Comparative Studies on Antioxidant Activity of Ten Medicinal Plants Collected From the Ilam District of Nepal

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CONFLICT OF INTEREST: None

ABSTRACT

Methanol extracts of ten medicinal plants collected from Ilam district were prepared and a preliminary phytochemical screening was performed. Phytochemical test revealed that selected plant samples contain alkaloids, flavonoids, terpenoids, glycosides, quinones, reducing sugars, coumarin, polyphenols, and saponins. The antioxidant properties of ten samples were evaluated by using DPPH free radical scavenging assay and their inhibitory concentration for 50% inhibition (IC_{50} values) for antioxidant properties were calculated. Among all ten studied samples, IC_{50} values of extracts of barks of *Atrocarpus lakoocha* (41.42±3.1 µg/mL) and flowers of *Woodfordia fructicosa* (41.89±2.5 µg/mL) were very close to that of standard ascorbic acid (38.74±2.5 µg/mL). Similarly, flowers of *Rhododendron arboreum* (45.55±2.2 µg/mL), roots of *Vetiveria zizanoids* (46.22±2.0 µg/mL) and rhizomes of *Mirabilis jalapa* (48.99±3.0 µg/mL) also showed strong activity against DPPH free radicals. These results showed that these plants could be the potential sources of natural antioxidants in the food and/or pharmaceutical industry.

Keywords: DPPH, Extracts, Free radicals, Phytochemical screening

1. INTRODUCTION

Nature has been a source of various medicinal agents for thousands of years. Since ancient times, natural products obtained from plants have played a vital role in the effective treatment of various ailments, including the diseases caused by oxidative stress (Kamble & Gacche 2019; Sen &

Samanta 2015; Parveen *et al.* 2015). It is estimated that Nepal harbors over 10,000 species of medicinal plants (Kunwar & Bussmann 2008). Some of them are used in traditional medicine, and some are still not explored scientifically for their medicinal values. So, Nepal is an important site to explore the biological and pharmaceutically active components of plants.

Oxygen is a vital component of a living organism to metabolize and use dietary nutrients to produce energy (Venditti *et al.* 2013). Although oxygen is the essential component for living beings, it is a highly reactive atom that can become a part of potentially damaging molecules commonly called free radicals (Phaniendra *et al.* 2015). These free radicals contain unpaired electrons that are unstable and capture electrons from other substances to neutralize themselves and thousands of free radical reactions can occur within few seconds on the primary reaction (Balasaheb & Pal 2015; Lushchak 2014). These free radicals are highly reactive chemicals that are capable of attacking the healthy cells of the body. They may lead to cell damage, disease, and several disorders (Bhattacharya 2015). The damage of cells caused by free radical appears to be a major contributor to aging and diseases like cancer, heart disease, a decline of brain function, a decline of the immune system, diabetes, liver disease, etc. (Dizdaroglu 2015; Wang *et al.* 2015).

The induced free-radicals can be neutralized by antioxidants (Sanchez 2017). Antioxidants can promote body cells and tissues from continuous threats by the damage caused by free radical and reactive oxygen species produced during normal oxygen metabolism or induced by exogenous damage. The body makes some of the antioxidants called endogenous antioxidants (Mironczuk *et al.* 2018). However, the body relies on external sources to obtain the rest of the antioxidants it needs that are supplied from fruits, vegetables, and grains and are commonly called dietary antioxidants. The majority of antioxidant activity is due to flavons, isoflavones, flavonoids, anthocyanins, coumarins, lignans, and catechins (Asante *et al.* 2016; Mamta *et al.* 2014; Tungmunnithum *et al.* 2018).

The present study was carried out to compare the antioxidant potency of locally available ten medicinal plants such as *Vetiveria zizanoids, Cissampelos pareira, Artocarpus lakoocha, Melia azedarach, Cyanodon dactylon, Lycopodium clavatum, Woodfordia fructicosa, Rhododendron arboreum, Mirabilis jalapa* and *Drymaria diandra* from Ilam district of Nepal. The selected plants are commonly used as traditional medicines in various forms by the local people. To the best of our knowledge, no reports are available on the comparative study of antioxidant activity of such plants from the Ilam district. However, the plants' antioxidant activity from the districts other than Ilam is available (Bhandari & Rajbhandari 2014; Subba & Paudel 2014; Maharjan & Baral 2013; Pathak & Niraula 2019).

2. MATERIALS AND METHODS

2.1 Plants Materials

Roots of *Vetiveria zizanoides,* rhizomes of *Cissampelos pareira* and *Mirabilis jalapa*, the bark of *Artocarpus lakoocha, Melia azederach* and *Lycopodium clavatum,* the flower of *Woodfordia fructicosa* and *Rhododendron arboreum* and aerial parts of *Cynodon dactylon* and *Drymaria diandra* were collected from Ilam district in May 2016 based on of their ethnomedicinal importance. The taxonomic identification of plants was authenticated by Prof. Dr. Ram Prasad Choudhary, Central Department of Botany, Kirtipur, Kathmandu. The collected plant materials were cleaned, shade dried, powdered, and stored in airtight plastic bags until used for further experiment. The name of plants, parts used, and their

Table 1: List of plants, parts used and therapeutical uses

Code	Local Name	Scientific Name	Parts used	Altitude (m)	Therapeutical use	References
M1	Khas or khus grass	*Vetiveria zizanoids*	Roots	1200-1300	Mouth ulcer, malaria, acidity, bone fracture	Pareek and Kumar 2013
M2	Gujargano	*Cissampelos pareira*	Rhizomes	1200-1300	The menstrual problem, uterine bleeding, threatening miscarriage	Thapa *et al.* 2013
M3	Badahar	*Artocarpus lakoocha*	Barks	1300-1400	Liver disease, purulent matter, pimples	Nesa *et al*. 2015
M4	Bakaino	*Melia azedarach*	Barks	1350-1400	Intestinal worm, blood purification, skin disease	Azam *et al*. 2014, Al-Rubae, 2009
M5	Dubo	*Cyanodon dactylon*	Whole parts	1200-1300	Bronchitis, piles, asthma, UTI, toothache	Ashokkumar *et al*. 2013
M6	Nagbeli	*Lycopodium clavatum*	Barks	1200-1300	The digestive disorder, anti-inflammatory, constipation,	Orhan *et al.* 2013
M7	Shayari	*Woodfordia fructicosa*	Flowers	1300-1400	Ulcer, urinary problem, wounds	Das *et al*. 2007
M8	Laligurans	*Rhododendron arboreum*	Flowers	1200-1300	Diarrhoea, dysentery	Rawat *et al*. 2017, Madhvi *et al*. 2019
M9	Lankeshari	*Mirabilis jalapa*	Rhizomes	1350-1450	Asthma, allergy, wound healing	Saha *et al*. 2020
M10	Abhijit	*Drymaria diandra*	Aerial parts	1100-1250	Headache, cerebral stimulant, relief cough	Mandal *et al*. 2009

2.2 Chemicals and Equipment

The majority of solvents and chemicals were of laboratory grade. The solvent used was methanol (Thermo Fischer Scientific India Pvt. Ltd., Mumbai), and reagents DPPH (Tokyo chemical industry Co. LTD, Japan), ascorbic acid (Wako pure chemical Industry, Co. LTD, Japan) for an antioxidant test. Extracts were prepared using a rotary evaporator (Buchi RE 111), and the absorbance was measured using a UV-Visible spectrophotometer (Thermo Fisher Scientific, Genesystem-10-5).

2.3 Extraction

The extraction of chemical constituents of dried and powdered plant materials (100 g each) was carried out with dehydrated methanol (250 mL) by the process of cold percolation. The solvents from plant extracts were removed by evaporation with a rotary evaporator at high pressure maintaining temperature lower than the respective solvent's boiling point and left for drying to solid semi-solid mass.

2.4 Phytochemical Screening

The phytochemical screening method was based on the standard protocol used for different reagents, producing different colours with the plant extract (Harborne 1998).

2.5 Antioxidant Activity Test

The antioxidant activity of extract of ten plants and standard (ascorbic acid) was assessed based on the radical scavenging effect of the stable 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical activity following the standard protocol with some modifications (Pathak & Niraula 2019; Nemkul *et al.* 2018).

2.6 Preparation of the 0.2 mM DPPH Solution

1,1-diphenyl-2-picrylhydrazyl (DPPH) has a molecular weight of 394.32 gm/mol. Thus, 100

mL of 0.2 mM solution of DPPH was prepared by weighing the 7.886 mg of the DPPH carefully, dissolving it in methanol, and finally maintaining the volume to 100 mL. The prepared purple-coloured DPPH solution was kept at -20 °C until used for further experiment.

2.7 Measurement of DPPH free Radical Scavenging Activity

Different concentrations (12.5, 25, 50, 75 and 100 µg/mL) of plant extracts and ascorbic acid (positive control) were prepared in methanol with serial dilution method on the clean and clear test tubes. The sample volume of each extract and ascorbic acid was taken (2 mL) in the Eppendorf tube. To this sample volume, 2 mL of the 0.2 mM DPPH solution was added. The tubes were shaken vigorously for uniform mixing. These tubes were allowed to stand in the dark for half an hour. The control was prepared as above but without the plant extract or ascorbic acid. The absorbance was taken on a UV-Visible spectrophotometer (Thermo Fisher Scientific, Genesystem-10-5) at 517 nm. Methanol was used to collect the baseline on the spectrophotometer. After measuring the absorbance values, the free radical scavenging activity was calculated using the following formula (Pathak *et al.* 2020; Ali *et al.* 2018).

% radical scavenging activity = [(Abs.control – Abs.sample)/Abs.control]×100%

where; Abs.control = absorbance of the control solution (1mL MeOH + 0.5 mL DPPH).

Abs.sample = absorbance of the sample

The standard graph of concentration (x-axis) versus the percentage of free radical scavenging activity (y-axis) of each sample was plotted. The IC_{50} value of each extract and sample was calculated from the different equations obtained from each plot. The IC_{50} value is the concentration of the sample to inhibit 50% of DPPH free radicals. The sample has a higher percentage of radical scavenging activity exhibit lower IC_{50} value (Patil *et al.* 2009). The IC_{50} values of each extract were compared to that of the standard taken (ascorbic acid). The value closest to ascorbic acid is considered to have the best antioxidant property (Dhanani *et al.* 2017).

3. RESULTS AND DISCUSSION

3.1 Extractive Values and Phytochemical Analysis

The plant materials (100 g each) were extracted with methanol by using a cold percolation technique. The results of the yield of the extract of different plants are shown in table 2. The highest amount of extract was obtained from the flower of *Rhododendron arboreum* and the lowest amount of extract was from *Cynodon dactylon.* The phytochemical screening of the extracts (Table 2) of different plants revealed secondary metabolites like alkaloids, flavonoids, terpenoids, glycosides, quinines, reducing sugars, polyphenols, saponins, etc. Alkaloids were present in almost all extracts except in *W. fructicosa* and *R. arboreum.* Flavonoids were absent in *C. dactylon, L. clavatum* and *D. diandra*. Terpenoids, glycosides, and quinones were present in six plant extracts, and reducing sugars were present in seven extracts, as shown in table 2. Polyphenols were present in five extracts. Saponins were present in *C. pareira, A. lakoocha,* and *M. azedarch* only. These phytochemical screening results showed that the collected plant materials could be the potential source of bioactive constituents.

Table 2: The yield and result of phytochemical screening of methanol extracts of different plants

Plant Name	Yield (%)	Alkaloids	Flavonoids	Terpenoids	Glycosides	Quinones	Reducing sugars	Poly phenols	Saponins
Vetiveria zizanoids	7.51	+	+	+	+	+	+	+	–
Cissampelos pareira	10.13	+	+	+	–	+	+	–	+
Artocarpus lakoocha	15.89	+	++	–	–	+	+	+	+

Melia azedarach	13.7	+	+	+	–	+	–	–	+
Cynodon dactylon	5.25	+	–	–	+	+	–	–	+
Lycopodium clavatum	6.55	+	–	–	+	+	+	–	–
Woodfordia fructicosa	9.79	–	+	–	+	–	+	+	–
Rhododendron arboreum	17.08	–	+	+	–	–	+	+	–
Mirabilis jalapa	11.96	+	+	+	+	–	+	+	–
Drymaria diandra	13.22	+	–	+	–	–	–	–	–

(+) indicates present and (-) indicates absent

3.2 Antioxidant Activity Test

The free radical scavenging activity of ten plants extracts was determined using DPPH, a very stable free radical having purple colour. When free radical scavengers are added, DPPH is reduced, and its colour is changed to yellow based on the efficacy of antioxidants. Among the ten extracts, only seven extracts (M1, M2, M3, M4, M7, M8 and M9) changed the purple colour of the DPPH solution into yellow. So, three samples (M5, M6 and M10) were discarded from the preliminary test, and the further tests were carried out on seven samples only.

The absorbance values of the control taken (1 mL MeOH + 0.5 mL DPPH), ascorbic acid and seven samples at different concentrations (0, 12.5, 25, 50, 75 and 100 µg/mL) were measured by spectrophotometer at 517 nm. The graph of absorbance versus concentration of ascorbic acid is shown in figure 1.

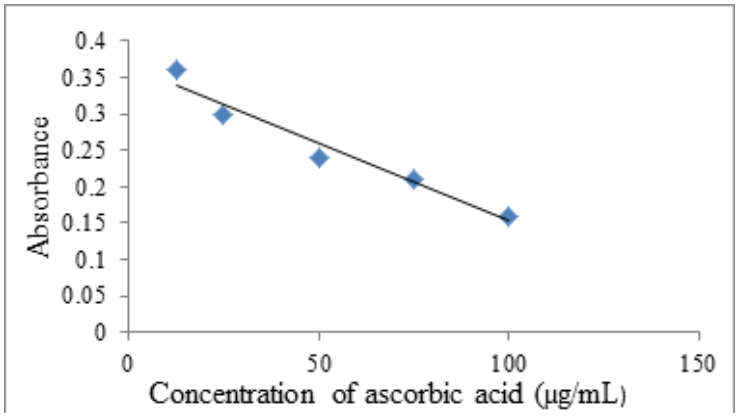

Fig. 1: Graph of absorbance vs concentration of ascorbic acid

The absorbance values were used to calculate the percentage inhibitions of DPPH radicals against the samples. The calculated values of % free radical scavenging activity of each extract and ascorbic acid are given in table 3. IC_{50} values of each samples were calculated using the plot's equations between % free radical scavenging versus concentration of samples. The comparison of % free radical scavenging activity of seven extracts against ascorbic acid are shown in figure 2, figure 3 and figure 4.

Table 3: Percentage radical scavenging activity of samples and ascorbic acid at different concentrations

Concentration (µg/mL)	% free radical scavenging activity							
	M1	M2	M3	M4	M7	M8	M9	Ascorbic acid
0	0	0	0	0	0	0	0	0
12.5	42.9	29.1	45.2	32	45	43.1	41.3	48.1 ± 2.5
25	53.2	35.4	56.1	37.1	56.3	53.4	51.4	57.0 ± 3.2
50	759	39	63.7	39.5	62.9	59.5	57.9	65.3 ± 2.8
75	64.8	42.2	68.5	44.8	68.3	65.9	63.7	70.5 ± 3.0
100	72	49.2	74.8	51.5	74	72.2	69.1	77.0 ± 3.5

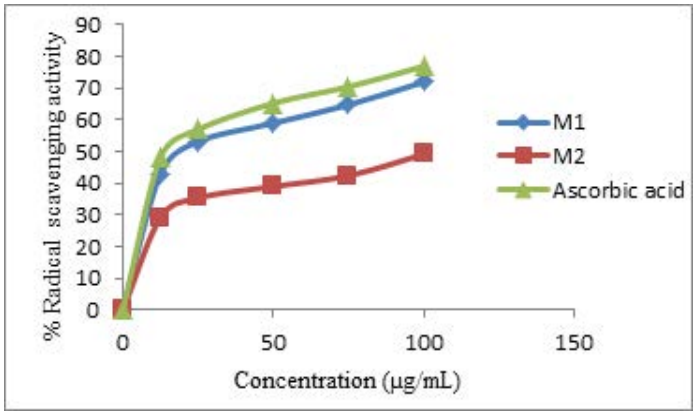

Fig. 2: Comparison of % radical scavenging between M1, M2 and ascorbic acid.

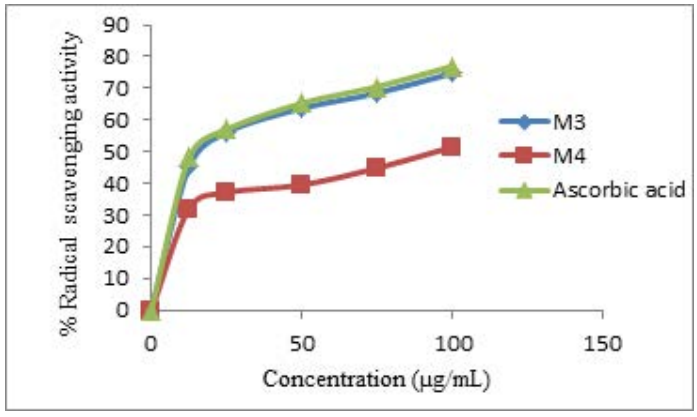

Fig. 3: Comparison of % radical scavenging between M3, M4, and ascorbic acid.

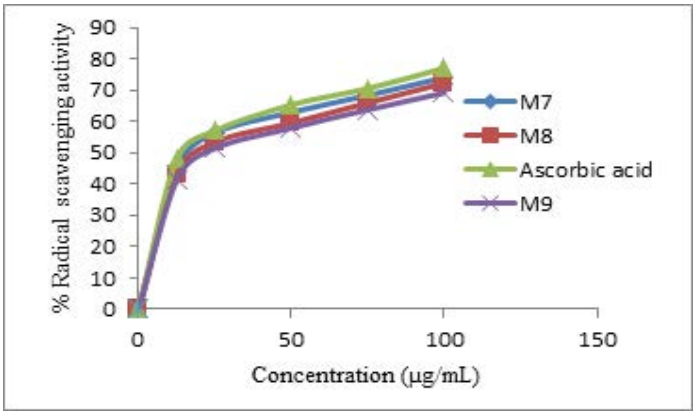

Fig. 4: Comparison of % radical sc avenging between M7, M8, M9, and ascorbic acid.

The IC_{50} values were calculated from the graph plotted between percentages of radical scavenging activity against extracts concentration. The results of the IC_{50} values are presented in table 4.

Table 4: IC_{50} values of different plant extracts and ascorbic acid

S.N.	Code	Sample	IC_{50} value (µg/mL)
1	-	Ascorbic acid	38.74± 2.5
2	M1	*Vetiveria zizanoids* (roots)	46.22± 2.0
3	*M2*	*Cissampelos pareira* (rhizomes)	90.80± 2.8
4	*M3*	*Artocarpus lakoocha* (barks)	41.42± 3.1
5	*M4*	*Melia azedarch* (barks)	85.07± 2.7
6	*M7*	*Woodfordia fructicosa* (flowers)	41.89± 2.5
7	*M8*	*Rhododendron arboreum* (flowers)	45.55± 2.2
8	*M9*	*Mirabilis jalapa* (rhizomes)	48.99± 3.0

Table 4 shows that IC_{50} values of extracts of barks of *Atrocarpus lakoocha* (41.42±3.1 µg/mL) and flowers of *Woodfordia fructicosa* (41.89± 2.5 µg/mL) were very close to that of standard ascorbic acid taken (38.74± 2.5 µg/mL). Previous studies from Singhatong *et al.* (2010) and Borah *et al.* (2016) also reported that *A. lakoocha* contained important antioxidants and polyphenolic compounds (Singhatong *et al.* 2010; Borah *et al.* 2016). Choi *et al.* (2010) isolated gallic acid from *W. fruticosa.* Sufficient gallic acid present in this plant may be responsible for the better antioxidant property (Choi *et al.* 2010). This exciting result shows that these two plant extracts are a potential source of antioxidants that the human being can exploit.

Similarly, IC_{50} values of flowers of *Rhododendron arboreum* (45.55±2.2 µg/mL), roots of *Vetiveria zizanoids* (46.22±2.0 µg/mL),and rhizomes of *Mirabilis jalapa* (48.99±3.0 µg/mL) were also near to that of ascorbic acid. This result indicates that these three plants are also a good source of antioxidants. Barks of *Melia azedarch* (IC_{50} value 85.07±2.7 µg/mL) and rhizomes of *Cissampelos pareira* (IC_{50} value 90.80±2.8 µg/mL) showed a mild antioxidant property. It may be due to the absence of polyphenolic compounds, although they have flavonoids as shown in the result of phytochemical screening (Table 2). Generally, the polyphenolic compounds and flavonoids present in the extracts are more responsible for showing antioxidant activity (Cao *et al.* 1997; Nemkul *et al.* 2018). Extracts of *Cynodon dactylon, Lycopodium clavatum* and *Drymaria diandra* do not show the antioxidant property as their extracts lack both flavonoids and polyphenols. The study explored that out of ten collected plants; seven plant samples can be useful for treating burning health issues based on their remarkable antioxidant property(Mandal *et al.* 2009; Orhan *et al.* 2007).

4. CONCLUSION

The phytochemical screening of methanol extract of all the selected plant materials revealed different classes of compounds like polyphenols, alkaloids, flavonoids, terpenoids, saponins, reducing sugar, glycosides, and quinones. Among the ten collected plant samples, methanol extracts of bark of *Atrocarpus lakoocha* and flower of *Woodfordia fructicosa* were the most potent in antioxidant activity. Flowers of *Rhododendron arboreum,* roots of *Vetiveria zizanoids* and rhizomes of *Mirabilis jalapa* also showed mild antioxidant property. Therefore, these plants could be the sources of natural antioxidants for developing drugs that cure diseases due to oxidative stress in the body.

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