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Research Article

COMPARATIVE STUDY OF POLYPHENOL, FLAVONOID, AND ANTIOXIDANT ACTIVITY OF VARIOUS MEDICINAL PLANTS COLLECTED FROM DIFFERENT ALTITUDES

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ABSTRACT

Objectives: The main aim of this investigation is to explore the folklore medicinal flora located at Terai and the lesser Himalayan region of Nepal and has a comparative study on phenol, flavonoid, and antioxidant activity that would bring forth a factual basis for its use in the traditional therapy of various health ailments.

Methods: Folin-Ciocalteu and Colorimetric aluminum chloride methods were used for the estimation of total polyphenolic and flavonoid content, respectively. Moreover, antioxidant activity was determined by DPPH radical scavenging activity.

Results: Among 21 plants collected, *Artemesia vulgaris* depicted higher (53±0.03 mg GAE/g) and *Mimosa pudica* (3.7±0.04 mg GAE/g) depicted lower phenolic content whereas the highest flavonoid content is observed in *Syzygium cumini and* the lowest value in *Mentha piperita.* Almost all the collected specimen demonstrated antioxidant activity, among which *Eupatorium adenophorum* and *Rhododendron anthopogon* demonstrated lower and higher antioxidant activity, respectively. Moreover, phenol and flavonoid content showed a weak correlation with the antioxidant activity indicating the major antioxidant to be different compounds other than phenols or flavonoids.

Conclusion: The present study confirms the antioxidant activity of the collected plant specimen and defends its ethnobiological use as a possible natural antioxidant. Furthermore, the result of antioxidant properties encourages their application in medicinal health, functional food, and biopharmaceutics.

Keywords: Phenol, Flavonoid, Antioxidant.

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INTRODUCTION

Numerous aromatics, spices, and medicinal plants are being abundantly used by the modern analyst to develop natural antioxidant formulations for food, cosmetic, pharmaceutical, and therapeutic applications [1]. An antioxidant neutralizes the free radicals or inhibits the oxidation of other substances. During various physiological and biochemical processes, environmental pollutions, UV and gamma radiation, as well as physical stress, free radicals, and other reactive oxygen species are generated in the human body [2,3]. Reactive oxygen species (ROS) and nitrogen (RNS) species act as pro-oxidants that either induces oxidative stress or inhibit the antioxidant activity and cause damage to proteins, lipid peroxidation, and fragmentation of deoxyribonucleic acid strand [3]. The process ultimately leads to cellular injury, aging, cancer, and hepatic, neurodegenerative, cardiovascular, and renal disorders [4,5].

The human body consists of superoxide dismutase, glutathione peroxidase, and non-enzymatic antioxidants such as Vitamins E and C, thiol antioxidants, carotenoids, and other compounds as an endogenous antioxidant enzyme that can reduce oxidative stress [6] and therefore, help in maintaining the ideal cellular activity [7,8]. Although under the ameliorative behavior of free radicals, these defensive endogenous antioxidants are insufficient, thus, directing the supply of dietary antioxidants [8,9]. Phenolic acids, polyphenols, and flavonoids are the common natural antioxidant products found in plants and supplements that protect plants, fruits, vegetables as well as human beings from oxidative damage [1].A cautious selection of the physicochemical characteristics of polyphenols is crucial as the bioavailability and chemical reactivity of phenols and flavonoids strongly affect its activity against ROS [4].

In recent decades, the physiological finding of ideal natural antioxidants from edible materials such as spices and herbs that can readily be absorbed, quench free radicals and cause metal inactivation are increasing [6,10]. There is a considerable quest for natural antioxidant molecules such as phenol, flavonoids obtained from plants as presently accessible artificial antioxidants, such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone, and gallic acid esters are reported to have genotoxic and carcinogenic effects [6,11]. Therefore, the investigations are mostly focused on congenital plant phytochemicals that are natural, non-synthetic and can not only counteract free-radical-induced oxidative stress and protect the human body from oxidative cell damage, diabetes, inflammation, and asthma but also be active in cardioprotective antitumor and antibacterial activities to a greater or lesser extent [2,11,12] as well as can overcome the side effects of synthetic antioxidants.

In developing countries, most of the populations rely on folklore medicine and are benefited from Ayurveda, a traditional medicinal herbal treatment for healthcare [13]. Nepal is a lavish country having an abundance of flora and fauna because of the altitudinal variation, climatic differences, varied topography, and abundant ecological habitats [14]. However, there is still limited scientific exploration and a lack of scientific proof on justifying the ethnomedicinal uses of Nepalese medicinal plants present in different altitudes. Therefore, the present investigation focuses on the collection of medicinal plants from Terai and the lesser Himalayan region of Nepal and studies its potential antioxidant behavior via DPPH radical scavenging activity. Furthermore, the study attempts to establish the correlation between phenolic and flavonoid content with the DPPH radical scavenging activity of 22 plant extracts that might be an encouraging source of natural antioxidants.

METHODS

Plant material collection and sample preparation

Twenty-two different traditional medicinal plants from Terai and lesser Himalayas of Nepal including areas such as Nepalthok, Khurkot, Dhulikhel, Sindhuli, Hetauda, Parsa, Pathlaiya, Niijgadh, and Bardibash were collected and evaluated in this study. Plant material was gathered near October and authenticated according to their scientific name by the national herbarium and Plant Laboratories, Godawari, Lalitpur. Plant material was washed, dried, and powdered by a mechanical grinder at room temperature. The powdered herbal samples (20 g each) were suspended and extracted in 250 mL of 80% methanol (v/v) through decoction process as shown in Fig. 1 for three times and evaporated until the dark-colored solvent is obtained to extract oils, volatile organic compounds, and other various chemical substances present in the plant materials. The herbal samples were mashed for maximum dissolution of the sample to the solvent used. The extract was then filtered and dried using a vacuum rotary evaporator. Thus, concentrated samples were subsequently weighed and kept at 4°C until use.A list of the names of the medicinal plants collected with their family name and parts of the plant used for the extraction process is expressed in Tables 1 and 2.

Fig. 1: Extraction of crude plant specimen via decoction process

Determination of total polyphenolic compounds

The total phenolic content (TPC) of the extracted sample was determined using the Folin-Ciocalteau assay following the methods mentioned in Settharaksa *et al.* 2012 where gallic acid was used as a standard antioxidant [15]. The extracted samples (12.5 µL) were mixed with 50 µL of water and 12.5 µL of Folin-Ciocalteau reagent for 6 min and then 125 µL of 7% sodium carbonate (Na_2CO_3) along with 100 µL of water was added. After the incubation at room temperature for 90 min, the absorbance was measured at 760 nm and all the determinations were performed in triplicates. Total phenolic content was reported as mg/g of GAE in reference to a standard curve (y = 0.0101x +0.0622 and R^2 = 0.9891).

Determination of total flavonoid content

Total flavonoid content (TFC) was determined by the Colorimetric aluminum chloride method as described in Mundhe *et al.,* 2011 [16]. Aliquots of each plant extracts were separately mixed with 1.5 mL of methanol, 0.1 mL $AlCl_3$ (10%), 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The solutions were kept at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm.A standard calibration curve was prepared by using known concentrations of quercetin at 415 nm. The total flavonoid in the test samples was calculated from the standard calibration curve with quercetin (0–100 µg/mL) which was again used for quantification in triplicates. The regression equation and R^2 for calibration curve arey = 0.004x+0.0376 and R^2 = 0.9887.

The result is expressed in mg quercetin equivalent/g of sample (mg Qu/g).

DPPH radical scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined using the method described in Brand Williams *et al.* 1995 with some modifications and by the use of synthetic radical DPPH assay [17]. One milliliter of the methanol extracts of the plants (400, 200, 100, 50, and 25 mg/mL) was added to 2 mL of a solution of DPPH radicals. The mixture was shaken continuously and allowed to stand for 30 min at room temperature. The absorbance (Abs sample) of the resulting solution was measured at 517 nm using a UV-Vis spectrophotometer and converted into a percentage of antioxidant

Table 1: Total phenolic content expressed as mg GAE/g sample and total flavonoid content as mg QE/g sample

S. No.	Plants	Sample	Parts used	Total phenolic content (mg GAE/g sample)	Total flavonoid content (mg QE/g sample)
1.	*Artemesia vulgaris*	A	Leaves	53±0.03	44.43±0.01
2.	Lemon peel	B	Fruit peel	52±0.03	10.18±0.02
3.	*Terminalia chebula*	C	Roots	46.05±0.04	28.5±0.01
4.	*Eclipta alba* (Bhringraj)	D	Whole plant	44.3±0.03	42.43±0.03
5.	*Ocimum sanctum*	E	Leaves	44±0.01	27.43±0.03
6.	*Malus domestica*	F	Fruit peel	34.25±0.02	33.81±0.04
7.	*Aegle marmelos*	G	Leaves	30.7±0.02	18.18±0.03
8.	*Amaranthus leucocarpus* (Latte)	H	Roots	30.55±0.04	22.87±0.03
9.	*Moringa oleifera leaf*	I	Leaves	29.4±0.02	14.75±0.04
10.	*Rhododendron anthopogon*	J	Leaves	29.35±0.02	42.3±0.01
11.	*Persea americana* (Avocado)	K	Leaves	29.05±0.04	44.62±0.02
12.	*Citrus aurantium* (Orange peel)	L	Fruit Peel	28.5±0.01	30.12±0.02
13.	*Brassica juncea* (Sarso)	M	Leaves	19.6±0.03	20.68±0.04
14.	*Moringa olifera*	N	Whole plant	18.6±0.01	12.75±0.04
15.	*Achyranthes aspera* (Dattiwan)	O	Roots	17.9±0.01	14.75±0.03
16.	*Colocasia esculenta* (Jangali Kakalo)	P	Leaves	17.5±0.03	6.62±0.03
17.	*Eupatorium adenophorum*	Q	Leaves	15.55±0.01	35.06±0.01
18.	*Euphoria wallichi*	R	Whole plant	13.4±0.04	26.62±0.04
19.	*Syzygium cumini* (Jamun)	S	Leaves	12.8±0.03	51.5±0.01
20.	*Asparagus racemosus* (Kurilo)	T	Stem	11.15±0.04	43.5±0.03
21.	*Mentha piperita* (Mint)	U	Leaves	5.1±0.03	1.93±0.02
22.	*Mimosa pudica* (Touch me not)	V	Whole plant	3.7±0.04	4.37±0.01

Each value is expressed as a mean±SD (n=3)

Table 2: Comparison of IC50 values of plant samples with ascorbic acid

S. No.	Plants	Family	Ascorbic acid (IC50)	IC_{50}
1.	*Artemesia vulgaris*	Asteraceae	45.88	92.81481
2.	Lemon peel	Rutaceae		179.6622
3.	*Terminalia chebula*	Combretaceae		85.4
4.	*Eclipta alba*	Asteraceae		130.0971
5.	*Ocimum sanctum*	Lamiaceae		249.64
6.	*Malus domestica*	Rosaceae		19.63636
7.	*Aegle marmelos*	Rutaceae		128.0769
8.	*Amaranthus leucocarpus*	Amaranthaceae		288
9.	*Moringa olifera* leaf	Moringaceae		36.5
10.	*Rhododendron anthopogan*	Ericaceae		17.72727
11.	*Persia americana*	Lauraceae		18.4231
12.	*Citrus aurantium*	Rutaceae		93.9474
13.	*Brassica juncea*	Brasicaceae		57.91411
14.	*Moringa olifera*	Moringaceae		78.78571
15.	*Achyranthes aspera*	Amaranthaceae		303.5484
16.	*Colocasia esculenta*	Araceae		273.1818
17.	*Eupatorium adenophorum*	Asteraceae		412.439
18.	*Euphoria wallichi*	Euphorbiaceae		59.50495
19.	*Syzygium cumini*	Myrtaceae		21.10092
20.	*Asparagus racemosus*	Asparagaceae		129.5
21.	*Mentha piperita*	Lamiaceae		58.12903
22.	*Mimosa pudica*	Fabaceae		92.81481

activity (AA) or percentage radical scavenging activity. The % radical scavenging capacity was calculated using the formula:

$$\text{Radical scavenging (\%)} = [(A_o - A_s)/A_o] \times 100$$

Where A_o = Absorbance of the control (DPPH solution + ethanol)
A_s = Absorbance of the test sample.

The radical scavenger activity was demonstrated in terms of the number of antioxidants required to alleviate the initial DPPH absorbance by 50%. The IC_{50} value for each sample was determined graphically by plotting the DPPH radical scavenging activity as a function of the sample concentration where ascorbic acid was used as standards. All determinations were achieved in triplicates and the plant extracts used for DPPH radical scavenging activity are shown in Fig. 2.

Statistical analysis

The data are presented as mean value ± standard deviation in triplicates for each of the above experiments. An standard calibration curves were prepared for the quantitative estimation of phenol, flavonoids, and antioxidants in the extracts, and all the statistical analysis was carried out through the Microsoft Excel program and Origin 2019b 64bit.

Fig. 2: Plant extracts for DPPH radical scavenging activity

RESULTS AND DISCUSSION

Determination of total polyphenolic and flavonoid content

In the present study, the bioactive phytochemicals that have effective immunological, pharmacological, and antioxidant activities, namely, total phenolic and flavonoid content were determined. TPC and TFC were estimated by extrapolation from the calibration curve (Figs. 3 and 4) prepared from gallic acid and quercetin as standard concentrations, respectively, are shown in Table 1. *Mimosa pudica* (touch me not) depicted the lowest phenolic, 3.7±0.04 mg GAE/g content whereas *Artemisia vulgaris,* belonging to a family Asteraceae commonly called mugwort and locally called as Titepati in Nepal manifested higher phenolic (53±0.03 mg GAE/g) and relatively higher flavonoid (44.43±0.01 mg QE/g) content than another specimen considered except *Persia americana* (44.62±0.02 mg QE/g) and *Syzygium cumini* (51.5 ±0.01 mg QE/g). Even so, Pandey *et al*., 2017 represented higher TPC (86.62 ± 0.04 mg GAE/g) and TFC (81.31 ± 0.53 mg Qu/g) relatively to this investigation [18]. The differences in the result might be related to the different extraction process adapted and also due to different environmental conditions of the selected plant. Although disparities in the result are found in other several kinds of literature, this plant is already proved to have greater health benefits and is commonly used in the treatment of menstrual problems, irregular periods, menopause, and premenstrual syndrome and acts as a sedative and anthelminthic agent [19]. The presence of well-known phenolic compounds, such as caffeic acid, neochlorogenic acid, and ferulic acid in *A. vulgaris* might be responsible for the high level of phenolics [20]. The study performed by Baral *et al.,* 2013, showed a total phenolic concentration of 10.9 GAE/100 g dried plant material of *R. anthopogon* methanolic extract [21]. In comparison to the result of Baral, TPC of the methanolic extract of *R. anthopogon* is better in our study (29.35±0.02 mg GAE/g). Traditionally, leaves and flowers of *R. anthopogan* are not only used as an antioxidant but also are being used to treat sore throats and counteract water-earth illness, remove headaches and back pain, cure cold, blood disorders, bone disease, potato allergies, vomiting, stimulate appetite, and relieve liver disorders in the higher Himalayan region of Nepal where its abundancy is quite high [21].

In this analysis, the content of flavonoids varied from 1.93±0.02 to 51.5±0.01mg QE/g extract where *Syzygium cumini* demonstrated higher while *Mentha piperita* demonstrated the lower flavonoid content than other plant species considered from the Terai region. Analogous TFC of *Mentha piperita* is also demonstrated in Attanosova *et al.,*

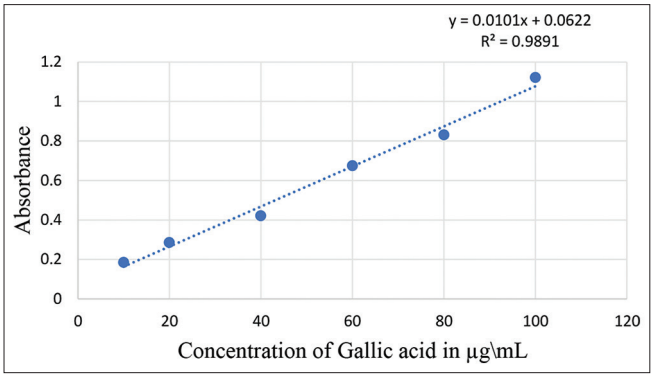

Fig. 3: Calibration curve of standard gallic acids

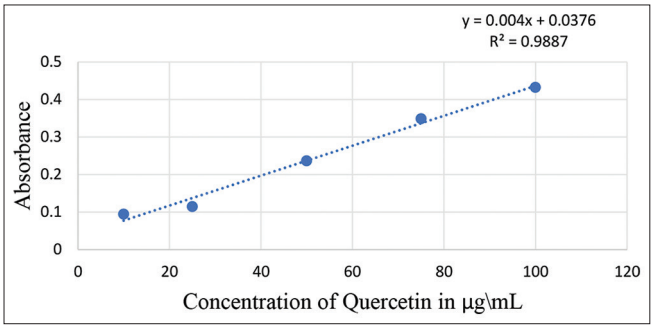

Fig. 4: Calibration curve of standard quercetin

2011 [11]. The presence of the flavonoids and phenolics such as ferulin and catechin is the main reason behind the antioxidant activity of *Syzygium cumini* [22]. The vast disparities in result between flavonoid and phenolic compound are found in lemon peel, *Syzygium cumini*, *Asparagus racemosus*, *Moringa olifera* leaf, and *Terminalia chebula*. *Eclipta alba* (Bringraj), *Malus domestica* (Apple Peel), *Citrus Aurantium* (Orange peel), and *Brassica juncea* (Sarso) manifested comparable antioxidant polyphenols, namely, phenols and flavonoid content.Eclipta alba (Bringraj),an herbaceous false daisy and a commercially attractive plant with excellent hepatotoxic and antidiabetic activities [23] showed TPC of 44.3±0.03 mg GAE/g and TFC of 42.43±0.03 mg Qu/g in this test indicating that these phytochemicals are likely to be responsible for the free-radical scavenging activity. Comparatively, higher values of TPC (52.5±0.64 mg GAE/g), as well as TFC values (144.62±1.62 mg Qu/g) in *Eclipta* species, are shown in Khurshid *et al.* 2020 [24].

It is reported that polyphenols and flavonoids are used for the prevention and cure of many diseases associated with free radicals and possess a broad spectrum of chemical and biological activity [11,20]. Flavonoids and phenols are considered as an important secondary metabolite containing at least one hydroxyl group that acts as free-radical scavengers and inhibitors of the hydrolytic and oxidative enzyme are known to have a profound anti-inflammatory action and health beneficial effects [8,11,25]. The presence of these metabolites in all the plant species though in different amounts is a significant finding of the present study.The phenolic compounds are also known to contribute toward quality and nutritional value through modifying color, taste, aroma, and flavor [26] whereas flavonoids are reported to be accountable for the chelating activity which helps in the reduction of oxidative stress [20].

The absorbance of gallic and quercetin at different concentrations is tabulated in Table 3 whereas the comparative bar diagram for total phenolic and flavonoid content is shown in Fig. 5. Each plant used is labeled from sample A to V for simplicity in making a bar graph.

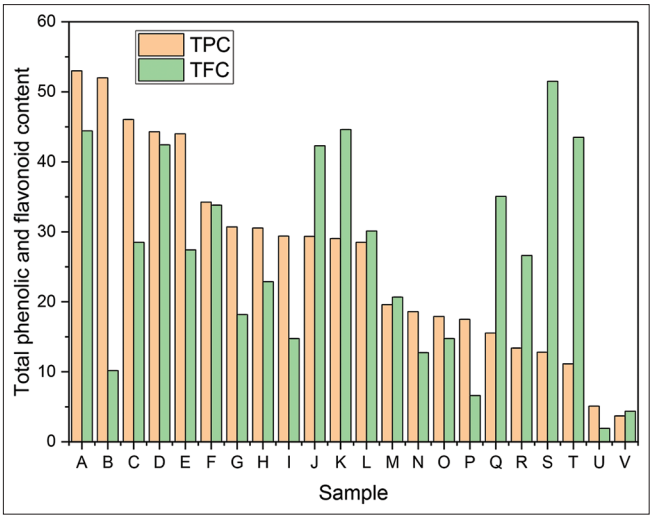

Fig. 5: Bar diagram representing total phenolic and total flavonoid content of the plant specimen

Table 3: Absorbance of standard gallic acid and standard quercetin at different concentration

Conc. of gallic acid µg/mL	Absorbance	Conc. of quercetin µg/mL	Absorbance
10	0.185	10	0.094
20	0.285	25	0.114
40	0.421	50	0.236
60	0.674	75	0.348
80	0.831	100	0.432
100	1.121		

DPPH radical scavenging activity

Antioxidant therapy is gainingimportance as it is used in the treatment of several metabolic diseasesand the development ofmass scientific programs havestarted with the objective of investigating medicinal propertiesof plants for their potential antioxidant properties [27]. The total antioxidant capacity of the extracts was determined with a synthetic DPPH assay by taking ascorbic acid as a standard solution where the scavenging activity is based on the capacity of an antioxidant to donate hydrogen or an electron to DPPH radical. DPPH itself is deep purple color and gets decolorized or converted to yellow by the respective reduction of DPPH radical to hydrazine, DPPH-H form [28]. Hence, more discoloration of DPPH solution denotes the stronger antioxidant potential of plant extract [29]. The pairing of an electron of radical results in the decrease of absorbance at 517 nm that are related to the potentiality of the antioxidants. Based on the standard graph plotted between concentration and free-radical scavenging activity and regression equation, the IC_{50} value of each extract was calculated which is represented in Table 2 which were then compared with standard ascorbic acid.

According to Jadid *et al.*, 2017, IC_{50} values ranging between 50 and 100 mg/mL are considered to exhibit intermediate antioxidant activity while extracts with IC_{50} value ranging between 10 and 50 mg/mL are considered to possess strong antioxidant activity and IC_{50} value >100 µg/mL showed weak antioxidant activity [30]. In this response, *Eupatorium adenophorum* with a highIC_{50}value412.439 represent low antioxidant activity, followed by *Achyranthes aspera* (Dattiwan), 303.5484, *Amaranthus leucocarpus* (Latte), 288, whereas *Rhododendron anthopogan* exhibited low IC_{50} value 17.72 before *Persia americana,* 18.42 *Malus domestica*, 19.63, *Syzygium cumini,* 21.10092,

Moringa olifera with 36.5 IC_{50} value, demonstrating about the highest antioxidant activity. Eshwarappa *et al*., 2014, exhibited the strong radical scavenging activity of the methanol extracts of *Syzygium cumini* similar to this study with IC_{50} value ranging between 10 and 50 mg/mL [27]. While Navarro *et al*., 2018, reported less IC_{50} value (4.54) of the peel extracts of *Malus domesticus*compared to this one [31]. Interestingly, plant samples F, I, J, and K have lower IC_{50} value as compared to ascorbic acid (45.88 mcg/mL) demonstrating the highest scavenging activity compared to ascorbic acid. Kumawat *et al*., 2012, and Jadid *et al*., 2017, also demonstrated greater antioxidant properties of plant extract than ascorbic acid [30,32].

Meanwhile, methanol is considered a major polar solvent as it can extract flavanols alkaloids, polyphenols, and saponins from plant materials in a high amount [30]. Some reports have also shown that the extracts of medicinal plants through methanol possess good pharmaceutical activity. The reducing ability of the methanol extract designates the presence of some compounds in the plant extracts which can contribute an electron and could react with free radicals to convert them into more stable products to terminate radical chain reactions. The only restriction found in DPPH activity is its solubility in organic solutions that cause a hurdle for the calculation of hydrophilic antioxidants [33]. Besides, the chemical structure of antioxidants also greatly influences the reaction of DPPH with antioxidants. Apart from this, *Mentha piperita*, *E. wallchi*, *Brassica juncea*, and *Moringa olifera* leaf act as potential antioxidant showing their half inhibitory maximum concentration value close to ascorbic acid as summarized in given Table 2.

Except for antioxidant activities the apple peel, Dattiwan, *Moringa olifera,* and other collected flora have healing abilities for various ailments and even some chronic diseases [31,34]. Several investigations are progressively carried out to isolate bioactive compounds from various medicinal plant parts included here because of their wide range of applications in pharmaceutical as well as in therapeutic activities. Therefore, phytomedicinal herbal plants are reliable in utilizing it as an alternative way in the medicinal field due to its affordable cost.

Relationship between antioxidants with total phenolics and total flavonoids

To correlate if the total phenolic content contributes to the antioxidant activity or not correlation analysis was carried out between TPC and TFC with antioxidant activity separately. In this research analysis, though extracts show high phenolic content with a high radical scavenging activity, good correlations could not be found among them. The antioxidant activity showed a weak correlation with phenols (Fig. 6 and 7).A linear regression analysis having the equation of y=3.4932x and R^2=0.3804 failed to demonstrate a direct correlation between radical scavenging activity and phenolic content of extracts. This low correlation between total phenols and DPPH scavenging activity suggests that the major antioxidant components might not be phenolics, but could be sterols, ascorbic acid, carotenoids, or pigments which are also responsible to contribute towards antioxidant property [20]. There was no correlation between total flavonoids and radical scavenging activity (Fig. 6). This lack of correlation between scavenging activity and phenols and flavonoids is also demonstrated in kinds of literature by Ghasemi *et al*., 2009, Kaur 2014, and Ghimire *et al.,* 2011 [20,35,36]. It is well-known that the flavonoids and other phenolic compounds have a certain structure, particularly hydroxyl position and number in the structure of a molecule that vastly affect the proton donating and radical scavenging activity [20]. Furthermore, the extracts are not only composed of phenol and flavonoids but are a mixture of different complex compounds possessing distinct activities. Besides, the Folin-Ciocalteu method used for the calculation of phenolic content is not considered an absolute measurement for the determination of the number of phenolic compounds [36]. The relationship of phenol, flavonoid, and IC_{50} values is also shown in the bar diagram (Fig. 8).

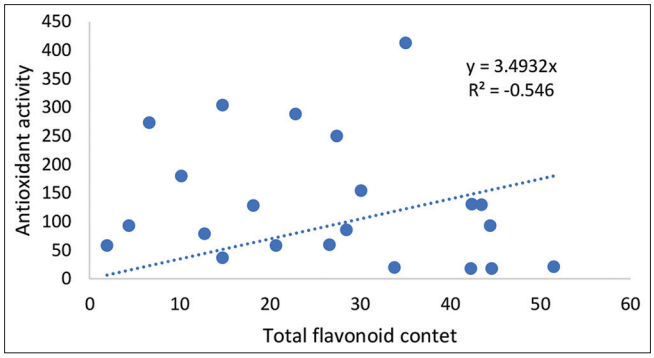

Fig. 6: Linear correlation between the antioxidant activity and total flavonoid content of the methanol extracts of 22 medicinal plants from different altitudes of Nepal

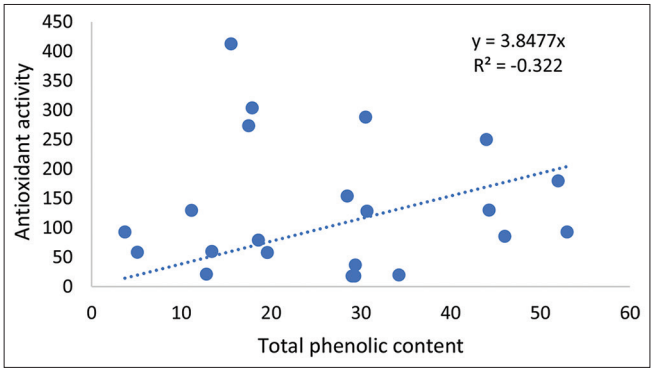

Fig. 7: Linear correlation between the antioxidant activity and total phenolic content of the methanol extracts of 22 medicinal plants collected from different altitudes of Nepal

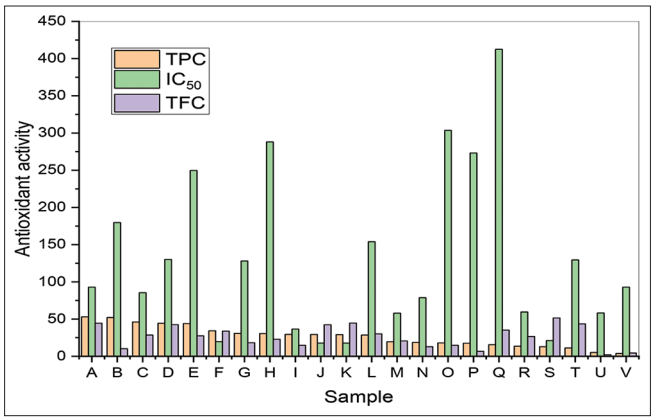

Fig. 8: Bar diagram representing the relationship of flavonoid, phenol, and IC50 value for antioxidant activity of the plant specimen

In contrast to this study, Kratchanova *et al*., 2010, have reported a high correlation between phenolic content and antioxidant activity [37]. In this study, only a few species such as *A. vulgaris*, *T. chebula* and *M. domesticus* showed a good correlation of antioxidant activity to phenolic content with an estimated coefficient of determination of R^2 = 0.96, thus indicating that the high DPPH activity may be related to the phenolic compounds in these plants.

S. cumuni, M. piperita, Mimosa pudica, and *E. wallichi* though exhibited strong antioxidant activity they did not contain concomitantly high phenolic compounds. High antioxidant activity with low phenolics or flavonoids can be attributed to individual phenolic or non-phenolic compounds with specific high antioxidant activity.

Similarly, *C. aurantium*, which has low phenolics and flavonoids revealed higher antioxidant activity. This might indicate the presence of active compounds of different polarities in this plant. Apart from phenol and flavonoids, various bioactive compounds such as Vitamin C, coumarins, terpenes, and quinoline have been reported in extracts of *C. aurantium* as reported by Suryawanshi, JAS 2011 that might be responsible for its high antioxidant property [38].

CONCLUSION

The toxicological effect of synthetic antioxidants in food pharmaceuticals and cosmetics has renewed the interest in uncovering natural antiradical from several plant species with traditional medicinal significance portraying pharmacological and phytochemical effects.

In conclusion, the present investigation on different medicinal plants found in the Terai region manifested a very good content of phenol and flavonoid along with certain degrees of antioxidant activity. In particular, the *Malus domestica,Moringa oleifera,Rhododendron anthopogon*, *Persia americana,Syzygium cumini,* and *Mentha piperita*proclaimed themselves as a promising source of natural antioxidants and as a possible prophylactic agent in the pathogenesis of some common metabolic disorders that would be effectively enhanced in absence of these oxidoreductive agents. However, the total phenolic and flavonoid content revealed a weak correlation with the antioxidant activity of the investigated plant. Hence, the comprehensive effect of individual bioactive compounds involved in the antioxidant activity of specific plants required for their use as pharmacological and phytochemical agents in food, cosmetics, and pharmaceutical industries should be studied. Furthermore, it would be fascinating to know the chemical composition along with the mechanism of action of the antioxidants present in the plant extract for novel drug formulation.

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AUTHORS CONTRIBUTIONS

All the authors contributed equally to this investigation and manuscript preparation.

CONFLICT OF INTERESTS

The authors declare no conflict of interest in the publication.

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