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Research Article

EVALUATION OF ANTIOXIDANT POTENTIAL AND QUANTITATIVE ESTIMATION OF PHENOLIC AND FLAVONOID CONTENT IN SOME SELECTED NEPALESE MEDICINAL PLANTS

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ABSTRACT

Objective: The aim of this study is to evaluate the antioxidant potential, determination of total phenolic and flavonoid content in nine selected medicinal plants *Spondias pinnata*, *Melia azedarach, Ageratina adenophora, Urtica dioica, Curcuma longa, Bauhinia variegata, Elaeocarpus angustifolius Blume, Achyranthes aspera,* and *Psidium guajava* from Kavre district of Nepal using *in vitro* studies.

Methods: Methanolic plant extracts were prepared by cold percolation method. The methanol extract of nine medicinal plants collected from Kavre district of Nepal, was screened for assessing bioactive phytoconstituents followed by antioxidant property, total phenolic, and flavonoid content. Different plants collected were powdered and extracted with methanol, concentrated by a rotatory evaporator and analyzed for the presence of phytochemicals. The antioxidant potential of the plant extracts was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

Results: The phytochemical analysis of methanolic extracts of all nine medicinal plants displayed the presence of various secondary metabolites such as alkaloids, flavonoids, polyphenols, saponins, and quinones. The extract of *S. pinnata* showed the highest percentage of radical scavenging activity up to 87.94±1.88 with 50% inhibitory concentration (IC_{50}) 17.51±1.27 µg/mL, followed by *B. variegata*, 80.63±1.06 with IC_{50} value 26.55±2.61 µg/mL. The standard, ascorbic acid has IC_{50} value of 20.13±1.17 µg/mL. Further, the ethyl acetate fraction of *S. pinnata* showed the maximum percentage of radical scavenging (85.92±1.37) with IC_{50} value of 46.95±1.17 µg/mL. Moreover, *S. pinnata* displayed the highest total phenolic content (TPC**)** 48.26±1.23 mg GAE/g (milligram gallic acid equivalent per gram) extract while the highest flavonoid content was displayed by *Melia azedarach* 41.07±1.53 mg QE/g (milligram quercetin equivalent per gram) extract measured by the Folin–Ciocalteu reagent method and aluminum chloride colorimetric method.

Conclusions: The preliminary results of this study have put forward the extract of *S. pinnata* showed the highest percentage of radical scavenging activity and *S. pinnata* displayed the highest TPC while the highest flavonoid content was displayed by *Melia azedarach* methanolic extracts although the further studies are needed to assess its mechanism of action.

Keywords: Antioxidant, Total phenolic content, Total flavonoid content, Phytochemicals.

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INTRODUCTION

Due to its greatly varied geographical and climatic conditions of Nepal, there is huge biodiversity of plants with medicinal and aromatic values. Nepal, being located at the portion of Central Himalayas, has contributed about 10% medicinal plants of expected 7000 species of flowering plants [1]. Kavre district of Nepal is rich in biodiversity due to climatic variation and possesses varied medicinal plants. In developing countries, medicinal plants are potent sources of medicine to treat various diseases. The people (approximately 80%) living in rural areas of underdeveloped countries still depend on medicinal plants for their basic health care [1]. Synthetic drugs are effective to cure various diseases, but in the long run, they show harmful side effects and they are expensive too. Hence, the drug development from the natural product is promising as plants show different bioactivity to cure ailments due to the presence of bioactive compounds.

Oxidative stress needs electron pairing for their stability and thus pair with biological macromolecules such as protein, DNA, lipids and even with healthy human cells, thereby gives rise to different diseases [2]. To scavenge these free radicals, synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, and tertiary butylated hydroquinone are being used. However, these commercially available antioxidants have side effects and are toxic *in vivo*. Hence, there is an increased in interest for safer antioxidants from natural sources to cure different diseases [3,4]. Glutathione is master antioxidant in human body have ability to neutralise and eliminate the free radical generated by oxidative stress [5].

Natural antioxidants, derived from plants, are secondary metabolites which scavenge the free radicals generated from the oxidation process in different cells and check different oxidative stress-related maladies. Natural antioxidants are less expensive having lower toxicity and side effects. In general, phenolic acids and flavonoids have been considered to show the bioactivity for scavenging free radicals. However, there might be other secondary metabolites which act as antioxidants. Natural antioxidants are less expensive having lower toxicity and side effects [6].

Nepal is enrich with several climatic conditions, geographical variations, and immense variety of plants with potential antidiabetic activities, but no effort has been made to seek more safe and efficient antioxidant and α-amylase inhibitors from the natural sources so far. People of Nepal have been using medicinal plants for many years for treatment and cure of different diseases. Therefore, it is urgent to identify, explore and preserve the antioxidant with a quantitative estimation of flavonoid and phenolic content natural resources of Nepal. Hence, the present study mainly focused to evaluate the antioxidant potential of methanol extract of nine selected medicinal plants from Kavre district of Nepal, to examine for the antioxidant potential and to determine the total phenolic and flavonoid content in the plant extracts quantitatively.

METHODS

Collection and identification of plant samples

Different parts of nine medicinal plants were collected from the farmland of Panchkhal Municipality, Kavre, Nepal; the plants were

collected in the summer of May/June 2017. The plants were identified by Prof. Dr. Mohan Sivakoti and Prof. Dr. Sangeeta Rajbhandari, Central Department of Botany, Tribhuvan University, Kathmandu, Nepal.

Extract preparation

Cleaned parts of plants were dried under shade at room temperature. Dried samples were chopped into pieces and then powdered using a mechanical grinder. Dried powder (100 g) was mixed separately in 400 mL methanol. The flasks were sealed tightly, and extraction was done for 72 h with occasional shaking. The obtained extracts were filtered and concentrated in a rotary evaporator. The yield of each fraction was determined and all the extracts were stored at 4°C in a refrigerator until analyses.

Preliminary phytochemical analysis

The method employed for phytochemical screening was based on the standard protocol of Harbone *et al*. with some modifications [7].

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

Antioxidant capacity was measured by the use of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The ability of different plant extracts to scavenge DPPH free radical was performed by the standard protocol adopted by Jamuna *et al*. [8]. Ascorbic acid of the same concentrations as that of plant extract prepared was used as standard and its absorbance was measured at 517 nm. Distilled water was used as a blank correction. The percentage of the DPPH free radical scavenging activity was calculated using the following equation:

$$\text{radical scavenging}(\%)=\left[\frac{A_{0-}A_s}{A_o}\right]\times 100$$

where,

A_0 = Absorbance of the control (DPPH solution + methanol)
A_s = Absorbance of test sample

The 50% inhibitory concentration (IC_{50}) value was indicated as an effective concentration of the sample that required to scavenge 50% of the DPPH free radicals. IC_{50} values were calculated using the inhibition curve by plotting extract concentration versus the corresponding scavenging effect.

Total phenolic content (TPC)

The TPC of all selected plant extracts was estimated using Folin–Ciocalteu reagent using gallic acid as standard based on the oxidation-reduction reaction. The total phenol content determination was performed with the help of the standard procedure given by Kim *et al*. (2007) with few modifications [6,9]. TPC content was expressed in milligram of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract using the formula; C = cV/M where, C = TPC compounds in mg/g, in gallic acid equivalent (GAE), c = concentration of gallic acid established from the calibration curve in mg/mL, V = Volume of extract in mL, M = Weight of plant extract. The linear correlation coefficient (R^2) value and regression equation were obtained from the gallic acid calibration curve. The regression equation was employed to calculate the concentration of each extract. Using the regression equation, the concentration of each extract was calculated. Thus, with the calculated value of the concentration of each extract, the TPC was calculated.

Total flavonoid content (TFC)

TFC of the selected plant extracts were determined by aluminum chloride colorimetric method using quercetin as standard with few modifications [10]. The TFC in plant extracts was expressed as milligram of quercetin equivalent per gram of dry weight (mg QE/g) of extract. TFC was expressed in milligram of quercetin equivalent per gram of dry weight (mg QE/g) of extract using the formula; C = cV/M where, C = TFC compounds in mg/g, in quercetin equivalent (QE), c = concentration of quercetin established from the calibration curve in mg/mL, V = Volume of extract in mL, M = Weight of plant extract. The linear correlation coefficient (R^2) value and regression equation were obtained from the quercetin calibration curve.

The crude methanol extract (7 g) of potent antioxidant *Spondias pinnata* was dissolved in 150 mL of distilled water; it was stirred well to make it dissolved completely. This was the mother solution, which was partitioned off successively by other solvents of different polarities, i.e., hexane, dichloromethane (DCM), and ethyl acetate. To the mother solution, 200 mL of hexane was added. It was then continuously shaken in separating funnel and let it stand until two layers were completely distinguishable. Hexane portion was then separated with an aqueous portion. The aqueous portion is further partitioned with DCM and ethyl acetate. Hence, finally hexane fraction, DCM fraction, and ethyl acetate fraction were obtained. In subsequent stages, each of the fractions was analyzed separately for the antioxidant property.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The identification of major secondary metabolites of methanolic extracts was carried out to determine the presence or absence of the different phytoconstituents. The results were evaluated by visual inspection as a change in color or precipitation. Table 1 shows the presence or absence of phytoconstituents in nine different plant extracts.

All the nine selected plant extracts were found rich in secondary metabolites. *Melia azedarach* possessed all the phytochemicals which were undertaken for investigation. Hence, due to the presence of such secondary metabolites *M. azedarach* be a good source of such secondary metabolites [11]. *S. pinnata* and *Psidium guajava* were found good sources of coumarin as a secondary metabolite.

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

In vitro antioxidant study of an extract of different plants was performed using methanolic extracts. Fig. 1 showed the concentration dependent free radical scavenging activity of plant extracts. All the plant extracts showed the concentration-dependent increasing in radical scavenging capacity. Among nine medicinal plant extracts, three plants extracts, namely, *Ageratina adenophora, Urtica dioica,* and *Achyranthes aspera* were found least antioxidant and thus are the poor sources of natural antioxidant compounds. The greatest DPPH radical scavenging potency of with a minimum inhibitory concentration (IC_{50}) value was recorded for *S. pinnata* (17.51±1.27 µg/ml), followed by *Bauhinia variegata* (26.55±2.61 µg/ml), *Melia azedarach* (62.04±1.55 µg/ml), *P. guajava* (70.91±2.61 µg/ml), *Curcuma longa* (83.50±6.42 µg/ml), and *Elaeocarpus angustifolius Blume* (104.23±3.58 µg/ml). This assay is a simple and widely used and most acceptable technique to evaluate the antioxidant potency of plant extracts. The antioxidants are the chemical compounds of the plant which are capable of enacting the visually noticeable quenching of the stable purple-colored DPPH radical to the yellow-

Table 1: Phytochemical analysis of plants extracts

Compounds	Sample								
	SP	MA	PG	AD	UD	BV	AA	EAB	CL
Reducing sugars	−	+	−	−	−	+	+	+	+
Polyphenols	+	+	+	−	−	+	+	+	−
Alkaloids	+	+	−	+	+	+	−	+	−
Glycosides	−	+	+	−	−	−	−	+	+
Quinones	+	+	+	+	+	+	+	+	−
Saponins	−	+	−	−	−	−	+	+	+
Coumarins	+	+	+	−	+	+	−	−	−
Flavonoids	+	+	+	−	−	+	−	−	+
Terpenoids	+	+	+	+	+	+	−	+	−

SP: *Spondias pinnata*, MA: *Melia azedarach*, AD: *Ageratina adenophora,* UD: *Urtica dioica,* CL: *Curcuma longa,* BV: *Bauhinia variegata*, EAB: *Elaeocarpus angustifolius* Blume*,* AA: *Achyranthes aspera*, PG: *Psidium guajava,* − for absence and + for presence

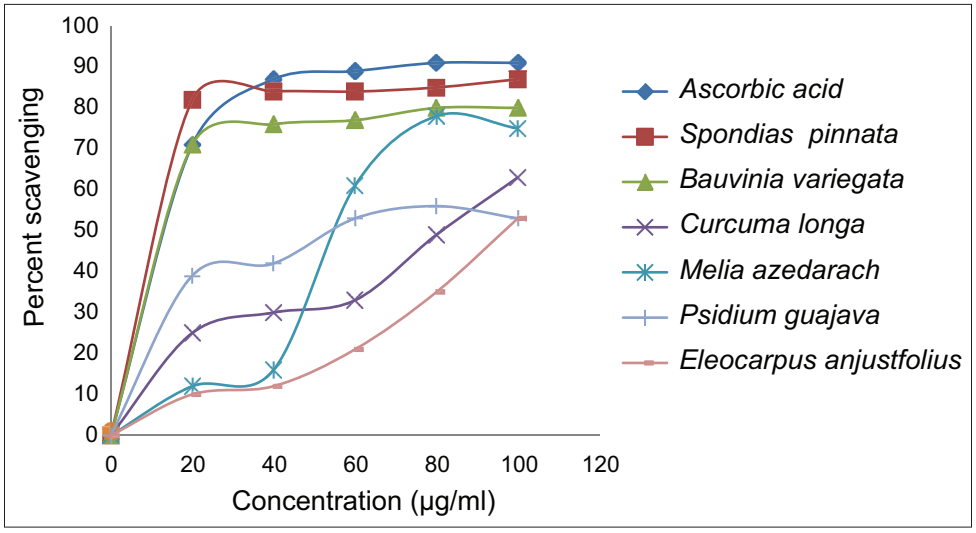

Fig. 1: Scavenging activity of ascorbic acid and selected medicinal plant extracts results expressed as the mean ± standard deviation (n=3) at a concentration of 20, 40, 60, 80, and 100 µg/ml

colored DPPH. The antioxidant potential of *S. pinnata* methanol extract was investigated in the search for new bioactive compounds from natural resources. Phytochemical analysis showed that the polyphenols were found in plant extract and act as reducing agents and antioxidants by the hydrogen donating property of their hydroxyl groups. These polyphenols are responsible for the observed antioxidant activity in this study.

The IC_{50} values of some plants which showed antioxidant property were calculated and tabulated in Table 2. IC_{50} value is the concentration of ascorbic acid or plant extracts to scavenge 50% of DPPH free radicals.

The percent scavenging and IC_{50} of *S. pinnata* showed that the extract is the potent source of the natural antioxidant compound. Hence, it was further fractioned to examine the antioxidant property of different fractions in different solvents based on the polarity and individual fractions were subjected for an antioxidant activity where results are presented in the line graph given in Fig. 2.

Ascorbic acid was used as standard and its IC_{50} value was 20.13±0.89 µg/mL. In this study, *S. pinnata* and *Bauhinia variegata* showed significant antioxidant property with IC_{50} 17.51±1.27 µg/mL and 26.55±2.61 µg/mL respectively. The ethyl acetate fraction of *S. pinnata* showed the maximum scavenging (85.92±1.37) with IC_{50} value 46.95±1.17 µg/mL. Table 3 showed the DCM and hexane fraction of *S. pinnata* has moderate inhibitory effect. The DCM and hexane fraction of *S. Pinnata* showed a moderate inhibitory effect. Similarly, the different fractions of *S. pinnata* showed the moderate antioxidant properties as well. *S. spinnata* and *B. variegata* showed the significant antioxidant property which is supported by previous studies where IC_{50} values for scavenging were 24.48±2.31 µg/mL and 6.48±0.08 µg/mL for *S. spinnata* and *B. variegata,* respectively [2,12]. Previous studies showed that DPPH free radical scavenging activity of different fractions of *S. pinnata* was found to be concentration-dependent, i.e., scavenging property increases with the increase in concentration and the finding of the present study is supported by the recent result not only in *S. pinnata* but also in rest of the plant extracts. Sharma *et al*. and Manik *et al*. concluded the fact about the potentiality of *B. variegata* and *S. spinnata* to act as a natural antioxidants [12,13]. The antioxidant activity of *Lepidium sativum* ethanol extract showed IC_{50} values of 162.4±2.3, 35.29±1.02, 187.12±3.4, and 119.32±1.5 µg/ml in terms of DPPH which is comparable to the antioxidant activity showed by the plant extracts in the present study [14].

TPC

A calibration curve was constructed using gallic acid as standard. The TPC in each plant extracts was estimated with the help of calibration curve. The result is shown in Table 4.

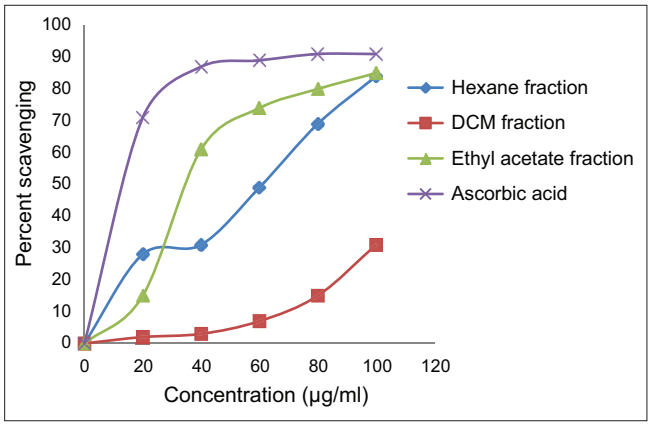

Fig. 2: Percent scavenging of different fractions of *Spondias pinnata*

Table 2: Half maximal inhibitory concentration values for antioxidant activity of different plants extracts

Serial number	Name of plant extracts/standard	IC_{50} (µg/mL), mean±SD
1.	Ascorbic acid (standard)	20.13±0.89
2.	SP	17.51±1.27
3.	BV	26.55±2.61
4.	CL	83.50±6.42
5.	MA	62.04±1.55
6.	EAB	104.23±3.58
7.	PG	70.91±2.61

SP: *Spondias pinnata*, BV: *Bauhinia variegata*, CL: *Curcuma longa*, MA: *Melia azedarach*, EAB: *Elaeocarpus angustifolius Blume*, PG: *Psidium guajava*, SD: Standard deviation, IC_{50}: Half maximal inhibitory concentration

Table 3: IC_{50} values of different fractions of methanol extract of *Spondias pinnata*

Name of plant samples/standard	IC_{50} (µg/mL), mean±SD
Ascorbic acid (standard)	20.13±0.89
Hexane fraction	57.31±2.25
DCM fraction	185.54±2.63
Ethyl acetate fraction	46.95±1.17

DCM: Dichloromethane, SD: Standard deviation, IC_{50}: Half maximal inhibitory concentration

Table 4: Total phenolic content of different plant extracts

Name of plants	Absorbance			TPC (mg GAE/g)			
	A1	A2	A3	C1	C2	C3	Mean±SD
SP	0.742	0.761	0.744	47.44	49.67	47.67	48.26±1.23
CL	0.54	0.601	0.554	28.78	31.78	26.56	29.04±2.62
Eleocarpus anjustifolius	0.402	0.351	0.39	9.67	4.00	8.33	7.33±2.96
BV	0.620	0.572	0.630	33.89	28.56	35.00	32.48±3.44
MA	0.461	0.410	0.451	16.22	10.56	15.11	13.96±2.99
AA	0.401	0.350	0.373	9.56	3.89	6.44	6.63±2.84
PG	0.55	0.673	0.662	26.11	39.78	33.89	33.26±6.86

GAE: Gallic acid equivalent, SD: Standard deviation, SP: *Spondias pinnata*, BV: *Bauhinia variegata*, CL: *Curcuma longa*, MA: *Melia azedarach*, PG: *Psidium guajava*, AA: *Achyranthes aspera*, TPC: Total phenolic content

Table 5: Total flavonoid content of different plant extracts

Name of plants	Absorbance			TFC (mg QE/g)			
	A1	A2	A3	C1	C2	C3	Mean±SD
SP	0.123	0.133	0.129	8.39	10.00	9.35	9.24±0.089
CL	0.199	0.222	0.215	20.65	24.35	23.23	22.74±1.89
BV	0.106	0.099	0.109	5.65	4.52	6.13	5.53±0.82
MA	0.333	0.315	0.329	42.26	39.35	41.61	41.07±1.53
AA	0.086	0.093	0.081	2.42	3.55	1.61	2.52±0.97
PG	0.132	0.139	0.145	9.84	10.97	11.94	10.92±1.05

SP: *Spondias pinnata*, BV: *Bauhinia variegata*, CL: *Curcuma longa*, MA: *Melia azedarach*, PG: *Psidium guajava*, AA: *Achyranthes aspera*, SD: Standard deviation, QE: Quercetin equivalent, TFC: Total flavonoid content

The TPC of extract of all selected medicinal plants showed varied results ranging from 6.63±2.84 to 48.26±1.23 mg GAE/g in *Achyranthes aspera and Spondias pinnata,* respectively. The total phenol content of the rest of the plant extract lied between these two extremes. The extract of *Ageratina adenophora* and *U. dioica* showed poor antioxidant property with not significance TPC. Similarly, *Achyranthes aspera showed poor* antioxidant property was also a moderate source of TPC. On the other hand, those plant extracts (*S. pinnata* and *B. variegata)* which showed potent antioxidant property also have appreciable TPC. The present findings agree with the view that DPPH scavenging activity and phenolic content of the plant extract are related as an antioxidant property of plant extract is directly correlated with its phenolic content [12]. A high correlation between antioxidant capacities and their TPCs indicated that phenolic compounds were a major contributor to antioxidant activity of these plant extracts.

TFC

TFC of the selected plants' extracts was determined by aluminum chloride colorimetric method taking quercetin as standard. The absorbance of quercetin (standard) was recorded from the spectrophotometer and the result is tabulated in Table 5.

The TFC of different selected medicinal plant extracts was found and the results revealed that the TFC varied from 2.52±0.97 mg QE/g in *Achyranthes aspera to* 41.07±1.53 mg QE/g in *Melia azedarach.* All the remaining plants extract showed the TFC in between two extremes; however, *U. dioica, Elaeocarpus angustifolius* Blume*,* and *Ageratina adenophora* are the moderate sources of TFC. Sharma *et al*. and Hazra *et al*. have suggested that greater flavonoid content of plant extract could be directly correlated with higher free radical scavenging property [12]. The present study showed that the antioxidant activity is not only due to the presence of flavonoid and phenolic compounds but also the presence of some other organic compounds that act as reducing agents.

CONCLUSIONS

The DPPH radical scavenging activities and subsequently the IC_{50} values of methanolic extracts of the selected plants showed a varied degree of antioxidant property; of which, *S. pinnata* showed appreciable percent scavenging followed by *B. variegata*. The highest percent scavenging showed by *S. pinnata* has IC_{50} value 17.51±1.27 µg/ml while the standard, ascorbic acid has 20.13±0.89 µg/ml. Further, the ethyl acetate fraction of *S. pinnata* showed the maximum scavenging (85.92±1.37) with IC_{50} value 46.95±1.17 µg/ml. The greater antioxidant property on them is credited to bioactive secondary metabolites especially phenols but not flavonoids. Hence, these plants could be the potential substitutes of synthetic antioxidants. Although, some medicinal plants showed a significant antioxidant property, they cannot directly be referred for pharmaceutical usage. Further extensive phytochemical and pharmacological investigation must be done to explore the mechanism of action and to isolate and characterize lead compounds that are responsible for pharmacological properties. This study demonstrates that these medicinal plants could be a potential source of natural antioxidants. Further studies need to be conducted to identify alkaloids and phenolic compounds that are correlated with the antioxidant activity of *S. pinnata* as well as their synergistic interactions.

Statistics

All the analyses were carried out in triplicate, and the results are expressed as mean ±SD.

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AUTHORS' CONTRIBUTIONS

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

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