7.5 mL of distilled water. This mixture was added with 1 mL of 35% sodium carbonate solution followed by diluting with 10 mL of distilled water. Thus, obtained blue-colored mixture was thoroughly agitated and incubated in the dark at room temperature for 30 minutes. Following that, an untreated blank containing all reagents except gallic acid was used to detect absorbance at 725 nm. The calibration curve was created using the absorbance values at various gallic acid concentrations. The total tannin contents were calculated similarly to the total phenolic and total flavonoid contents.

2.8. Calculation of TPCs, TFCs, and TTCs. The concentration of flavonoids, phenolics, and tannins was determined by using calibration curves, which were generated by plotting the concentration of the standards on the x-axis and absorbance on the y-axis. The data were linearly fitted, and the coefficient of determination (R^2) was found to be between 0.9753 and 0.9997.

2.9. Determination of the Antioxidant Activity. The antioxidant activity of the ten plant extracts and the standard (ascorbic acid) was evaluated by following the methodology of Alabri et al. [33] and Shyur et al. [34], which is based on the ability of 2,2-diphenyl-1-picrylhydrazyl (DPPH) to scavenge free radicals. Different concentrations of the plant extracts (15–500 μg/mL) and control ascorbic acid (15–500 μg/mL) were prepared in methanol. Then, 1 mL of 0.1 mM DPPH solution was added to 1 mL of plant extract. The tubes were briskly shaken for even mixing and incubated in the dark at room temperature for 30 minutes. Methanol was taken to collect the baseline on the spectrophotometer (UV-spectrophotometer 1800). The absorbance of the samples was measured at 517 nm. The DPPH free radical scavenging activity was calculated in terms of percentage inhibition as follows:

$$\%\ \text{Inhibition} = \frac{A_o - A_s}{A_o} \times 100\%, \tag{2}$$

where, A_o is the absorbance of the control (ascorbic acid) and A_s is the absorbance of the sample.

2.10. Determination of Antimicrobial Activity. The plant extracts were tested for antimicrobial activity using the agar well diffusion method. The Gram-positive bacterial strains, including *Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC11778, and Gram-negative bacterial strains, including *Escherichia coli* ATCC 15922 and *Salmonella typhimurium* ATCC 14028, were grown in nutrient agar media. Using a sterile cork borer (6 mm), wells were created on agar plates and correctly labeled. With the aid of a micropipette, 40 μL of the plant extract's working solution (10 mg/mL) was then added to the appropriate wells. In a different well, the solvent DMSO was also tested for its activity as a control. A 50 mg/mL neomycin solution was employed as a positive control. Plant extracts were then allowed to diffuse through the media by leaving the plates with the lid closed for 30 minutes, and the plates were incubated at 37°C. The plates were examined for the zone of inhibition (ZoI) around the wells after incubating for 18–24 hours.

2.11. Brine Shrimp Bioassay. The brine shrimp bioassay for each methanolic extract was carried out by following procedures used by Olowa and Nuñeza [35]. New-born brine shrimp larvae were utilized in the brine shrimp bioassay for biological screening. A table lamp (100 watts) was used to illuminate for 48 hours while maintaining a temperature of 30°C to facilitate the hatching of brine shrimps (*Artemia salina*) using roughly 50 mg of brine shrimp eggs in a beaker. After hatching, active nauplii that were free of eggshells were collected from the hatching chamber's brighter area and used for the toxicity test. Then, 10 mature brine shrimps were added to test tubes containing different concentrations of plant extracts as shown in Table 2, and the survivors were counted after 24 hours.

2.12. Statistical Analyses. Statistical analyses of the data were carried out using R (version 4.2.1) and R Studio (version 2022.07.1). The variables on which analyses were performed are TPC, TFC, TTC, IC_{50} (for antioxidant activity), and LC_{50} (for cytotoxicity). The correlation was investigated and principal component analysis was performed as well. To find a suitable method for correlation (e.g., Pearson, Kendall, Spearman), a test of normality was deemed necessary. Initially, the Shapiro-Wilk normality test, and for validation, the Anderson-Darling normality test were conducted on the variables TPC, TFC, TTC, $IC_{50,}$ and LC_{50}, to check for normality, skewness, and kurtosis. If the data were found to be normally distributed Pearson product-moment correlation was evaluated, or else Kendall rank correlation was evaluated. Principal component analysis (PCA) was performed to reduce the dimensionality of the dataset, by producing new uncorrelated variables and selecting the leading two that bring the most variance to the data.

3. Results

3.1. Phytochemical Screening. The highest extract yield was obtained from the leaves of *B. albiflora* (35.07%), and the lowest yield was obtained from the bark of *O. indicum* (9.23%). Table 3 shows the results of the phytochemical analysis of methanol extracts from ten different plants. Glycosides, flavonoids, phenolic compounds, terpenoids, and tannins were found in all extracts. Alkaloids were present in all extracts except in *K. pinnata*. Likewise, steroids and saponins were present in all extracts except in *C. wallichii*.

3.2. Total Phenolic Contents. The total phenolic contents were estimated by employing gallic acid as a standard in the Folin-Ciocalteu reagent assay for each plant extract. On using the Folin-Ciocalteu reagent, plant extracts generate a blue complex that can be detected at 760 nm by a visible-light spectrophotometer. Different concentrations of gallic acid, i.e., 125, 250, 500, and 1000 μg/mL were used to generate a calibration curve. The phenolic contents of ten medicinal plants were found between 14.94 and 229.89 mg GAE/g (Table 4). The phenolic content of *A. tomentosa* was found to be maximum, i.e., 229.89 mg GAE/g.

3.3. Total Flavonoid Contents. For the determination of total flavonoid contents, quercetin was used for constructing the standard calibration curve. The absorption was taken at 510 nm with 125, 250, 500, and 1000 μg/mL of quercetin. The total flavonoid contents of the ten plants were found between 66.67 and 900 mg QE/g (Table 4). Here, *E. acuminata* was found to contain the maximum amount of flavonoids

Table 2: The number of survived brine shrimp nauplii after treatment with plant extracts and their percentage mortality.

Plant extract	Concentration (μg/mL)	Total number of surviving nauplii after 24 h	% Mortality
O. indicum	10	26	13
	100	24	20
	1000	20	33
K. pinnata	10	22	27
	100	14	53
	1000	9	70
P. vallatoria	10	25	17
	100	18	40
	1000	11	63
E. acuminata	10	23	23
	100	11	63
	1000	0	100
C. serratum	10	22	27
	100	12	60
	1000	7	77
C. wallichii	10	26	13
	100	20	33
	1000	10	67
A. tomentosa	10	20	33
	100	11	63
	1000	0	100
D. integrifolia	10	25	17
	100	22	27
	1000	7	77
B. albiflora	10	18	40
	100	14	53
	1000	0	100
C. zeylanicum	10	25	17
	100	18	40
	1000	3	90

Table 3: Phytochemical screening of medicinal plants collected in Nepal.

Plant extracts	Class of compounds									
	Glycosides	Flavonoids	Alkaloids	Phenolic compounds	Terpenoids	Steroids	Carbo-hydrates	Saponins	Tannins	Volatile oils and fats
O. indicum	+	+	+	+	+	+	+	+	+	+
K. pinnata	+	+	−	+	+	+	+	+	+	+
P. vallatoria	+	+	+	+	+	+	−	+	+	+
E. acuminata	+	+	+	+	+	−	+	+	+	+
C. serratum	+	+	+	+	+	+	−	+	+	+
C. wallichii	+	+	+	+	+	−	+	−	+	−
A. tomentosa	+	+	+	+	+	+	−	+	+	−
D. integrifolia	+	+	+	+	+	+	−	+	+	−
B. albiflora	+	+	+	+	+	+	−	+	+	+
C. zeylanicum	+	+	+	+	+	+	−	+	+	+

"+" = Present and "−" = Absent.

(900 mg QE/g) followed by *A. tomentosa* (833.33 mg QE/g). The flavonoid contents of the other plants were found to be moderate, except those of *O. indicum*, *C. wallichii,* and *P. vallatoria,* which were found to be low.

3.4. Total Tannin Contents. For the determination of tannins, gallic acid was used for constructing the standard curve. The absorption was taken at 725 nm. Using the Folin-Ciocalteu method, the total tannin contents of the plant extracts were determined and expressed in terms of gallic acid equivalents. Table 4 lists the total tannin contents of the plant extracts. The methanolic extract of all plants shows high tannin content. Notably, *E. acuminata, C. serratum, A. tomentosa, D. integrifolia, B. albiflora,* and *O. indicum* contain a high quantity of tannins at 150, 140, 168, 116, 114, and 116 mg GAE/g, respectively.

Table 4: Total phenolic contents, total flavonoid contents, total tannin contents, and antioxidant activity (IC_{50} values of ten plant species and ascorbic acid).

Plant extracts	Total phenolic contents (mg GAE/g)	Total flavonoid contents (mg QE/g)	Tannin contents (mg GAE/g)	IC_{50} values (μg/mL)
O. indicum	70.11	66.67	116	147.39
K. pinnata	62.07	366.67	96	57.16
P. vallatoria	68.97	66.67	80	253.53
E. acuminata	163.22	900	150	24.83
C. serratum	97.70	766.67	140	32.91
C. wallichii	14.94	66.67	42	313.20
A. tomentosa	229.89	833.33	168	7.89
D. integrifolia	55.17	400	116	78.29
B. albiflora	103.45	200	114	137.75
C. zeylanicum	73.56	500	90	39.90
Ascorbic acid (control)	—	—	—	15.62

3.5. Antioxidant Activity. The antioxidant activities of the plant extracts were determined using the DPPH assay. The IC_{50} value of the standard ascorbic acid was found to be 15.62 μg/mL (Table 4). The free radical scavenging action of methanol extracts of plants are found to be in order as *A. tomentosa* (7.89 μg/mL) > *E. acuminata* (24.83 μg/mL) > *C. serratum* (32.29 μg/mL) > *C. Zeylanicum* (39.90 μg/mL) > *K. pinnata* (57.16 μg/mL) > *D. integrifolia* (78.29 μg/mL) > *B. albiflora* (137.75 μg/mL) > *O. indicum* (147.39 μg/mL) > *P. vallatoria* (253.53 μg/mL) > *C. wallichii* (313.20 μg/mL).

3.6. Evaluation of Antimicrobial Activity. Table 5 shows the antimicrobial activity, in terms of zone of inhibition, of the different plant extracts. *A. tomentosa* and *P. vallatoria* showed the best antimicrobial activity against *S. aureus* and *B. cereus* with ZoI of 19 mm and 18 mm, respectively. These two plants also exhibited the highest inhibition against *E. coli* and *S. typhimorium* with ZoI 20 and 18 mm, respectively. Overall, the extracts showed more activity against Gram-negative bacteria than Gram-positive bacteria. Neomycin (50 mg/mL) was used as a positive control, while DMSO was used as a negative control. The presence of alkaloids and polyphenols in medicinal plant materials is likely to be responsible for antimicrobial activity [36].

3.7. Brine Shrimp Bioassay for Toxicity Analysis. The results of the brine shrimp bioassay displayed that *A. tomentosa, B. albiflora E. acuminata*, *C. serratum*, and *C. zeylanicum* were bioactive with LC_{50} values of 33.11, 33.11, 50.12, 63.09, and 107.15 μg/mL, respectively. Table 6 summarizes the LC_{50} values of the different plant extracts. The extract of *O. indicum*, whose LC_{50} was found to be 63095.73 μg/mL, is not considered cytotoxic. Table 2 shows the number of survived brine shrimp nauplii after treatment with plant extracts and the percentage mortality.

3.8. Statistical Analyses

3.8.1. Correlation. As shown in Tables 7 and 8, both the Shapiro-Wilk test and the Anderson-Darling test have indicated that all data except LC_{50} is normally distributed (having a p-value greater than 0.05).

Table 5: In vitro antimicrobial activity of plant extracts against different bacterial strains.

Plant extract (100 mg/mL)	Zone of inhibition (mm) (including 6 mm well size)			
	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus	*B. cereus*	*E. coli*	*S. typhimurium*
O. indicum	16	14	12	13
K. pinnata	11	12	14	14
P. vallatoria	19	18	18	18
E. acuminata	13	12	16	16
C. serratum	16	15	17	17
C. wallichii	6	6	6	6
A. tomentosa	19	18	20	20
D. integrifolia	15	12	18	16
B. albiflora	16	12	16	14
C. zeylanicum	14	13	11	11
Neomycin (50 mg/mL)	32	27	30	32
DMSO	6	6	6	6

Table 6: LC_{50} values of different plant extracts.

Plant extract	LC_{50} (μg/mL)
O. indicum	63095.73
K. pinnata	199.50
P. vallatoria	251.18
E. acuminata	50.12
C. serratum	63.09
C. wallichii	524.81
A. tomentosa	33.11
D. integrifolia	213.80
B. albiflora	33.11
C. zeylanicum	107.15

Such a phenomenon as seen in the case of LC_{50} is common for cytotoxic assay data. Kendall rank correlation coefficient was evaluated for the data pairs due to the non-normality of LC_{50} (Table 9).

The Kendall correlation rank coefficient has shown that a great number of pairs exhibit significant correlations. Only the correlations between LC_{50} and TTC, and LC_{50} and IC_{50} are nonsignificant. Among significant correlations, LC_{50} and

Table 7: Shapiro-Wilk test for normality.

	TPC	TFC	TTC	IC_{50}	LC_{50}
W	0.87039	0.88447	0.97758	0.85526	0.37185
p-value	0.101	0.1468	0.9509	0.06707	$1.181e-07$

Table 8: Anderson-Darling test for normality.

	TPC	TFC	TTC	IC_{50}	LC_{50}
A	0.65508	0.43735	0.18386	0.63846	3.1582
p-value	0.06074	0.2336	0.8803	0.06737	$1.162e-08$

Table 9: Kendall rank correlation coefficient.

	TPC	TFC	TTC	IC_{50}	LC_{50}
TPC		0.51 *	0.58 *	−0.60 *	−0.67 * *
TFC	0.51 *		0.58 *	−0.83 * *	−0.63 *
TTC	0.58 *	0.58 *		−0.58 *	-0.48^{ns}
IC_{50}	−0.60 *	−0.83 * *	−0.58 *		0.63^{ns}
LC_{50}	−0.67 * *	−0.63 *	-0.48^{ns}	0.63^{ns}	

^{ns}Nonsignificant; * significant within $\alpha = 0.05$; * * significant within $\alpha = 0.01$.

TPC, and IC_{50} and TFC are highly statistically significant (i.e., within 99% C.I.).

3.8.2. Principal Component Analysis. The scree plot of the principal component analysis (Figure 2) shows that only two components have an eigenvalue greater or equal to 1. Therefore, for further analysis only principal components (PCs) 1 and 2 were considered as they account for the majority of variance in data as depicted.

Figure 3 shows that all the variables except IC_{50} and LC50 are positively correlated to PC1. Additionally, only IC_{50} and TFC are positively correlated to PC2.

Tables 10 and 11 provide the data of the principal component analysis.

4. Discussion

For the development of new drugs, plants constitute a significant source of potential drug molecules. The free radical scavenging molecules, including phenolic acids, flavonoids, tannins, and other substances that are present in many plants have been extensively studied [37]. The antioxidant properties of medicinal plants are primarily due to phenolic and flavonoid compounds [7]. They are present in both edible and nonedible plants and are responsible for anticancer, antibacterial, antiviral, anti-inflammatory, and antioxidant activities [38]. Flavonoids are secondary metabolites of plants and are known to have beneficial effects on human health, and more than 4000 flavonoids have been identified [39]. Studies have shown that tannins possess anticarcinogenic, antimutagenic, and antimicrobial properties [40]. They are also a unique group of plant phenolic compounds with anti-ischemic and endothelium-dependent vasorelaxant properties [41]. So, this study was focused on the investigation of

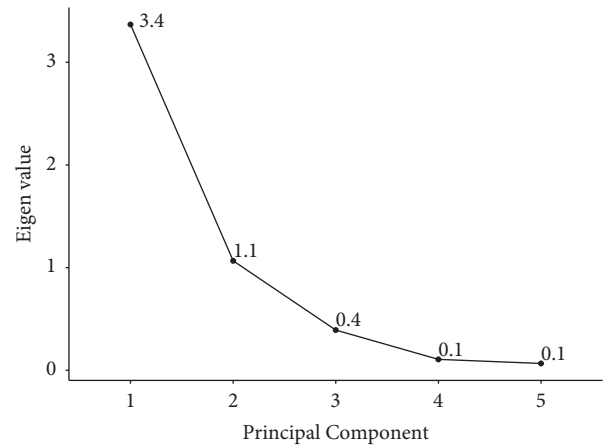

Figure 2: Scree plot of principal component analysis.

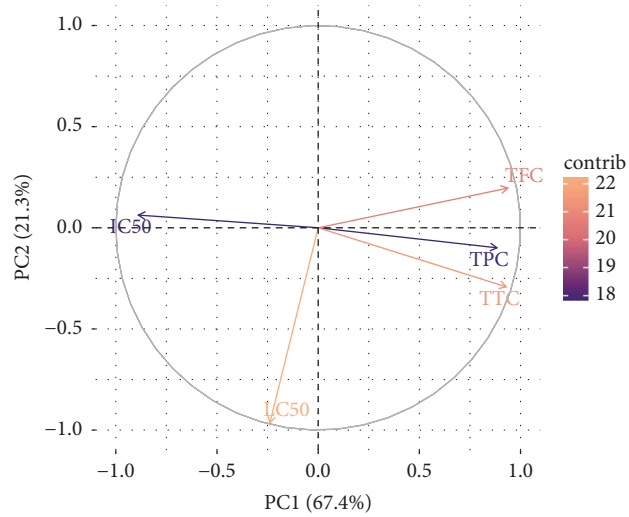

Figure 3: Principal component plot.

Table 10: Principal components.

	PC1	PC2	PC3	PC4	PC5
Standard deviation	1.835	1.033	0.626	0.326	0.258
Eigenvalue	3.369	1.067	0.392	0.106	0.066
Percentage of variance	67.37	21.34	7.84	2.12	1.33

Table 11: Factor loading data of variables with PC1 and PC2.

	PC1	PC2
TPC	0.482	−0.096
TFC	0.511	0.190
TTC	0.506	−0.282
IC_{50}	−0.484	0.061
LC_{50}	−0.130	−0.933

phytochemicals and biological activities of a number of Nepalese medicinal plants, namely, *O. indicum*, *K. pinnata*, *P. vallatoria*, *E. acuminata*, *C. serratum*, *C. wallichii*, *A. tomentosa*, *D. integrifolia*, *B. albiflora*, and *C. zeylanicum*. Phytochemical analysis showed positive for alkaloids, tannins, terpenoids, saponins, volatile oils, and

fats, and these phytochemicals have potential antioxidant activity. The methanol extract of plants showed strong antioxidant activity with IC_{50} values of 7.89, 24.83 μg/mL, 32.29 μg/mL, 39.90, and 57.18 μg/mL for *A. tomentosa*, *E. acuminata*, *C. serratum*, *C. zeylanicum*, and *K. pinnata*, respectively.

A previous study on *O. indicum* showed TPC of 118.84 ± 0.62 μg GAE/mg and TFC of 81.42 ± 0.62 μg QE/mg, and antioxidant activity was reported as 9.42 ± 0.04 μg/mL from methanol extract [42]. In our study, the TPC, TFC, and TTC for *O. indicum* were found to be 70.11 mg GAE/g, 66.67 mg QE/g, and 116 mg GAE/g, respectively, and antioxidant activity was found to be 147.39 μg/mL. The flavonoids, namely, baicalin, baicalein, and chrysin were isolated from *O. indicum* [43]. As reported in previous studies, *K. pinnata* showed TPC of 5.538 ± 0.005 mg GAE/g, TFC of 0.242 ± 0.001 mg QE/g, and TTC of 0.019 ± 0.001 mg TAE/g from ethanol extract, and antioxidant activity was reported as 37.28 μg/mL from bark-stem extracts [44]. In our study, *E. acuminata* (IC_{50} = 24.83 μg/mL) and *C. serratum* (IC_{50} = 32.29 μg/mL) showed strong radical scavenging activity. The antioxidant activity of the methanolic extract of *C. serratum* in previous studies was reported as 0.125–1.0 mg/mL [45]. As reported, the methanolic extract of *B. albiflora* exhibited antioxidant activity with an IC_{50} value of 243.8 μg/mL and the ethanolic extract of this plant showed a TPC of 110.9 μg/mL and TFC of 42.8 μg/mL [46]. While, in our study, *B. albiflora* showed a TPC of 103.45 mg GAE/g, TFC of 200 mg QE/g, and IC_{50} value of 78.29 μg/mL. Similarly, as reported methanolic extract of leaves of *C. wallichii* exhibited a TPC of 22.59 ± 0.90 mg GAE/g and TFC of 716.58 ± 0.06 mg QE/g [47]. In our study, *C. wallichii* showed the TPC and TFC of 14.94 mg GAE/g and 66.67 mg QE/g, respectively. The variations in the amounts of polyphenols, flavonoids, and tannins can be attributed to the fact that the content of phenolic compounds is impacted by various factors, including the geographical and climatic circumstances of the location where the sample are gathered, the extraction technique, solubility, and the kind of solvent used [48]. The presence of polyphenols, which are among the most active antioxidant components of plants and effective donors of hydrogen to the DPPH radical, was found to be related linearly with antioxidant activity. The majority of this activity is therefore attributable to the presence of polyphenols [49]. The plant extracts were biologically screened by using the brine shrimp bioassay. The ability to kill laboratory-cultivated brine shrimps is used in a brine shrimp lethality test to investigate the toxicity of medicinal plants [50, 51]. The method determines the LC_{50} values for different extracts [52], and the lower the LC_{50} value, the greater the toxicity. A previous study of cytotoxicity on *O. indicum* showed that LC_{50} value of 251.2 μg/mL [53], while *K. pinnata* exhibited an LC_{50} value of 51.88 ± 0.52 μg/mL [54]. In our study, leaves of *E. acuminata* (LC_{50} = 50.12 μg/mL) and roots of *C. serratum* (LC_{50} = 63.09 μg/mL) were found to be highly active against brine shrimps among all selected plant extracts. *A. tomentosa and P. vallatoria* demonstrated the best antimicrobial activity against both Gram-positive and Gram-negative bacteria as determined by measuring the inhibitory zone surrounding the agar well filled with a plant extract. The presence of numerous active components in the plants may be the cause of their antimicrobial activity. To isolate and characterize the bioactive components and create new antimicrobial drugs, more research in this area is required.

Despite the normal distribution exhibited by all variables apart from LC_{50}, Kendall's rank correlation was calculated instead of Pearson's product-moment correlation coefficient. Kendall's correlation was thought to be a better fit because the variables lack a strict quantitative relationship. The use of Pearson's correlation can be found in the literature; however, it is not appropriate as a rank-based approach, which is a better model.

Based on correlations, a key takeaway is the highly significant negative correlation between LC_{50} and TPC, which demonstrates greater levels of lethality with the increase in TPC. Also, the highly significant negative correlation between IC_{50} and TFC demonstrates high TFC and decreases IC_{50} value. Hence, indicating the increased drug potency when TFC is increased. Furthermore, the significant negative correlation of TTC with IC_{50} and nonsignificant correlation with LC_{50} provides us the explanation that an increased level of TTC has an increase in drug potency due to a decrease in IC_{50} along with a lack of significant relationship with lethality.

Principal component analysis was conducted to break down this dataset for the reduction in dimensionality. Through the principal component analysis, five principal components were identified, but only two, which have an eigenvalue greater than 1, were chosen for further study as they contribute the most to the dataset [55]. These two principal components were responsible for 88.71% of the variance of data. Based on the factor loading score of the principal component analysis, it can be inferred that PC1 is primarily a measure of TFC and TTC, as these variables have a positive factor loading greater than 0.5. It is seen that a decrease in LC_{50} and IC_{50} shows an increase in PC1, this however is not statistically significant due to the factor loading score of less than 0.5. In the case of PC2, it appears that it is a measure of lethality, due to its strong negative correlation with LC_{50}.

5. Conclusions

Medicinal plants are abundant sources of diverse biologically active secondary metabolites, which provide ample opportunities for new drug leads. This study was focused on the evaluation of phytochemicals and biological activities of ten medical plants collected from different locations in Nepal. The study showed the great therapeutic potential of *A. tomentosa, E. acuminata, C. serratum*, and *K. piñnata* as antioxidant and antibacterial activities with high contents of tannins, phenolics, and flavonoids. Hence, extensive research can be carried out in these plants focusing on the bioassay-guided isolation of the active phytoconstituents followed by their *in vivo* studies to validate their traditional uses as well as to authenticate their candidacy in the future drug discovery process.

Abbreviations

DMSO: Dimethyl sulfoxide
DPPH: 2,2-Diphenyl-1-picrylhydrazyl-hydrate
FCR: Folin-ciocalteu reagent
GAE/g: Gallic acid equivalent per gram
IC_{50}: Half-maximum inhibitory concentration
LC_{50}: Lethal concentration 50%
TFC: Total flavonoid content
TPC: Total phenolic content
TTC: Total tannin content
QE/g: Quercetin equivalent per gram
ZOI: Zone of inhibition.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Keshav Ranabhat and Kamal Prasad Regmi performed research; Sarwesh Parajuli performed statistical analysis; Ranjita Thapa, Arjun Prasad Timilsina, and Sarwesh Parajuli wrote the manuscript; Shantel Fleming, Bishnu P Regmi, Akkal Dev Mishra, and Saurav Katuwal edited the manuscript; Bishnu P Regmi and Khaga Raj Sharma supervised the project. Keshav Ranabhat and Kamal Prasad Regmi contributed equally.

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