

ESTIMATION OF PHENOLIC CONTENT, FLAVONOID CONTENT, ANTIOXIDANT, AND ALPHA-AMYLASE INHIBITORY ACTIVITY OF SOME SELECTED PLANTS FROM SIRAHA DISTRICT NEPAL

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Received: 28 December 2019, Revised and Accepted: 25 January 2020

ABSTRACT

Objectives: The purpose of this research was to estimate the phenolic content, flavonoid content, antioxidant, antibacterial, and α -amylase inhibitory activity of some selected plants such as *Anethum sowa*, *Trigonella foenum-graecum*, *Lepidium sativum*, *Cuscuta reflexa*, *Eclipta alba*, *Leucas cephalotes*, and *Tinospora cordifolia* collected from Siraha district of Nepal using *in vitro* studies.

Methods: Methanol extracts of these medicinal plants were prepared by cold percolation method. Preliminary phytochemical screening was performed by color differentiation method. Total phenolic and flavonoid content were estimated by Folin-Ciocalteu reagent method and aluminum chloride colorimetric method. Antioxidant potential was evaluated by 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay. Furthermore, the α -amylase enzyme inhibitory activity was studied using starch as a substrate, pancreatic α -amylase as the enzyme, and acarbose as standard.

Results: Phytochemical screening showed that the plant extracts were found a rich source of secondary metabolites. The phenolic content estimation showed *T. foenum-graecum* 939.764 \pm 0.01, *L. sativum* 551.63 \pm 0.02, *A. sowa* 306.34 \pm 0.06, *L. cephalotes* 233.19 \pm 0.03, *T. cordifolia* 211.76 \pm 0.02, *E. alba* 202.67 \pm 0.02, and *C. reflexa* Roxb. 145.09 \pm 0.09 mg milligram gallic acid equivalent per gram. The flavonoid content estimation showed *T. cordifolia* 852.07 \pm 0.11, *L. sativum* 553.81 \pm 0.05, *E. alba* 322.13 \pm 0.02, *A. sowa* 329.02 \pm 0.05, *L. cephalotes* 164.93 \pm 0.02, and *C. reflexa* Roxb. 146.37 \pm 0.00 mg milligram quercetin equivalent per gram. The antioxidant potential showed by *E. alba* IC₅₀ 33.48 \pm 0.82 μ g/ml and the values ranged from *E. alba* 33.48 \pm 0.82 to *A. sowa* 47.62 \pm 1.09 μ g/ml. The α -amylase inhibitory activity showed by *A. sowa* 76.78 \pm 2.00–*E. alba* 777.36 \pm 9.66 μ g/ml. The result of brine shrimp toxicity showed LC₅₀ value >1000 μ g/ml. Among the seven plant extracts, only the plant extract of *E. alba* showed a zone of inhibition 14 mm against *Staphylococcus aureus*.

Conclusions: The plant extract of *T. foenum-graecum* showed the highest phenolic content, and *T. cordifolia* showed the highest flavonoid content. The highest antioxidant potential exhibited by *E. alba* and the highest α -amylase inhibition activity showed by *A. sowa*. The plant extract of *E. alba* showed moderate antibacterial activity against *S. aureus*. All plant extracts were found non-toxic against brine shrimp larvae although further study is needed to assess its mechanism of action.

Keywords: Antioxidant, α -Amylase, Gallic acid, Quercetin, Plant extract.

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INTRODUCTION

Nepal is rich in all the three levels of biodiversity, namely, species diversity, genetic diversity, and habitat diversity. In Nepal, the use of different parts of several medicinal plants to cure specific diseases has been in vogue since ancient times [1]. The use of herbal medicines in Asia represents a long history of human interaction with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infection diseases. Phytochemical from plants as safe and broadly effective alternatives with the less adverse effect is beneficial for biological activities such as anticancer, antimicrobial, antioxidant, analgesic, and wound healing [2,3]. Oxidation reaction can produce the free radicals, which starts chain reactions that damage the cells. Antioxidants are natural or synthetic substances that may prevent or delay oxidative cell damage [4]. Antioxidant plays the role on prevention of diabetes and other diseases too [5]. The antioxidant from natural sources neutralizes the radicals formed in the human body by oxidative stress. The plants secondary metabolites have pharmacological properties [6,7]. Today, numerous antimicrobial agents exist to treat a wide range of infections. The development of new anticancer and antimicrobial therapeutic agents from natural sources is one of the fundamental goals in medicinal chemistry [8].

Phenolic and flavonoid compounds are the main class of secondary metabolites in the plants and are divided into phenolic acids and polyphenols. The antioxidant activity of phenolic and flavonoid compounds is attributed to the capacity of scavenging free radicals, donating hydrogen atoms, electrons, or chelate metal cation [9]. Medicinal plants have been using since many years for the primary cure of different diseases in all most all countries of the world. Among the several diseases, diabetes is the seven leading causes of death. Hence, the search of potent natural compounds with high antioxidant potential is an urgent need. Due to the chronic hyperglycemic disturbances of carbohydrate, fat, and protein metabolism resulting from defect in insulin secretion, its action, and both causes diabetes mellitus [5]. The number of diabetes patients will be increased from 171 million from the year 2000 to 366 million or more by the year 2030 [5]. Mainly diabetes is of two types, Types 1 and II. Type 1 diabetes is due to insulin-deficient causes when pancreatic β -cell does not produce insulin, and Type II is insulin resistance. There are several factors of causing diabetes, although both genetic and environmental factors such as obesity, changing lifestyle eating habits, and lack of exercise play a significant role [5]. Long-term using oral hypoglycemic agents and insulin secretion agents lead to increase blood sugar level, drug resistance and affects the immune system of the body. To avoid such problems, the natural antidiabetic agents can be used for good management of diabetes [10]. The phytoconstituents reported to

to each of them and then again incubated for 15 min at 37°C. After incubation, the enzymatic reaction was quenched with 800 µl of HCl (0.1 M). Then, 1000 µl of iodine reagent was added, and the absorbance was measured at 630 nm. Then assay was carried out in triplicates. Percentage of enzyme inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = 1 - [\text{Abs2} - \text{Abs1} / \text{Abs4} - \text{Abs3}] \times 100$$

where, Abs1=Absorbance of incubated mixture containing plant extract, starch, and amylase, Abs2=Absorbance of incubated mixture containing plant extract and starch, Abs3=Absorbance of incubated mixture containing starch and α-amylase, Abs4=Absorbance of incubated solution containing starch only.

The standard graph was plotted by taking the concentration on the X-axis and percentage inhibition on the Y-axis. With the help of this graph, IC₅₀ value of each sample was calculated. The species having the lowest IC₅₀ is considered to have the best α-amylase inhibition property.

Antibacterial activity

Antibacterial activity of the plant extracts was performed by the agar well diffusion method. Effectiveness of antimicrobial substance was evaluated by determination of zone of inhibition (ZOI) by adopting the protocol Cavalieri *et al.* [16].

Brine shrimp bioassay

Brine shrimp, *Artemia salina* is a tiny crustacean. The eggs of brine shrimp are readily available at low cost, and they remain viable for years in the dry state. On being placed in a brine solution, the eggs hatch within 48 h providing large number of larvae. These larvae are used in this assay for toxicity test. This method is rapid, inexpensive, simple, and in-house approach for screening and monitoring physiologically active plant extracts. It determines the LC₅₀ value (S) (µg/ml) for the crude extract (s). Compounds/extracts having LC₅₀ values <1000 ppm (µg/ml) are considered as pharmacological active. The assay was carried out by adopting the standard protocol of Meyer *et al.* [17].

RESULTS AND DISCUSSION

Quantitative estimation showed that the plant extracts are the rich sources of secondary metabolites with high yield percentage. The results of the yield percentage are shown in Table 2.

Phytochemical screening

The results of the phytochemical analysis are shown in Table 3. Most of the plant extracts are found as the rich source of secondary metabolites such as alkaloids, coumarins, flavonoids, glycosides, polyphenols, flavonoids, quinones, reducing sugars, and saponins.

Antioxidant activity

The antioxidant activity of each plant extract was measured using DPPH free radical. DPPH is scavenged by antioxidants through the donation of proton, forming the reduced DPPH. DPPH solutions show a strong absorbance band at 517 nm, appearing as deep violet color. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm.

The DPPH radical assay was performed for each plant extract using ascorbic acid as standard according to the standard procedure. In this assay, the mixture of DPPH with different concentrations of extract solution and ascorbic acid was separately incubated at room temperature, and absorbance was recorded at 517 nm by a spectrophotometer. The comparison of percentage radical scavenging between different plant extracts and ascorbic acid as standard is shown in Fig. 1.

The antioxidant potential of the plants extracts is influenced by the various factors and largely depends on both the composition of the extract and the analytical test system. The number of the

plant constituents had been proven for the free radical scavenging or antioxidant activities, among the phenolic compounds are very important for the plant constituents. Total phenolic compounds of the plants and antioxidant activities have mutual relationships due to the presence of the hydroxyl group, which has the scavenging ability. By donating an electron, a phenolic compound can also scavenge the hydrogen peroxide and neutralize it to the water. The antioxidant potential of different plants was determined using DDPH assay and ascorbic acid as a standard compound (IC₅₀ 39.85±0.025µg/ml). Maximum antioxidant potential was found in *Eclipta alba* (IC₅₀ 33.48±0.823) and minimum in *Leucas cephalotes* (IC₅₀ 52.86±1.80). These results are shown in Table 4. Similarly, results found that all the extracts exhibited maximum percentage of free radical scavenging activity at a dose of 100 µg/ml are shown in Table 5 from the literature review it is found that the antioxidant potential radical scavenging of *Cuscuta reflexa* has 76.38 and *L. cephalotes* has 98.09±0.69 [18,19].

Antibacterial activity

The ZOI showed by plant extracts on particular bacteria was measured for the estimation of their antimicrobial activity. The methanolic extract of *Anethum sowa*, *Trigonella foenum-graecum*, *Lepidium sativum*, *C. reflexa*, *L. cephalotes*, and *Tinospora cordifolia* did not show any ZOI at 100 mg/ml. However, in the literature survey, it is found that the *L. sativum* shows the positive result [20]. The extract of *E. alba* was found to be resistant against *Escherichia coli*, whereas the same extract

Table 2: Percentage yield of different plant extracts

Plant sample	Quantity of sample (g)	Quantity of extract (g)	Percentage yield
<i>Anethum sowa</i>	80	11	13.75
<i>Trigonella foenum-graecum</i>	80	9	11.25
<i>Lepidium sativum</i>	80	12	15
<i>Cuscuta reflexa</i>	100	12	12
<i>Eclipta alba</i>	100	14	14
<i>Leucas cephalotes</i>	100	10	10
<i>Tinospora cordifolia</i>	120	15	12.5

Table 3: Phytochemical screening of methanol extract

Group of compounds	AB1	AB2	AB3	AB4	AB5	AB6	AB7
Alkaloid	+	+	+	+	+	-	+
Coumarins	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Glycosides	+	-	-	+	-	-	+
Polyphenols	-	+	-	+	+	+	+
Quinones	-	+	+	+	+	-	-
Reducing sugars	-	+	-	-	+	+	+
Saponins	+	-	+	+	-	-	+
Terpenoids	+	-	+	+	+	+	+

+: Presence and -: Absence, AB1: *Anethum sowa*, AB2: *T. foenum-graecum*, AB3: *Lepidium sativum*, AB4: *Cuscuta reflexa*, AB5: *Eclipta alba*, AB6: *Leucas cephalotes*, AB7: *Tinospora cordifolia*

Table 4: Comparison of IC₅₀ values of different plant extracts with standard ascorbic acid

Plant sample/standard	IC ₅₀ (µg/ml)
Ascorbic acid	39.85±0.025
<i>Anethum sowa</i>	47.62±1.095
<i>Trigonella foenum-graecum</i>	45.71±0.53
<i>Lepidium sativum</i>	41.88±2.38
<i>Cuscuta reflexa</i>	47.25±1.44
<i>Eclipta alba</i>	33.48±0.823
<i>Leucas cephalotes</i>	52.86±1.80
<i>Tinospora cordifolia</i>	36.08±3.024

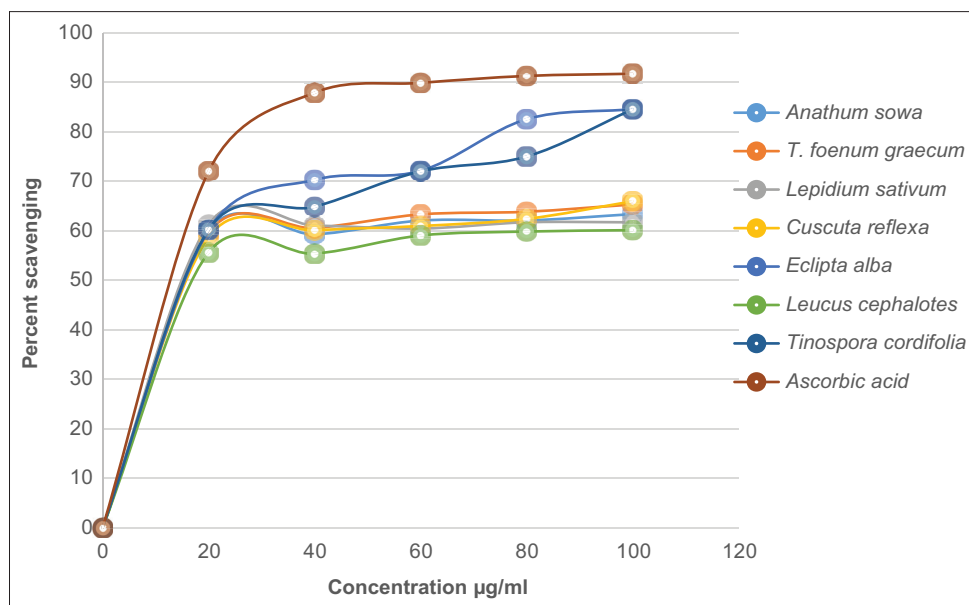


Fig. 1: Comparison of radical scavenging between ascorbic acid and plant extracts

Table 5: Percentage of radical scavenging with different concentration of plant extracts

Concentration (µg/ml)	Absorbance			Percent scavenging			Mean	Mean±standard deviation
	Abs1	Abs2	Abs3	S1	S2	S3		
<i>Anethum sowa</i>								
20	1.066	1.008	1.072	59.2507	61.46789	59.0214	59.9133	59.91±0.82
40	1.073	1.065	1.059	58.9831	59.28899	59.5183	59.2635	59.26±0.26
60	1.01	1.015	0.953	61.39144	61.20031	63.5703	62.0540	62.05±1.31
80	1.007	0.965	1.004	61.50612	63.1162	61.6208	62.0795	62.07±0.89
100	1.012	0.898	0.966	61.31498	65.6727	63.07339	63.35372	63.35±2.19
<i>Trigonella foenum-graecum</i>								
20	1.078	1.049	1.05	58.79205	59.9006	59.86239	59.5183	59.51±0.02
40	1.046	1.018	1.034	60.0152	61.0853	60.4740	60.5247	60.52±0.35
60	0.963	0.943	0.975	63.1880	63.952	62.7293	63.2900	63.29±0.7
80	0.932	0.92	0.986	64.3730	64.8318	62.3088	63.837	63.83±1.45
100	0.907	0.905	0.911	65.3287	65.4052	65.1785	65.30326	65.30±0.133
<i>Lepidium sativum</i>								
20	1.012	0.996	1.032	61.3149	61.9266	60.5504	61.24	61.24±1.12
40	1.007	1.074	0.972	61.50612	58.94495	62.84404	61.04	61.04±3.18
60	1.06	1.059	0.987	59.4801	59.5183	62.27064	60.44	60.44±2.24
80	1.049	0.93	1.026	59.90061	64.4495	60.77982	61.7	61.7±2.99
100	0.969	0.98	1.057	62.9587	62.5382	59.5948	61.69	61.69±2.4
<i>Cuscuta reflexa</i>								
20	1.114	1.065	1.065	57.415	59.2889	59.2889	58.66	58.66±1.08
40	1.016	1.101	1.015	61.16208	57.9128	61.20031	60.09	60.09±1.88
60	1.006	1.038	1.02	61.54434	60.3211	61.0091	60.95	60.95±0.613
80	0.927	1.023	1.001	64.56422	60.894	61.7354	62.398	62.39±1.92
100	0.943	0.868	0.862	63.9526	66.8195	67.04893	65.9403	65.94±1.72
<i>Eclipta alba</i>								
20	1.046	1.04	1.04	60.0152	60.2446	60.244	60.16	60.16±0.132
40	0.817	0.758	0.758	68.7691	71.0244	71.0244	70.27	70.27±1.3
60	0.708	0.741	0.741	72.935	71.6743	71.6743	72.09	72.09±0.72
80	0.485	0.443	0.443	81.46024	83.06575	83.06575	82.53	82.53±0.92
100	0.435	0.388	0.388	83.371	85.1682	85.168	84.56	84.56±1.032
<i>Leucas cephalotes</i>								
20	1.145	1.156	1.156	56.23089	55.8104	60.244	55.59	55.59±0.24
40	1.146	1.053	1.146	56.19266	59.7477	71.0244	55.37	55.37±2.05
60	1.143	1.036	1.037	56.30734	60.397	71.674	59.03	59.03±2.36
80	1.123	1.014	1.015	57.07187	61.238	83.0657	59.83	59.83±2.39
100	1.104	1.013	1.013	57.79817	61.2767	85.1678	60.11	60.11±2.008
<i>Tinospora cordifolia</i>								
20	1.145	1.07	0.906	56.23089	59.0976	65.3669	60.23	60.23±4.67
40	0.859	1.053	0.848	67.1636	59.7477	67.5841	64.83	64.83±4.40
60	0.711	0.772	0.715	72.8211	70.489	72.6682	71.99	71.99±1.30
80	0.7	0.558	0.705	73.24159	78.669	73.0504	74.98	74.98±3.19
100	0.601	0.057	0.558	77.02599	97.821	78.6697	84.50	84.50±1.56

was not resistant against *Staphylococcus aureus*. Results obtained from the antibacterial screening of different extracts are tabulated in Table 6.

The extract of *E. alba* showed high growth inhibition against *S. aureus*, whereas the rest of the plant extract were found inactive against these microorganisms.

Table 6: Antimicrobial activity of plant extracts

Plant sample (extracts)	Bacteria	ZOI (mm) of extracts at 100 mg/ml	ZOI (mm) of chloramphenicol as control at 100 mg/ml
<i>Anethum sowa</i>	<i>E. coli</i>	-	14
	<i>S. aureus</i>	-	18
<i>Trigonella foenum-graecum</i>	<i>E. coli</i>	-	14
	<i>S. aureus</i>	-	18
<i>Lepidium sativum</i>	<i>E. coli</i>	-	14
	<i>S. aureus</i>	-	18
<i>Cuscuta reflexa</i>	<i>E. coli</i>	-	14
	<i>S. aureus</i>	-	18
<i>Eclipta alba</i>	<i>E. coli</i>	-	14
	<i>S. aureus</i>	14	18
<i>Leucas cephalotes</i>	<i>E. coli</i>	-	14
	<i>S. aureus</i>	-	18
<i>Tinospora cordifolia</i>	<i>E. coli</i>	-	14
	<i>S. aureus</i>	-	18

(-) = No effective antibacterial activity, ZOI: Zone of inhibition.
E. coli: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*

Table 7: Phenolic content in different plant extracts

Plant sample (extract)	TPC mg GAE/g
<i>Anethum sowa</i>	306.34±34
<i>Trigonella foenum-graecum</i>	939.76±0.01
<i>Lepidium sativum</i>	551.63±0.02
<i>Cuscuta reflexa</i>	145.09±0.01
<i>Eclipta alba</i>	202.67±0.02
<i>Leucas cephalotes</i>	233.19±0.03
<i>Tinospora cordifolia</i>	211.76±0.02

Total phenolic and flavonoid content

The result of phenolic content is displayed in Table 7. The total phenolic content of all plant extracts showed varied data ranging from 145.09 to 939.76 mg GAE/g in *C. reflexa* Roxb. and *T. foenum-graecum*, respectively. The *T. foenum-graecum* has the highest total phenolic content, followed by *L. cephalotes* and *T. cordifolia*. The result showed similar results, as reported earlier 269.5±0.5 mg GAE/100 g of total phenolic content in the *E. alba* [21].

The result of flavonoid content is shown in Table 8. Among the seven plants extract *T. cordifolia* showed the presence of the highest flavonoid contents, whereas the *C. reflexa* showed the least TFCs and other plants showed the flavonoids contents in between them. The results of the present study are found comparable to that of previously reported 142±0.15 mg QE/100 g of total flavonoid in *E. alba* [22]. Antioxidant properties of flavonoids depend on their structure, particularly hydroxyl position in molecule although quantitative determination of flavonoid compounds in plant extracts is influenced by their structural complexity, diversity, nature of analytical assay method, selection of standard, and presence of interfering substances.

In vitro α-amylase inhibition activity

The mode of α-amylase inhibition of plant's extract was evaluated by determining the IC₅₀ values, adopting the standard protocol given by Bernfeld [22]. Alpha-amylase inhibitory properties are presented in Fig. 2.

The result of IC₅₀ of α-amylase inhibition activity of plant extract is shown in Table 9. The IC₅₀ values of different plant extract along with standard acarbose were evaluated and found that the value range from 76.78 µg/ml to 777.36 µg/ml. The extract of *A. sowa* having IC₅₀ value 76.78±2.00 µg/ml was found to be potent toward the antidiabetic activity. The previous study reported that the aqueous leaves extracts of *P. americana* possess hypoglycemic activity [23]. Similarly, different fractions of *R. ellipticus* fruits were reported for its antidiabetic activity on alloxan-induced diabetes and glucose tolerance tests in rats [24].

Toxicity test

All the plant extracts showed LC₅₀ >1000 µg/ml indicating the non-toxic effect. This method provides preliminary screening data that can be backed up by more specific bioassays once the active compounds have been isolated.

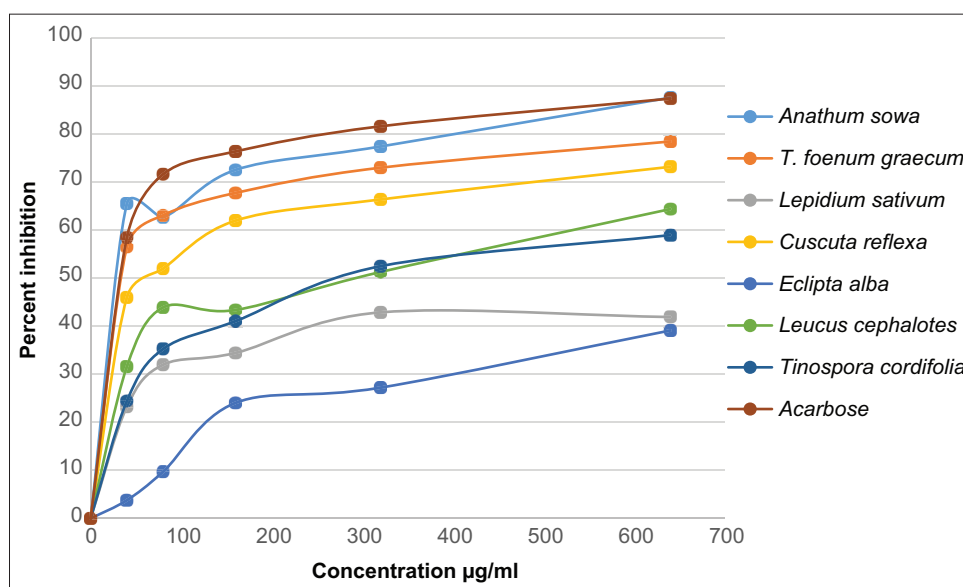


Fig. 2: Percent α-amylase inhibition of plant extract in comparison to standard acarbose

AQ3

Table 8: Flavonoid content in different plant extracts

Plant sample (extract)	TFC mg QE/g
<i>Anethum sowa</i>	329.023±0.050
<i>Trigonella foenum-graecum</i>	231.3817±0.0413
<i>Lepidium sativum</i>	553.819±0.058
<i>Cuscuta reflexa</i>	146.372±0.00936
<i>Eclipta alba</i>	322.138±0.025
<i>Leucas cephalotes</i>	164.9306±0.020
<i>Tinospora cordifolia</i>	852.071±0.110

CONCLUSIONS

The present research provides some scientific support for their traditional use for diabetes management and other different diseases. From the phytochemical investigation, it can be concluded that plant extracts are the good sources of secondary metabolites. Radical scavenging activities of the methanolic extract of the selected plants showed a varied degree of antioxidant property, of which *E. alba* was found to have high antioxidant properties. The total phenolic contents were found high in *T. foenum-graecum* having value 939.76±0.01mg GAE/g, and least can be seen *C. reflexa* Roxb. (145.09±0.01). Similarly, the TFCs of *T. cordifolia* were found to be highest having value of 852.07±0.011 mg QE/g and least was found in *C. reflexa* (Roxb.) 146.372±0.096 mg QE/g, the greater antioxidant property on them is credited to secondary metabolites such as phenols and flavonoids. The most effective antidiabetic activity was found in the plant extract of *A. sowa*. The moderate and mild antidiabetic activity was shown respectively by the plant extracts of *T. foenum-graecum* and *C. reflexa* (Roxb.). The plant extract with lower IC₅₀ value will be greatly beneficial to reduce the rate of digestion and absorption of carbohydrate and thereby contribute to effective treatment of diabetes by decreasing hyperglycemia.

ACKNOWLEDGMENTS

The authors are thankful to the Central Department of Chemistry, Tribhuvan University, for providing laboratory services to conduct this research work. We would like to thank Prof. Dr. Ram Prasad Chaudhary and Prof. Dr. Mohan Siwakoti, Central Department of Botany, for the identification of collected plants.

AUTHORS' CONTRIBUTIONS

Dr. Khaga Raj Sharma analyzed the data and wrote the manuscript, whereas Numer Alam carried out the laboratory work. Both the authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest in publishing this research article.

AUTHORS' FUNDING

The authors themselves bear the publication fees of this paper.

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