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Phytochemical Analysis, Antioxidant and Antibacterial Activities of Some Medicinal Plants Growing in Nepal

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Abstract

Plants used in traditional medicines are the best options for the discovery, development, and delivery of new drugs. Hence the present study was conducted to evaluate the antioxidant and antibacterial activities, chemical screening, and estimation of phenolics, flavonoids, tannins, and sugars in different extracts of *Ampelocissus daivaricata, Brassaiopsis hainla, Eranthemum pulchellum* and *Ficus cunia.* These plants are commonly used in Nepalese traditional medicine to treat various diseases. In screening tests, phenolics, flavonoids, tannins glycosides, and reducing sugars were present in polar extracts. The higher amounts of phenolics (236.95±2.42 mg GAE/g extract), flavonoids (181.03±2.26 mg CE/g extract), and tannins (195.75±1.82 mg TAE/g extract) were present in the ethyl acetate extract of *F. cunia*. The higher amounts of sugars were present in the methanol extract (324.56 ± 0.299 mg GE/g extract) of *A. daivaricata.* In DPPH free radical scavenging assay, the ethyl acetate (24.64±2.23 µg/mL) and methanol extract of *F. cunia* (24.71±1.67 µg/mL) showed similar IC_{50} values followed by the methanol extract of *A. daivaricata* (IC_{50}:25.99±0.94µg/mL) and ethyl acetate extract of *B. hainla* ($IC_{50:}$ 29.26±1.07 µg/mL). The extracts having higher amounts of phenolics and flavonoids showed greater antioxidant activities. In the antimicrobial assay, all the extracts showed a weak and narrow spectrum of activities.Some of the extracts of *A. daivaricata, B. hainla,* and *F. Cunia* showed activity against gram-positive bacteria. Only a few extracts were active against gram-negative bacteria. The extracts of *B. hainla* and *F. cunia* showed activity against *C. albicans*. The findings of this study support to some extent, the traditional use of these plants to treat microbial infections. These plants could be the source of new drugs to manage oxidative stress.

Keywords: Antibacterial; Antioxidant; Phytochemicals; Plants; Traditional medicine.

Introduction

Nepal is rich in various floras of medicinal value. Traditional medicine is still very popular in the rural areas of Nepal, where only limited modern health facilities are available and people still rely on local medicinal plants to satisfy their primary healthcare needs. Overall, it is expected that about 75% of the rural Nepalese population and about 75–80% of the world's population still rely on herbal medicines derived from medicinal plants for their primary health care [1,2]. The World Health Organization has been promoting traditional medicines as a source of less expensive, comprehensive medical care, especially in developing countries as they play an important role in the primary health care

systems. Traditional medicine usually involves biological resources and the knowledge of the local healers regarding their medicinal use [3].

Traditional medicines are the best options for the discovery, development, and delivery of new drugs with enhanced performance, and still, many modern drugs are derived from natural products [4]. Natural products are not only considered direct sources of new pharmaceuticals but also provide unlimited opportunities for new drug leads because of their unmatched chemical diversity, structural complexity, and biological potency [5]. Plant-derived phytochemicals exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites. The production of these phytochemicals depends on the environmental conditions where they grow as well as genetics factors [6]. This chemical diversity, arising from the distribution of plant resources in different eco-geographic regions, has a direct impact on biological activities, which is very interesting to investigate [7].

In this study, we have selected four medicinal plants *A. daivaricata, B. hainla, E. pulchellum,* and *F. cunia. A. daivaricata* (Wall. ex M.A.Lawson) Planch, belongs to Vitaceae family. Traditionally, the root juice is used against snake or scorpion bites and root paste is applied externally on infected areas of skin [8,9]. *B. hainla* (Buch. -Ham. ex D. Don) Seem, which belongs to Araliaceae family. Roots are administered orally in dysentery [10]. *E. pulchellum* Andrews, belongs to Acanthaceae family. Extracts of leaf, stem, and root of this plant are utilized as antimicrobial and antiseptic agents [11]. *F. cinia* Buch. -Ham. ex Sm. (Synonym: *F. semicordata*) belongs to family Moraceae. The fruit, root, and leaf are used to cure constipation and indigestion. Roots are also used to prevent menstrual disorders, leaf decoction is taken orally to get relief from jaundice, leaf juice is applied externally for curing scabies, treating stomach disorders, wounds, leprosy, indigestion, liver disease, and skin diseases [12-15].

A comprehensive literature review revealed that *A. daivaricata* has not been investigated phytochemically and pharmacologically. Anthelmintic, thrombolytic, cytotoxic, anti-diarrheal, and antipyretic properties of *B. hainla* have been reported [16]. The antibacterial activity of silver nano particles of *E. pulchellum* has been reported and a new iridoid glucoside, eranthemoside has been isolated [17, 18]. Total phenolic, flavonoid, and tannin content as well as antioxidant, anticoagulant, antifungal, and hepatoprotective activities of *F. semicordata* have been reported [19-24]. In this paper, we aim to bridge the traditional medicinal uses of these plants with the scientific findings. Therefore, we evaluate the total phenolic, flavonoid, tannin, sugar content, and antioxidant and antibacterial activities of *A. daivaricata, B. hainla, E. pulchellum and F. cunia* that are often used in traditional medicine in Nepal.

Materials and Methods

Chemicals

All the solvents and chemicals used were of analytical grade. Gallic acid was purchased from Merck, Dramstadt, Germany. (±)-catechin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Company, USA. Tannic acid, D-glucose, and Anthrone were purchased from THOMAS BAKER, Mumbai. The chemicals like Folin–Ciocalteu phenol reagent (FCR), Aluminium chloride ($AlCl_3$), Sodium carbonate (Na_2CO_3), Sodium nitrite ($NaNO_2$), Sodium hydroxide (NaOH), Ferric chloride ($FeCl_3$) and solvents like n-hexane, dichloromethane, ethyl acetate, methanol, acetone were purchased from Thermo Fisher Scientific India, Pvt. Ltd. Muller Hinton Agar (MHA) was from Himedia Laboratories Company Ltd., India. Ciprofloxacin and Amphotericin were from Sigma-Aldrich Co. Ltd. Double distilled water

was used throughout the experiments.

Plant materials

The leaves of four different plants were collected from different eco-geographical regions in October-November 2018. The voucher specimens were deposited at RECAST. The collected materials were washed, shade dried and finally crushed to powder for further use. The names of collected plants, collection sites, vernacular names, and voucher number are presented in **Table 1**.

Table 1. Collected plants, collection sites, vernacular names, and voucher number.

Plants	Collection sites	Vernacular name	Voucher no.
A. daivaricata	Sunkhuwasabha	Pureni	AD-2018-JK
B. hainla	Dhading	Hati paile	BH-2018-MB
F. cunea	Dhading	Khanyo	FC-2018-MB
E. pulchellum	Nawalpur	Arklejhaar	EP-2018-AP

Extraction

The collected dried and powdered leaves of *A. daivaricata* (30 g), *B. hainla* (54 g), *E. pulchellum* (100 g), and *F. cunia* (78 g) were extracted successively with hexane (400 mL), dichloromethane (400 mL), ethyl acetate (300 mL) and methanol (300 mL) in a Soxhlet extractor for 6-7 hours until the last extract became colorless. The variable amount of dry leaves extracts used attributes to the number of samples collected. The remaining residue after extraction with methanol was refluxed with 50% aqueous methanol (200 mL) for 2 hours then allowed to cool and filtered. The respective extracts were concentrated under a rotary evaporator (BUCHI R-200, BUCHI V-800) at reduced pressure to obtain solid or semi-solid extracts. The dried extracts were kept in a fridge at -20 °C for further analysis.

Screening of Extracts for Phytochemicals

The extracts were screened for the presence of different classes of phytochemicals such as alkaloids, terpenoids, phenolics, flavonoids, tannins, saponins, and glycosides following the standard procedure described by Culie (1982) [25].

Estimation of total phenolic content

The total phenolic content in different extracts was estimated by using the Folin-Ciocalteu (FC) reagent as described by Waterhouse (2002) [26]. The calibration curve was constructed using gallic acid. Various concentrations of gallic acid solutions were prepared (10, 25, 50, 75 and 100 µg/mL). In a 20 mL test tube, 1 mL gallic acid solution of each concentration was added and then 5 mL 10 % FC reagent and 4 mL 7% sodium carbonate solution were added. The blue colored mixture was shaken well and incubated for 30 minutes at 40 °C in a water bath. Then, the absorbance was measured at 760 nm against a blank using Chemito UV-VIS spectrophotometer. Similarly, various concentrations of the extracts (100, 50, 25, and 12.5 µg/mL) were prepared. Following the procedure applied for gallic acid, absorbance for each concentration of the extract was recorded.

Estimation of total flavonoid content

The total flavonoid content in different extracts was estimated by using an aluminum-chloride reagent [27]. The calibration curve was constructed using (±)-catechin. Various concentrations of catechin solutions (10, 25, 50, 75 and 100 µg/mL) were prepared. In a 20 mL test tube, 1 mL of catechin solution of each concentration, 6.4 mL of double distilled water, and 0.3 mL of 5 % $NaNO_2$ solution were added. After 5 minutes, 0.3 mL of 10% $AlCl_3$ solution was added and waited for 1 minute. Then 2 mL of 1 M NaOH was added with shaking. The absorbance of the pink mixture was determined at 510 nm. Similarly, various concentrations of the extracts (100, 50, 25, and 12.5µg/mL) were prepared. Following the procedure applied to catechin, absorbance for each concentration of

the extract was recorded.

Estimation of total tannin content

The total tannin content in different extracts was estimated by using Folin-Ciocalteu method [28]. The calibration curve was constructed using tannic acid. Various concentrations of tannic acid solutions (10, 25, 50, 75, and 100 µg/mL) were prepared. In a 20 mL test tube, 1 mL tannic acid solution of each concentration was mixed with 8.4 mL of double distilled water, 0.5 mL of FC reagent (10% v/v), and 0.1 mL of sodium carbonate solution (7% w/v). The mixture was then shaken well and allowed to stand for 30 minutes and then absorbance was taken at 700 nm. Similarly, various concentrations of the extracts (100, 50, 25, and 12.5µg/mL) were prepared. Following the procedure applied to tannic acid, absorbance for each concentration of the extract was recorded.

Estimation of total sugar content

The total carbohydrate/sugar content in different extracts was estimated by using anthrone reagent [29]. Various concentrations of D-glucose (10, 25, 50, and 75 µg/mL) were prepared. An aliquot of 2 mL glucose of each concentration and 8 mL of freshly prepared anthrone reagent (200 mg of anthrone in 100 mL of ice-cold 95 % H_2SO_4) were mixed in a 15 mL test tube. The mixture was shaken well and heated for 8 minutes in a boiling water bath. Then cooled rapidly and the absorbance of the green color solution was measured at 630 nm against a blank containing all reagents except sugar. The amount of 100 mg of each extract was dissolved in methanol followed by the addition of 5 mL of 2.5 N HCl and subjected to hydrolysis by keeping it in a boiling water bath for 3 hrs. It was then cooled and neutralized with solid sodium carbonate, made the volume to 100 mL (1 mg/mL) and centrifuged. Serial dilution of supernatant was carried out to get the concentration of 100, 200, 400, and 600 µg/mL. Following the procedure applied to D-glucose, the absorbance of each concentration of the extract was measured at 630 nm against blank.

Calculation

The total phenolic, flavonoid, tannin, and sugar contents were calculated in all the extracts separately using the equation C = c V/m (1), Where, C = total content of phenolic/flavonoid/tannin/sugar compounds (mg/g), c = concentration of gallic acid/(±)-catechin/tannic acid/D-glucose established from the calibration curve in mg/mL, V = volume of extract (mL) and m = weight of plant extract.

Determination of antioxidant activity

The antioxidant activity of the extracts and ascorbic acid was determined using the DPPH free radical [30]. DPPH solution (0.10 mM), plant extracts and ascorbic acid solutions of different concentrations (5, 10, 20, 30, 40, 60, 80, and 100 µg/mL) were prepared in methanol. To 0.5 mL of ascorbic acid or extract, 2.5 mL of DPPH solution was added with shaking and was kept in the dark for 30 min. Then absorbance was measured at 517 nm. A control was prepared by adding 0.5 mL methanol instead of ascorbic acid or extract. The percentage of DPPH radical scavenging activity was calculated using the formula,

$$\% \text{ of radical scavenging } = \frac{Ac-As}{Ac} \times 100 \ldots\ldots \text{ (I)}$$

where, *Ac* is the absorbance of the control, *As* is the absorbance of the solution. IC_{50}value was calculated from the plotted graph of radical scavenging percentage against the concentration of ascorbic acid or extracts.

Determination of antibacterial and antifungal activities

The antimicrobial activities of the extracts

were evaluated by agar well diffusion method against one Gram-positive bacterium *S. aureus* (ATCC 25923), three Gram-negative bacteria, *S. typhi* (ATCC 14028), *E. coli* (ATCC25922), *S. sonnei* (ATCC25931) and a yeast species, *C. albicans* [31]. Mueller–Hinton agar was used for both bacteria and yeast. The extracts were prepared at a concentration of 100 mg/mL in 50% DMSO. Then 50 µL of the prepared extract was introduced into the agar well of 6 mm diameter seeded with the respective microorganisms. Negative control experiments were performed using an equivalent volume of 50% DMSO and positive control experiments were performed using a standard antibiotic, ciprofloxacin for bacteria and amphotericin for yeast. The plates were kept in the refrigerator at 4 °C for 4 hours. For bacteria, the plates were incubated overnight at 37 °C and for *C. albicans,* the plates were incubated at 28 °C for 48 hours in an inverted position. At the end of the incubation period, the clear inhibition zone of bacterial growth was observed around each well in the presence of different extracts/standard drugs that were measured.

Statistical analysis

For total phenolic, flavonoid, tannin, sugar content, and antioxidant activity determination, absorbance data were recorded as a mean of three determinations for different concentrations. The total content of phenolic, flavonoid, tannin, sugar, and IC_{50} values in DPPH assay was calculated from the regression equation of the calibration curve, y=mx+c, where y is the absorbance of extract, m is the slope from the calibration curve, x is the concentration of extract, and c is the intercept. The linear correlation coefficient (R^2) values were also calculated. From the calculated values of concentration of each extract, the total phenolic, flavonoid, tannin, and sugar content were calculated. All the data were presented as a mean±SD. The mean values were compared using one-way ANOVA. In the case where the results were statistically different ($p<0.05$), Tukey-Kramer multiple comparison test was performed using Microsoft Excel 2016.

Results and Discussion

Extractive values in different solvents

The extraction yield was found to be increased with solvent polarities. The highest amounts of extracts were obtained with methanol. This indicated that all the plants contain greater amounts of polar chemicals than the non-polar or less polar chemicals. The highest and the lowest amounts of extracts were obtained from the methanol (12.77%) and dichloromethane (0.93%) extracts of *F. cunia* respectively. The results are presented in **Table 2.**

Table 2. Percentage yields of various extracts.

Plants	Percentage Yield				
	Hexane	DCM	Ethyl acetate	Methanol	50% Methanol
A. daivaricata	2.80	1.57	3.99	10.96	6.33
B. hainla	1.53	2.60	2.33	4.27	7.73
E. pulchellum	1.79	1.91	1.24	2.36	2.14
F. cunia	1.16	0.93	4.19	12.77	2.94

Phytochemical screening

In phytochemical screening, most of the phytochemicals namely, polyphenols, flavonoids, tannins, terpenoids, alkaloids, saponins, quinones, glycosides, and reducing sugars were absent in hexane and dichloromethane extracts of all plants. Phenolics, flavonoids, and gallotannins were present in ethyl acetate, methanol, and 50% aqueous methanol extracts of all plants. Glycosides and reducing sugars were present in the methanol and 50% aqueous methanol extracts of all plants. Based on these phytochemical results, total phenolic, flavonoid, hydrolysable tannin and sugar content were estimated only for ethyl acetate, methanol and 50% aqueous methanol extracts of all plants.

Total phenolic content

The total phenolic content in different extracts was calculated from the calibration curve using regression equation $y = 0.0115x + 0.123$ and $R^2 = 0.9942$ and expressed as mg gallic acid equivalent (GAE) per gram of extract in dry weight. The results showed that higher amounts of phenolics were present in the ethyl acetate (236.95±2.42 mg GAE/g extract) and methanol extracts of (180.38±1.98 mg GAE/g extract) of *F. cunia*. Higher amounts of phenolics were also present in the methanol (179.8±0.469 mg GAE/g) and 50% aq. methanol extracts (179.3±0.391 mg GAE/g extract) of *A. daivaricata.*Other extracts contained relatively lower amounts of phenolics. The results are presented in Table 3. The literature data for total phenolic content for other plants was inaccessible except *F.semicordata*. The total phenolic content of ethyl acetate (34.65±0.11 mg GAE/g) and methanol (45.68±0.55 mg GAE/g) extracts of leaves collected from Andra Pradesh and total phenolic content in terms of catechol equivalent (38.05± 3.896 mg CE/g) in macerated leaf extract collected from Gujrat has been reported [20-22].The reported values were very low in comparison to our results.

Total flavonoid content

The total flavonoid content in different extracts was calculated from the calibration curve using regression equation Y= 0.0029x, R^2 = 0.9997, and expressed as mg catechin equivalent (CE) per gram of extract in dry weight. The results showed that higher amounts of flavonoids were present in the ethyl acetate (181.03±2.26 mg CE/g extract) and methanol extracts (103.87±2.88 mg CE/g extract) of *F. cunia*. The results are also presented in Table 3. The total flavonoid content of *F. semicordata* (296.16± 55.12 mg QE/g extract) reported was high in comparison to our results [22]. In our findings, the order of flavonoid content in *F. cunia* extracts was ethyl acetate (181.03±2.26 mg CE/g)>methanol (103.87±2.88 mg CE/g)>50% aq. methanol (43.19±2.79 mg CE/g). In other plants, the flavonoid content varies from (150.39 ± 0.28 mg CE/g) for methanol extract of *A. daivaricata* to (10.31±2.15 mg CE/g) for ethyl acetate extract of *E. pulchellum*. The variation in the content of phytochemicals depends on several factors. However, one of the important factors could be the standard compound used.

Total hydrolysable tannin content

The total tannin content in different extracts was calculated from the calibration curve using regression equation $y = 0.0037x + 0.0082$ and $R^2 = 0.9960$ and expressed as mg tannic acid equivalents (TAE) per gram of extract in dry weight. The results showed that higher amounts of tannins were present in the ethyl acetate (195.75±1.82 mg TAE/g extract) and methanol (122.99±2.24 mg TAE/g extract) extracts of *F. cunia*. Similarly, higher amounts of tannins were present in the ethyl acetate (106.72 ± 0.2075 mg TAE/g extract) and 50% aqueous methanol extracts (123.1 ± 0.393 mg TAE/g extract) of *A. daivaricata*. Other extracts contained relatively lower amounts of tannins. The results are presented in Table 3. In the leaf extract of *F. semicordata,* total tannin content has been reported (587.00± 65.69) which was very high in comparison to our results [22]. In other plants, tannin content varies from (123.1 ± 0.39 mg TAE/g) in 50% aq. methanol extract of *A. daivaricata* to (19.14±0.81 mg TAE/g) in methanol extract of *E. pulchellum*. In addition to other factors, generally, the content varies with the method applied for quantification. Gandhi et al. (2019) used Prussian blue colorimetric method to estimate the total tannin content, and we have used the FC method [22,39].

Total sugar content

The total sugar content in different extracts was calculated from the calibration curve using regression equation Y = 0.0085x, R^2 = 0.9983 and expressed as mg glucose equivalents (GE) per gram of dry extract. The results showed that higher amounts of sugars were present in the methanol (324.56 ± 0.299 mg GE/g extract) extract of *A. daivaricata*. Other extracts contained relatively lower amounts of sugars. The results are presented in **Fig. 1**. This indicated that the plant included in this study could be the source of bioactive polysaccharides. However, reports regarding sugar content in these plants were not available to date.

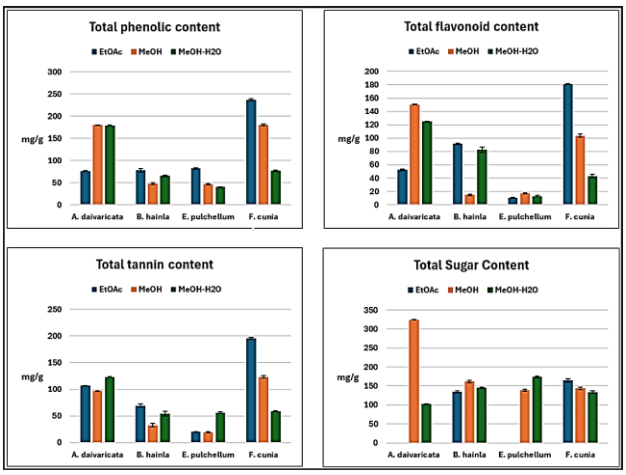

Fig. 1. Total phenolics, flavonoids, tannins, and sugar content of plant extracts.

DPPH antioxidant activity

In DPPH assay, all the tested extracts showed low IC_{50} values. However, the lowest IC_{50} values were shown by the ethyl acetate (24.64±2.23 µg/mL) and methanol extract (24.71±1.67 µg/mL) of *F. cunia*. In addition, the methanol extract of *A. daivaricata* (25.99±0.94 µg/mL), and ethyl acetate extract of *B. hainla* (29.26±1.07 µg/mL) showed low IC_{50} values. The results are presented in **Table 3**. Generally, the extracts having higher amounts of phenolics and flavonoids showed greater antioxidant activity. The results of this investigation indicated that there is a direct correlation between phenolics, flavonoid content, and antioxidant activity [40]. The antioxidative potential of these plant extracts indicated that the consumption of these plants may help in the management of oxidative stress to some extent. But they were not as potent as ascorbic acid. The literature data regarding the antioxidant activity of these plants were not available to date. This is the first report on the antioxidant potential of these plants.

Table 3. Antioxidant activities of plant extracts.

Plants	DPPH Assay IC_{50} (µg/mL)±SD (n=3)			
	EtOAc	MeOH	MeOH-H_2O	Ascorbic acid (standard)
A. daivaricata	37.44±1.04*	25.99±0.94*	33.04±0.25*	
B. hainla	29.26±1.07*	44.98±1.26*	>100	
E. pulchellum	36.76±1.27*	>100	46.86±1.55*	11.56±0.53
F. cunia	24.64±2.23*	24.71±1.67*	>100	

Mean values ± SD (*p< 0.05 against positive control, ascorbic acid).

Antimicrobial activity

In antimicrobial assay, some of the extracts *A. daivaricata, B. hainla,* and *F. cunia* were active against gram-positive bacteria, *S. aureus* with an inhibition zone ranging between 7-12 mm. Only a few extracts were active against gram-negative bacteria with inhibition zones ranging between 8-12 mm. All extracts were found to be inactive against *E. coli*, only the dichloromethane extract of *B. hainla* was active against *S. typhi.* Dichloromethane extract of *A. daivaricata* and methanol extract of *E. pulchellum* were active against *K. pneumonia.* Only the extracts of *B. hainla* and *F. cunia* showed activity against *C. albicans* with an inhibition zone ranging between 7-10 mm. The results are presented in **Table 4**. Gandhi et al. (2019) also reported the antifungal activity of methanol extract of the leaf of *F. semicordata* against *C. albicans* with an inhibition zone of 11 mm while our extract showed an inhibition zone of 8 mm. The greater activity of *F. semicordata* against *C. albicans* could be due to the presence

of greater amounts of flavonoids [22].

Table4. Antimicrobial screening of different extracts.

Plant extracts	Plants	Inhibition zone produced in mm against bacteria				
		S. aureus	*K. pneumonia*	*S. typhi*	*E. coli*	*C. albicans*
Hexane	*A. daivaricata*	7	-	-	-	-
	B. hainla	-	-	-	-	-
	E. pulchellum	-	-	-	-	-
	F. cunia	-	-	-	-	-
Dichlorometh ane	*A. daivaricata*	-	8	-	-	-
	B. hainla	10	-	11	-	-
	E. pulchellum	-	-	-	-	-
	F. cunia	9	-	-	-	-
Ethyl acetate	*A. daivaricata*	8	-	-	-	-
	B. hainla	10	-	-	-	10
	E. pulchellum	-	-	-	-	-
	F. cunia	10	-	-	-	7
Methanol	*A. daivaricata*	8	-	-	-	-
	B. hainla	-	-	-	-	10
	E. pulchellum	-	12	-	-	-
	F. cunia	12	-	-	-	8
50% aq. methanol	*A. daivaricata*	-	-	-	-	-
	B. hainla	-	-	-	-	-
	E. pulchellum	-	-	-	-	-
	F. cunia	-	-	-	-	-
Ciprofloxacin 10 µg/well	-	34	34	34	44	0
Amphotericin 10 µg/well	-	-	-	-	-	24

Zone of inhibition produced in mm including 6 mm well.

Plant extracts are a complex mixture of various phytochemicals, they show synergistic as well as antagonistic effects [41, 42]. The weak activity of our extracts could be due to the antagonistic effect where some phytochemicals reduce the effect of others. So, it is necessary to isolate and characterize the bioactive compounds. The findings of this study support to some extent, the traditional use of these plants to treat stomach disorders due to bacterial infections, skin rashes, and scabies due to fungal infections.

Conclusions

The present study highlights the total content of phenolics, flavonoids, tannins, and sugars as well as antioxidant and antibacterial properties of leaf extracts of four medicinal plants A. *daivaricata, B. hainla, E. pulchellum,* and *F. cunia.* The finding of this study revealed that all plants are the sources of phenolics, flavonoids, tannins, and sugars. However, the extracts of *A. daivaricata* and *F. cunia* are rich sources of these compounds. In DPPH free radical scavenging assay, all the tested extracts showed radical scavenging potencies. The ethyl acetate and methanol extract of *F. cunia,* methanol extract of *A. daivaricata,* and ethyl acetate extract of *B. hainla* showed low IC_{50} values. These extracts contain higher amounts of phenolics and flavonoids. Thus, these plants could be the source of antioxidants to manage oxidative stress-induced health disorders. However, in statistical analysis, using one-way ANOVA followed by Tukey test, there were significant differences in IC_{50} values between the different extracts and standard ascorbic acid. In an antimicrobial assay, all the extracts showed a very weak and narrow spectrum of activity. Some extracts of *A. daivaricata, B. hainla,* and *F. cunia* were active against gram-positive bacteria. Only a few extracts of *A. daivaricata, B. hainla,* and *E. pulchellum* were active against gram-negative bacteria. Only ethyl acetate and methanol extracts of *B. hainla* and *F. cunia* showed activity against *C. albicans*. The weak activity of our extracts could be due to the antagonistic effect. The findings of this study support to some extent, the traditional uses of these plants. In conclusion, the under-utilized natural resources of Nepal can be used for the formulation of dietary supplements, nutraceuticals, or herbal medications. Toxicological study is also crucial before the formulation of herbal drugs. The chemical profile and biological properties of these plants are still lacking and need to be explored. Plant-derived drugs could be developed by scientific follow-up of well-known plants used in ethno-medicine.

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assay.

Author's Contribution Statement

Manish Baskota: Plant collection, Methodology, Data analysis, Writing: original manuscript, review and editing, **Ananda Pokhrel:** Plant collection, Methodology, Writing: review and editing **Janak Khatri:** Plant collection, Methodology, Writing: review and editing **Prakash Rimal:** Plant collection, Methodology, Writing: review and editing**, Meena Rajbhandari:** Conceptualization, Supervision, Data analysis, and Writing: review and editing

Conflict of Interest

The authors do not have any conflict of interest throughout this research work.

Data Availability Statement

The data supporting this study's findings are available from the corresponding authors upon reasonable request.

References

1. G. Ambu, R. P. Chaudhary, M. Mariotti, and L. Cornara.Traditional Uses of Medicinal Plants by Ethnic People in the Kavrepalanchok District, Central Nepal. *Plants*, 2020, 9, 759. (DOI: https://doi.org/10.3390/plants9060759)
2. D. Karki, D. Khadka, R. M. Kunwar, P. C. Aryal, H. Paudel, S. Bhatta, and S. Shi. Ethnomedicinal plants in Champadevi rural municipality, Okhaldhunga district, Nepal. *Journal of Ethnobiology and Ethnomedicine*, 2023,19(1), 58. (DOI: https://doi.org/10.1186/s13002-023-00627-y)
3. D. D. Soejarto. Biodiversity prospecting and benefit-sharing: perspectives from the field. *Journal of Ethnopharmacology*, 1996, 51(1-3), 1-15. (DOI: https://doi.org/10.1016/0378-8741(95)01345-8)
4. B. Patwardhan and R. A. Mashelkar.Traditional medicine-inspired approaches to drug discovery: can Ayurveda show the way forward? *Drug Discovery Today*, 2009, 14(15-16), 804-811.(DOI:https://doi.org/10.1016/j.drudis.2009 .05.009)
5. G. A. Cordel and M. D. Colvard. Natural products and traditional medicine: turning on a paradigm. *J Nat Prod.*, 2012, *75*(3), 514-525. (DOI:https://doi.org/10.1021/np200803m)
6. Galm, U. and Shen, B., Natural product drug discovery: The times have never been better. *Chemistry and Biology*, 2007, 14(10), 1098-1104. (DOI: https://doi.org/10.1016/j.chembiol.2007.10.004)
7. V. E. Tyler, Phytomedicines: back to the future, *Journal of Natural Products,*1999, 62(11), 1589-1592. (DOI:https://doi.org/10.1021/np9904049)
8. N. P. Manandhar. Ethnobotanical note on folklore remedies of Baglung district Nepal, Contributions *to Nepalese Studies*, 1993, 20(2), 183-196.
9. A. G. Singh and J. P. Hamal. Traditional phytotherapy of some medicinal plants used by Tharu and Magar communities of western Nepal, against dermatological disorders, *Scientific World*, 2013, 11(11), 81-89.
10. H. K. Badola and B. K. Pradhan. Plants used in healthcare practices by Limboo tribe in South-West of Khangchendzonga Biosphere Reserve, Sikkim, Indian *Journal of Traditional Knowledge,* 2013, 12, 355–69.
11. J. Sharma, R. D. Gaur, S. G. Gairola, R. M. Painuli, and T. O. Siddiqi. Traditional herbal medicines used for the treatment of skin disorders by the Gujjar tribe of Sub-Himalayan tract, Uttarakhand, Indian *Journal of Traditional Knowledge,*2013, 736-746.
12. R. M. Kunwar and R. W. Bussmann. Ficus (Fig) species in Nepal: a review of diversity and indigenous uses, *Lyonia*, 2006, 11(1), 85-97.
13. R. S. Rama Shankar, M. S. Rawat, S. Deb, and B. K. Sharma. Jaundice and its traditional cure in Arunachal Pradesh, *Journal of Pharmaceutical and Scientific Innovation*, 2012, 1(3), 93-97.

14. S. Gupta, and R. Acharya. Antioxidant and nutritional evaluation of BhuUdumbara (Ficus semicordataBuch. -Ham. ex Sm.) leaves and fruits: An extra pharmacopoeial drug of Ayurveda, AYU (Int Quarterly Journal of Research in Ayurveda), 2019, 40(2), 120-126. (DOI: 10.4103/ayu.AYU_289_18)
15. S. Gupta, R. N. Acharya, C. R. Harisha, V. J. Shukla,.and S. A. Hegde. Detailed pharmacognostical, phytochemical screening and DNA barcoding of leaves of Ficus semicordata Buch. -ham. ex Sm. (Bhumi Udumbara)—An extra pharmacopoeial drug of Ayurveda, *International Journal of Pharmacy Research*, 2020, 12(3),1123-31.(DOI: https://doi.org/10.31838)
16. S. A. Zishan, M. M. Uddin, M. Mohammad, M. E. Uddin, S. M. Azad, J. Naima,and S. S. Ibban.In vivo and In vitro Initiatives for the Examination of Pharmacological Properties of Brassaiopsis hainla Leaves, *Asian Journal of Research in Botany* 2023, 9(1), 15-28.
17. H. Fischer, W. Jensen, S. R. Jensen and B. J Nielsen. Eranthemoside, a new iridoid glucoside from Eranthemum pulchellum (Acanthaceae). *Phytochemistry*, 1987, 26(12), 3353-3354.
18. J. Prakash, H. Shekhar, S. R. Yadav, A. K. Dwivedy, V. K. Patel, S. Tiwari, and N. K. Vishwakarma. Green synthesis of silver nanoparticles using Eranthemum Pulchellum (Blue Sage) aqueous leaves extract: Characterization, evaluation of antifungal and antioxidant properties. *Biomedical and Biotechnology Research Journal (BBRJ)*, 2021, 5(2), 222-228.
19. N. Dhami. Ethnomedicinal uses of plants in Western Terai of Nepal: a case study of Dekhatbhuli Vdc of Kanchanpur District, *Medicinal plants in Nepal: an anthology of contemporary research*, 2008, 165-177.
20. D. N. Prasad, B. G. Rao, E. Sambasivarao, T. Mallikarjunarao and V. S. D Praneeth. Quantification of phytochemical constituents and in-vitro antioxidant activity of Ficus semicordata leaves extracts, *International Journal of Pharmacy and Pharmceutical Science,* 2012, 4, 619-622.
21. S. Ambreen, M. Tariq, M. S. Masoud, I. Ali, M. Qasim, A. Mushtaq and R. Asghar. Anticoagulant potential and total phenolic content of six species of the genus Ficus from Azad Kashmir, Pakistan. *Tropical Journal of Pharmaceutical Research* 2019,18(6), 1245-1251. (DOI: https://doi.org/10.4314/tjpr.v18i6.14)
22. A. J. Gandhi, V. J. Shukla, and R. N. Acharya. Qualitative, quantitative screening and antifungal study of *Ficus semicordata* Buch. -HAM. Ex Sm., *International Journal of Botanical Study* 2019, 4(6), 82-85.
23. S. Gupta. Ethnomedicinal claims of *Ficus semicordata* Buch. -Ham. ex Sm.: A review. *International Journal of Green Pharmacy,* 2018, 12(01). (DOI: https://doi.org/10.22377/ijgp.v12i01.1621)
24. S. Gupta, A. Ranade, S. Gayakwad, R. Acharya, and S. Pawar. Hepatoprotective activity of Ficus semicordata Buch. -Ham. ex Sm. leaves aqueous extract on D-galactosamine induced toxicity in HepG2 cell line, *Indian Journal of Natural Products and Resources*, 11(4), 239-243. (DOI: http://op.niscpr.res.in/index.php/IJNPR/article/view/27819/0)
25. I. Ciulei. Methodology for analysis of vegetable drugs, practical manuals on industrial utilization of medicinal and aromatic plant, Phytochemistry, 1982, 63(1), 97.
26. A. L. Waterhouse. Determination of total phenolics. *Current Protocols in Food Analytical Chemistry*, 2002, 6 (1), I1-1.
27. D. W. Barnum. Spectrophotometric determination of catechol, epinephrine, dopa, dopamine and other aromatic Vic-diols. *Analytical Chemistry*

Acta, 1977, 89(1), 157-166. (DOI: https://doi.org/10.1016/S0003-2670(01)83081-6)

28. R. Katoch. Methods for nutritional quality evaluation of food materials, *Analytical Techniques in Biochemistry and Molecular Biology*, 2011, 251-322. (DOI: https://doi.org/10.1007/978-1-4419-9785-2_13)
29. J. E. Hedge, B. T. Hofreiter and R. L. Whistler. Carbohydrate chemistry. *Academic Press, New York*, 1962,17, 371-80.
30. W. Brand-Williams. Use of a free radical method to evaluate antioxidant activity, *Food Science and Technology*, 1999, 28, 1231-1237.
31. S. Sapkota, A. Maharjan, S. Tiwari and M. Rajbhandari. Phytochemical Analysis, Antioxidant Potential and Antibacterial Activities of Different Anatomical Parts of *Hypericum cordifolium* Choisy, *The Scientific World Journal*, 2024, 1, 8128813. (DOI:https://doi.org/10.1155/2024/8128813)
32. N. R. Farnsworth, O. Akerele, A. S. Bingel, D. D. Soejarto and Z. Guo, Z. Medicinal plants in therapy, Bulletin *of the World Health Organization*, 1985,63(6), 965.
33. D. X. Kong, X. J. Li, and H.Y. Zhang. Where is the hope for drug discovery? Let history tell the future. *Drug Discovery Today*, 2009, 14(3-4), 115-119. (DOI: https://doi.org/10.1016/j.drudis.2008.07.002)
34. B. A. Graf, P.E. Milbury, and J.B. Blumberg. Flavanols, flavones, flavanones, and human health: epidemiological evidence, Journal *of Medicine and Food*, 2005, 8(3), 281-290. (DOI: https://doi.org/10.1089/jmf.2005.8.281)
35. L. J. Appel, T. J. Moore, and R. Obarzanek. A clinical trial of the effects of dietary patterns on blood pressure, New *England Journal of Medicine,* 1997, 336, 1117-1124. (DOI: https://doi.org/10.1056/NEJM199704173361601)
36. O. Weinreb, S. Mandel, T. Amit, and M. B. Youdim. Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases, *Journal of Nutritional Biochemistry,* 2004, 15(9), 506-516. (DOI: https://doi.org/10.1016/j.jnutbio.2004.05.002)
37. T. Okuda. Tannins, a new family of bio-active natural organic compounds (questions and answers), *Yakugakuzasshi: Journal of Pharmaceutical Society of Japan, 1995*, 115(2), 81-100. (DOI: https://doi.org/10.1248/yakushi1947.115.2_81)
38. K. T. Chung, T. Y. Wong, C. I. Wei, Y. W. Huang and Y. Lin, Y. Tannins and human health: a review, *Critical Review on Food Science and Nutrition,* 2010, 38(6), 421-464. (DOI: https://doi.org/10.1080/10408699891274273)
39. H. D. Graham. Stabilization of the Prussian blue color in the determination of polyphenols. *Journal of Agriculture and Food Chemistry*, 1992, 40(5), 801-805.
40. F. J. Pashkow. Oxidative stress and inflammation in heart disease: do antioxidants have a role in treatment and/or prevention? *International Journal of Inflammation*, 2011, 1, 514623. (DOI: https://doi.org/10.4061/2011/514623)
41. H. Brüssow. The antibiotic resistance crisis and the development of new antibiotics. *Microbial Biotechnology*, 2024, 17(7), e14510.
42. T. Efferth, and E. Koch. Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy. *Current drug targets*, 2011, 12(1), 122-132.