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Assessment of Phytochemicals, Antioxidant Activity, Total Phenolic and Flavonoid Contents of Selected Nepalese Medicinal Plants

Deepak Kumar Shrestha, Basanta Kumar Sapkota, Krishna Prasad Sharma
Tribhuvan University, Department of Chemistry, Butwal Multiple Campus, Butwal

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Abstract

Several medicinal plants have been used from the traditional period of times to cure different diseases but there is little scientific evidence. The phytochemicals of plants can reduce cardiovascular and other diseases. The present study analyzed the five different medicinal plants of the Gulmi and Rupandehi districts of Nepal using *in vitro* studies. They were *Crateva Unilocularis, Aegle marmelos, Nyctanthes arbor-tristis, Urtica dioica and Justicia adhatoda.* The antioxidant potential of the methanolic extract of plants was evaluated by DPPH radical scavenging assay, total phenolic content was determined by using the Folin-Ciocalteu method and total flavonoid content was determined by using the Aluminium chloride colorimetric method. Results revealed that the methanolic extract of plants contained phytochemicals such as alkaloids, flavonoids, polyphenols, saponins, quinones, terpenoids, etc. The extract of *Nyctanthes arbor-tristi* showed the highest % of radical scavenging activity up to 64.931±0.032% with an IC_{50} value of 70.506±1.55µg/ml followed by *Aegle marmelos* and the lowest in Urtica dioica. *Nyctanthes arbor-tristis* revealed the highest TPC (97.647±7.01mgGAE/g) and lowest in Urtica dioica. *Crateva unilocularis* had the highest TFC 31.99±2.345mgQE/g and followed by *Nyctanthes arbortristis* and lowest in *Justicia adhatoda*. These parameters were analyzed from the period 5 September 2021 to 10 October 2021.

Keywords: Antioxidant, Flavonoid, Phenolic compound, 2,2-Diphenyl-1-picrylhydrazyl.

Corresponding author, email: shresthadeepak854@gmail.com

Introduction

Nepal is situated in South Asia. It is a land-locked country that occupies 0.03 % and 0.3% land area of the World and Asia respectively and has total area 1,47,181 sq. km [1,2]. Nepal is ranked 25th and 11th positions in biodiversity richness in the world and Asia, respectively. Nepal is enriched with several climatic conditions, geographical variations, and an immense variety of medicinal plants has contributed about 10% medicinal plants of the expected 7000 species of flowering plants [2,3]. Gulmi district, a part of province no. 5 of Nepal, which altitude ranges from 465 m to 2690 m [4]. Rupandehi District is also part of Lumbini Province, its altitude ranges from 100m to 1229m from sea level. Natural products are the major sources of new drug discovery. About 90% of Nepalese populations, residing in rural areas are still using medicinal plants for their primary health care. Despite the widespread use of medicinal plants in Nepal, there are limited studies on phytoconstituents and their antioxidant activity [2]. Synthetic drugs in the long run, show harmful side effects and they are expensive too. Hence, drug development from the natural product is a promising field. Therefore, it is urgent to identify phytochemicals and explore the antioxidant with a quantitative estimation of flavonoid and phenolic content in the natural resources of Nepal. Hence, the present study mainly focused on medicinal plants found in Gulmi and Rupandehi districts. The oxidative stress inside body produces chronic diseases such as diabetes, heart disease, and cancer [5]. In oxidative stress, the balance between the formation of reactive oxygen species (ROS) and the amount of antioxidants in the body is destroyed which causes damage to cell components such as proteins, lipids, and nucleic acid and eventually leads to cell death [6]. ROS and reactive nitrogen species (RNS) are the main sources of free radicals which lead to serious disorders such as Alzheimer's disease, Parkinson's disease, and Strokes [7]. Although NO and superoxide radicals are involved in host defense, overproduction of these two radicals contributes to the pathogenesis of inflammatory diseases [8]. Antioxidants are compounds that hinder oxidative processes and thereby delay or prevent oxidative stress [9]. Increasing intake of antioxidants can prevent diseases and lower health problems. In the present context, many antioxidants are manufactured synthetically such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylated hydroxyquinone (TBHQ), and gallic acid ester. These synthetically produced antioxidants possess certain side effects and toxic effects. Natural products, mainly obtained from dietary sources provide a large number of

antioxidants that decreases oxidative injury [1]. Plants contain many phytochemicals that are useful sources of natural antioxidants, such as phenolic diterpenes, flavonoids, tannins, alkaloids and phenolic acids etc [10]. Polyphenols are the strong antioxidant in plant extracts [11]. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen and various free radicals. As per [12] by GC-MS analysis for different extract of bark of *Crateva religosa* suggest that this plant has vast variety of photochemical which can be used for various ailments and drug formulations in future. The ethnic claims of this plant were also verified by the present study. As per [13] the phytochemical analysis of methanolic extracts of all nine medicinal plants of Kavre district, displayed the presence of various secondary metabolites such as alkaloids, flavonoids, polyphenols, saponins, and quinones [13]. The methanolic 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 [13]. Moreover, *S. pinnata* displayed the highest total phenolic content (TPC**)** 48.26±1.23 mg GAE/g while the highest flavonoid content was displayed by *Melia azedarach* 41.07±1.53 mg QE/g [13],[14]. As per [15] bioactive properties were found strongly on 4 selected traditionally used medicinal plants with strong enzyme inhibition potential where α-Glucosidase and α-amylase inhibitory activities were investigated using in vitro model followed up by antioxidant and antimicrobial activities. The study showed that ethyl acetate fraction of *Melastoma melabathrium* (IC_{50} 9.1 ± 0.3 µg/mL) and water fraction *Acacia catechu* (IC_{50} 9.0 ± 0.6 µg/mL) exhibit strong α-glucosidase inhibition. Furthermore, to identify the metabolites within the fractions, they employed high-resolution mass spectrometry (LC-HRMS) and annotated 17 known metabolites [15]. As per [16] hydro-alcoholic extract of *Urtica dioica* shows positive results for antioxidant activity with IC_{50} value of 88.33 ± 2.88 µg/ml while standard ascorbic acid showed IC_{50} value of 2.8 ± 0.62 µg/ml [13]**.**

Table 1: Chosen medicinal plants list

Name of plants	Local Name	Utilized Parts	Family
Aegle marmelos	Bel	Raw fruits	*Rutaceae*
Nyctanthes arbor-tristis	Parijat	Leaves	*Oleaceae*
Urtica dioica	Sisnu	Leaves	*Urticaceae*
Crateva unilocularis	Siplikan	Buds and leaves	*Capparaceae*
Justicia adhatoda	Asuro	leaves	*Acanthaceae*

Extract of *Nyctanthes arbor-tristis* exhibited various pharmacological activities like hepatoprotective, antileishmanial, antipyretic, antihistaminic, antimalarial, antibacterial, anti-inflammatory, antioxidant, antiviral, and antifungal etc. because of the presence of glycosides and phenolic compounds [17]. *Gnidia glauca* and *D. bulbifera* contained significant amounts of phytochemicals with antioxidative properties [18]. Similarly, the highest radical scavenging activity of *Dioscorea bulbifera* fruit powder was found in vacuum drying ranged from 65.36% to 81.33% of the concentration of 200 µg/ml to1000 µg/ml, [19]. In vitro antioxidant activity of the *Aegle marmelos* plant extract revealed that both the extracts showed good antioxidant power with IC_{50} value ranges of 37.11±3.50 to 158.99±59.46 µg/ml for aqueous extract and 35.02±8.10 to 283.06 ± 135.80µg/ml and 35.02±8.10 to 283.06 ± 135.80µg/ml for alcoholic extract [20]. Polyphenols, especially flavonoids are the strong antioxidant in plant extracts [21]. Different extract of *Justicia adhatoda* by ultra UHPLC analysis revealed the presence of polyphenolic compounds and flavonoids which might be responsible for bioprotective activity. Among the five fractions (hexane, chloroform, ethyl acetate, n-butanol and aqueous), n-butanol and ethyl acetate exhibited significant antioxidant activity with minimum IC_{50} value (< 105.33 µg/ml) [22]. The present study was to analyze secondary metabolites, total phenolic, flavonoid contents, and antioxidant activity of five different medicinal plants. It helps to find scientific pieces of evidence of the medicinal value of plants and also help to further investigation.

Methods and materials

Assembling and identifying plant samples

Different parts of five different selected medicinal plants *Crateva unilocularis* from Gulmi district and other four plants viz *Aegle marmelos, Nyctanthes arbotristis ,Urticadiocia, Justicia adhatoda* were collected from different region of Rupandehi (**Table 1**). They were collected in the period of 1 march 2021 to 2 April 2021. Botanist Dr. Ananta Gopal Singh of BMC identified the plants.

Statistical evaluation

There were three copies of each experiment run. The mean ± standard deviation (SD) was used to express the results. Microsoft Excel 2016 was used for all statistical analysis. The IC_{50} values was calculated by using Microsoft Excel 2016.

Extract preparation after drying

The plant's raw fruits and leaves were gathered locally and processed. The components of the gathered plant were cleaned, then dried at room temperature in the

shade. They were ground into a powder using a mechanical grinder, stored at a low temperature in a clean plastic bag. By using the cold percolation process, extract was created. 400 ml of methanol and 150 g of dried powdered components were combined in separate, clean, dry conical flasks. Extraction was carried out in a flask that was firmly sealed for 72 hours while being frequently shaken and filtered. The resulting residue was once more steeped in methanol. Until the sample's methanol became colorless, the procedure was repeated. The rotary evaporator was used to concentrate the thusly produced filtrate. Different plants' solid methanolic extracts were prepared and kept in a refrigerator at 4°C until analysis [13].

Table 2. Methods used for data analysis

Parameters	Methods employed
Antioxidant activity	DPPH radical Scavenging assay
Total Phenolic content	Folin-Ciocalteu method
Total Flavonoid content	Aluminium chloride colorimetric method
UV visible Spetrophotometer	Aczel Pvt. Ltd, Model no: AUV-8S ID: 210702206

Chemicals and reagents needed

Analytical-grade chemicals were employed throughout. Fehling's solution, alpha-Naphthol, $FeCl_3$, $NaHCO_3$, $Bi(NO_3)_3$, KI, $HgCl_2$, Picric acid, dimethyl sulfide, sodium hydrogen phosphate, sodium carbonate, sodium chloride, sodium hydroxide, sodium nitroprusside, and distilled water are a few examples of substances that fall under this category. The 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, and the Folin-Ciocalteu reagent were bought from Qualigens Fine Chemicals in India. Meyer's reagent, Dragendorff's reagent, Molisch's reagent, and other reagents and solvents used in phytochemical analysis were made in the lab using chemicals of the laboratory reagent grade.

Table 3. Phytochemical screening of methanolic extract of different plant samples.

Phytochemicals	A.M	N.A	U.D	C.U	J.A
Alkaloids	+	+	+	+	++
Terpenoids	+	+	+	+	+
Coumarins	+	+	+	+	+
Flavonoids	+	+	-	+	++
Quinones	+	+	+	+	+
Polyphenols	+	+	-	+	+
Glycosides	+	+	-	+	+
Reducing sugar	+ +	-	-	-	-
Saponins	+	+	-	-	-
Tannins	+	+	-	+	+
Carbohydrates	+ +	-	+	-	-

A.M= *Aegle marmelos*, N.A= *Nyctanthes arbortristis*, U.D= *Urtica dioica*, C.U= *Crateva unilocularis*, J.A= *Justicia adhatoda*, and (-) for absence & (+) for presence.

Required apparatus

Hot air oven, mechanical grinder, digital weighing balance, cuvettes, burettes, pipettes, micropipettes, thermometer, condensers, beakers, conical flasks, test tubes, reagents bottles, stands, vial tubes, round bottom flasks, Rotary evaporator with water bath was used for the evaporation of solvents. Using a UV-visible spectrophotometer, absorbance for the DPPH assay and absorbance for total phenolic and flavonoid content were determined.

Phytochemical analysis

The method used for phytochemical screening was based on protocol put forward as per [23]. Basically phytochemical screening helps to identify secondary metabolites (bioactive compounds) present in plants. The analysis was done by the color reaction using different specific reagents [24]. The qualitative results are expressed as (+) for the presence and (−) for the absence of phytochemicals. The phytochemical screening was carried out to test for Alkaloids (Meyer's test, Dragendorff's test), Coumarins, Flavonoids, Quinones, Polyphenols, Glycosides, Reducing sugar, Saponins, Tannins, and carbohydrates (Molish's test) etc. respectively

Antioxidant activity measurement

DPPH radical scavenging activity

The protocol developed as per [25] and [26] was used to conduct the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging experiment. This test is a straightforward, popular, and widely accepted method to assess the antioxidant potential of plant extracts. Each plant sample's extract solution was diluted to a different concentration (10 µg/ml to 100 µg/ml) and mixed with 2 ml of DPPH solution (60 µM). For a 30-minute reaction, the mixture was left to stand in full darkness. Finally, a UV spectrophotometer was used to assess each plant sample's absorbance at 517 nm. Each sample's radical scavenging activity was determined using the formula below:

Radical scavenging (%) = $[(A_0 - A_1 / A_0) \times 100\%]$

Where A_0 is the absorbance of the control and A1 is the sample extract's absorbance. The test solution without the sample is the control. The standard was ascorbic acid. A similar process was used with ascorbic acid solutions that ranged in concentration from 10 to 100 µg/ml. Each sample's antioxidant activity was quantified using its IC_{50} (the concentration needed to suppress the production of DPPH radicals by 50%) value. The sample's effective concentration was defined as the 50% inhibitory

concentration (IC_{50}) value, which was needed to effectively scavenge 50% of the DPPH free radicals. By graphing the extract concentration versus the corresponding scavenging action, the inhibition curve was used to determine the IC_{50} values.

Total phenolic content determination

Using gallic acid as a reference and the oxidation-reduction reaction as a basis, the total phenolic content of the extract was calculated using the Folin-Ciocalteu technique. The method described as per [13] was used to determine the total phenolic content, with a few changes [5]. 5 ml of 10% Folin-Ciocalteu reagent were mixed with 1 ml of crude extract. 4 ml of 7% (w/v) sodium carbonate was mixed and shook after standing for 5 minutes. After 40 minutes of incubation, the mixture's absorbance at 760 nm was measured. The entire experiment was run in triplicate. Gallic acid was used as the standard in the creation of the calibration curve. The calibration curve was used to quantify the total phenolic content, and the results were represented as mg of gallic acid equivalent (GAE) per gram dry weight of extract using formula,

TPC= C×V/M where V= Volume of extract in ml, M=Weight of plant extract in mg, and C=Concentration of Galic Acid Obtained from Calibration Curve in mg/ml. The gallic acid calibration curve was used to derive the linear correlation coefficient (R^2) value and regression equation. Each extract's concentration was determined using the regression equation. The TPC was determined using the calculated value of each extract's concentration.

Total flavonoid content determination

With a small modification of protocol as per [11] and [27], the aluminum chloride colorimetric method was employed to determine the flavonoid concentration. 4 ml of distilled water and readily added 0.3 ml of 5% sodium nitrite were combined with 1 ml of each extract solution. 0.3 ml of 10% aluminum chloride was added after 5 minutes, and the mixture was let to stand for 6 minutes. After shaking well, 2 ml of 1 M sodium hydroxide and 2.4 ml of distilled water were added, bringing the total volume to 10 ml. The absorbance was then measured at 510 nm using a UV spectrophotometer. The standard used to create the calibration curve was quercetin. From the calibration curve, the total flavonoid concentration was determined and results were represented as mg of quercetin equivalent (QE) per gram dry extract weight.

Results

Extraction yield value

The extract of the selected plant samples was prepared in methanolic solvent by cold percolation method. The yield percent of the plant extract is given in **Table 4**.

Table 4: Methanolic extract of different plant samples' yield percentages.

Name of Plants	Yield% of extract (in methanol)
Aegle marmelos	12.42%
Nyctanthes arbo-tristis	10.28%
Urtica dioica	11.42%
Crateva unilocularis	10.14%
Justicia adhatoda	12.4%

Note: Extract Yield Percent is equal to [(Weight of Dry Extract/Weight of Crude Plant Sample) 100%].

Variations in the scavenging of DPPH radicals

Figure 1 displays the percentage of DPPH radical scavenging activity of various samples at various concentrations in methanolic solvent, whereas **Table 4** displays the IC_{50} value for the DPPH radical scavenging activity. With the least amount of inhibitory concentration and the highest DPPH radical scavenging activity (IC_{50}) value was found *Nyctanthes arbor-tristis* (IC_{50} value 70.506±1.55 µg/ml), *Aegle marmelos* has 99.872±1.27, *Justicia adhatoda* has103.146±1.33, *Crateva unilocularis* has 127.672±2.61 whereas *Urtica dioica* (IC_{50} value 179.103±3.58 µg/ml) Has relatively little DPPH radical scavenging efficacy in comparison to ascorbic acid standard (IC_{50} value 55.40±0.003 µg/ml).The antioxidants are the chemical elements found in plants that can effectively quench the stable purple DPPH radical, converting it to the more visible yellow DPPH.

Table 5. Antioxidant activity of Ascorbic acid

S.N	Concentration (ppm)	Absorbance			Mean ± SD	% Scavenging
		A1	A2	A3		
1	10	0.445	0.441	0.452	0.446 ± 0.005	9.712 ± 0.132
2	20	0.397	0.401	0.403	0.400 ± 0.003	19.01 ± 0.013
3	40	0.312	0.322	0.323	0.319 ± 0.006	35.38 ± 0.002
4	60	0.209	0.208	0.216	0.211 ± 0.004	57.12 ± 0.003
5	80	0.140	0.148	0.144	0.144 ± 0.004	71.42 ± 0.012
6	100	0.054	0.057	0.057	0.056 ± 0.001	88.47 ± 0.013
IC_{50}	55.40	Blank			0.494±0.001	

Table 6. percentage Scavenging (Antioxidant) activity of methanolic extract of different plants {result expressed as the mean ±SD (n=3)

Plants	Concentration (ppm)	10	20	40	60	80	100
Nyctanthes arbortristis	Mean% Scavenging	5.697±.089	26.031 ±0.12	32.514 ±0.33	44.007 ±0.45	57.563 ±0.33	64.931±0.032
Aegle marmelos	Mean % Scavenging	1.305±2.3	3.343±1.023	6.534±0.45	14.021±0.12	30.931±0.22	63.033±0.14
Crateva unilocularis	Mean % Scavenging	1.089±1.8	2.008±1.4	4.144±0.45	9.509±0.34	21.034±0.01	48.310±0.22
Urtica dioica	Mean% Scavenging	0.495±2.4	1.450±1.44	3.432±0.89	9.098±0.76	19.231±0.33	40.789±0.02
Justicia adhatoda	Mean % Scavenging	2.024±1.34	5.048±0.98	9.09±0.42	14.56±0.56	29.123±0.32	60.756±0.13

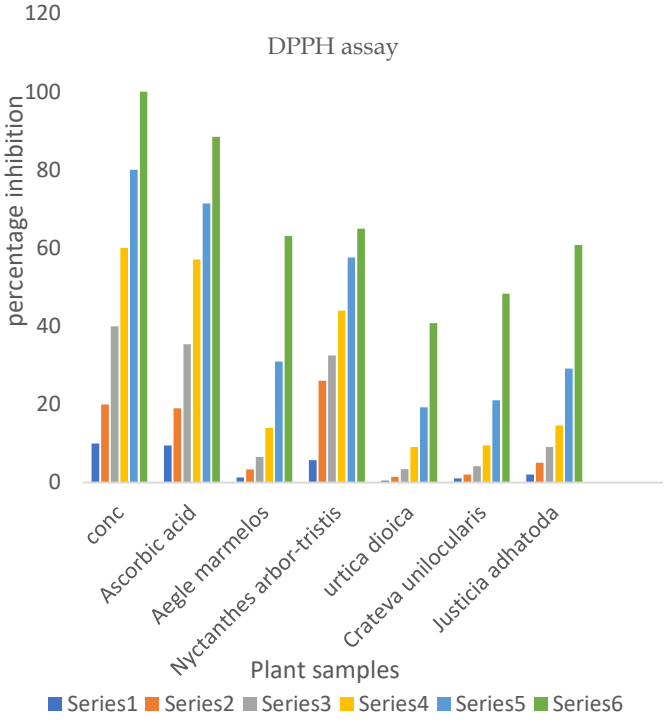

Figure 1. Percentage Scavenging of DPPH free radicals by methanolic extract of plants with reference to ascorbic acid, results expressed as the mean ± standard deviation (n=3) at a concentration of 10, 20, 40, 60, 80, and 100 µg/ml.

Table 7. Ascorbic acid and methanolic extract of several plant samples' DPPH radical scavenging activity, expressed in terms of IC_{50} value.

	Name of plant extracts	IC_{50}(µg/mL), mean ± SD
1.	Ascorbic acid (standard)	55.40 ±0.003
2.	*Aegle marmelos*	99.872 ±1.27
5.	*Nyctanthes arbortristis*	70.506 ±1.55
6.	*Urtica dioica*	179.103 ±3.58
7.	*Crateva unilocularis*	127.673 ±2.61
8.	*Justicia adhatoda*	103.146 ±1.33

Variation of Total phenolic content

By applying the Folin-Ciocalteu method and using gallic acid as a reference, the total phenolic content of methanolic extracts was calculated [13,5]. At 760 nm, the highest absorption was noted. Concentrated gallic acid solution (10-100 µg/ml) confirmed at 760 nm with a regression coefficient (R^2) of 0.9698 to Beer's Law (**Figure 2**). With the aid of a calibration curve using gallic acid as the reference, the total phenolic content was determined and expressed as mg GAE/g dry extract weight, total phenolic content was found highest in *Nyctanthes arbortristis* (97.647±7.01) mg GAE/g followed by *Aegle marmelos* (88.559.647±6.71), *Crateva unilocularis* (83.333±2.73),and lowest in *Justicia adhatoda* (64.126±1.368) respectively and was found lowest in *Urtica dioica* (24.36±1.33 mg GAE/g).

Table 8. Total phenolic content of different plant extract

Name of plants	Absorbance			TPC (mgGAE/g)			
	A1	A2	A3	C1	C2	C3	Mean±SD
A.M	1.645	1.646	1.624	88.919	88.973	87.78	88.559±.6.715
N.A	1.846	1.664	1.915	99.7831	89.8459	103.514	97.647±7.01
U.D	0.473	0.424	0.455	25.5676	22.9189	24.5946	24.36±1.33
C.U	1.533	1.496	1.596	82.8649	80.8649	83.333	83.333±2.73
J.A	1.215	1.167	1.177	65.67	63.081	63.621	64.126±1.368

GAE: Gallic acid equivalent, SD: Standard deviation,A.M: *Aegle marmelos*, N.A: *Nyctanthes arbortristis*,U.D: *Urtica dioica*, C.U= *Crateva unilocularis*,J.A= *Justicia adhatoda*, TPC: Total phenolic content, A_1 A_2, A_3= Absorbances triplicate, C_1, C_2, C_3= TPC triplicates

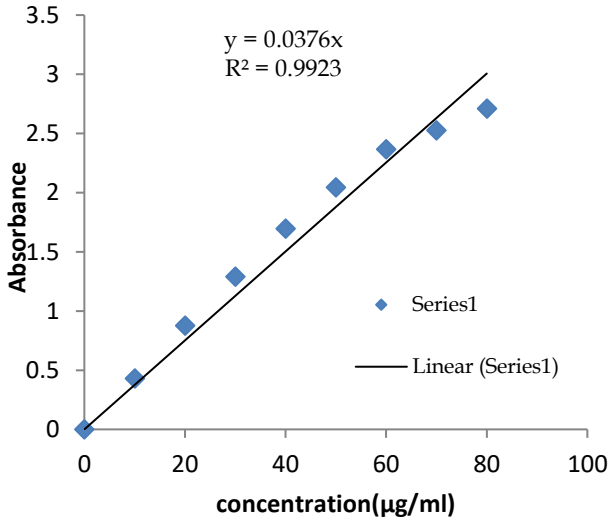

Figure 2: Calibration curve of gallic acid.

Table 9: Different plant extracts' total flavonoid content

Plants	Absorbance			TFC (mg QE/g)			
	A1	A2	A3	C1	C2	C3	Mean ± SD
Aegle marmelos	0.172	0.171	0.168	11.0968	11.0323	10.838	10.989 ± 0.134
Nyctanthes arbortristis	0.273	0.323	0.223	17.612	20.838	14.387	17.62 ± 3.225
Urtica dioica	0.172	0.171	0.168	11.0468	11.032	10.438	10.518 ± 5.231
Crateva unilocularis	0.435	0.505	0.487	28.064	32.580	31.419	31.99 ± 2.345
Justicia adhatoda	0.157	0.156	0.143	10.1290	9.936	9.108	9.724 ± 0.103

TFC=Total Flavonoid Content (n=3), SD= Standard deviation, Q.E=Quercetin equivalent

Variation in Total amount of Flavonoids

Using quercetin as a reference, the total flavonoid content of methanolic extracts was determined using the aluminum chloride colorimetric technique. With a regression coefficient (R^2) of 0.9729, the quercetin solution (10–100 µg/ml) obeyed Beer's Law at 510 nm (Figure 3). Total flavonoid content was found highest in *Crateva unilocularis* (31.99±2.345) QE/g, followed by *Nyctanthes arbortritis* (17.62±3.225) then *Aegle marmelos* (10.998±0.134), *Urtica dioica* (10.518±5.231) and lowest in *Justicia adhatoda* (9.724±0.103) mg QE/g.

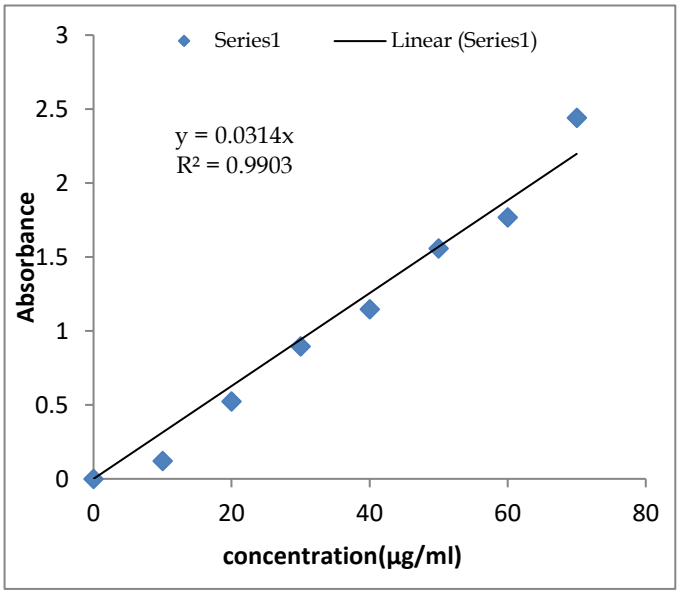

Figure 3. Calibration curve for quercetin

Conclusion

The DPPH radical scavenging activities and subsequently the IC_{50} values of chosen plants' methanolic extracts revealed various levels of antioxidant activity. *Nyctanthes arbortristis* showed highest percent scavenging, it has IC_{50} value 70.505±1.55 µg/ml while the standard, ascorbic acid has 55.40±0.003 µg/ml, followed by *Aegle marmelos.* The greater antioxidant property on them might be due to the presences of bioactive compounds such as phenolic ,terpenoids, flavonoid, saponins, tannin, alkaloids etc .The highest TPC was found in *Nyctanthes arbortristis* (97.647±7.01) followed by *A.marmelos* and lowest in *U.dioica*.While highest TFC is found in *Crateva unilocularis*(31.99±2.345) and lowest in *Justicia adhatoda*. Hence, these plants contain appreciable amount of antioxidants, TPC and TFC. These results gave some scientific proof for these indigenous medicinal plants. Even while some medical plants have substantial antioxidant properties, they cannot be used directly as drugs. To make these therapeutic plants a possible source of natural antioxidants, more thorough phytochemical and pharmacological research must be done.

Author's Contribution

D. K. Shrestha and B.K Sapkota designed research and performed the experiments. B.K.Sapkota, D. K.Shresth and K.P. Sharma analysed the data and K. P. Sharma reviewed the literature and edited the manuscript. All the authors contributed equally in drafting and revising the manuscript.

Competing Interests

We declare that authors have no conflict of interests of any kind.

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Ethical Approval and Consent:

All the ethical guidlines from ERB and NHRC are strictly followed. We ensure that there was no use of animal models and hazardous materials in this study.The plants used in this study were not endangered species or banned by the government. No people other than the

authors are involved in this research. The harm or discomfort for others during this research was nill.

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