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

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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 (IC₅₀ values) for antioxidant properties were calculated. Among all ten studied samples, IC₅₀ 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 (41.42±3.1 µg/mL) and 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 zizanoides, Cissampelos pareira, Artocarpus lakoocha, Melia azederach, Cyanodon dactylon, Lycopodium clavatum, Woodfordia fruticosa, 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 fruticosa 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 zizanoides* | 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 dac-tylon*  | 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 fruc-tiosa* | 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 stim-Mandal et al. ulant, relief cough                          | 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).

\[
\%\text{ radical scavenging activity} = \left[\frac{\text{Abs.} - \text{Abs. sample}}{\text{Abs. control}}\right] \times 100\%
\]

where: \(\text{Abs. control} = \text{absorbance of the control solution (1mL MeOH + 0.5 mL DPPH).}\)

\(\text{Abs. sample} = \text{absorbance of the sample}\)

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, quinones, 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.

| Plant Name        | Yield (%) | Alkaloids | Flavonoids | Terpenoids | Glycosides | Quinones | Reducing sugars | Polyphenols | Saponins |
|-------------------|-----------|-----------|------------|------------|------------|----------|-----------------|-------------|----------|
| Vetiveria zizanoids | 7.51      | +         | +          | +          | +          | +        | +               | +           | _        |
| Cissampelos pareira | 10.13     | +         | +          | +          | _          | +        | +               | _           | +        |
| Artocarpus lakoocha | 15.89     | +         | ++         | _          | _          | +        | +               | +           | +        |
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.

![Graph of absorbance vs concentration of ascorbic acid](image)

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.

| Plant Name          | Antioxidant Activity |
|---------------------|----------------------|
| Melia azedarach     | + + + - + - - +     |
| Cynodon dactylon    | + - - + + - - +     |
| Lycopodium clavatum | + - - + + + - +     |
| Woodfordia fructcosa| - + - + - + + -     |
| Rhododendron arboresum | 17.08 - + + - - + + - |
| Mirabilis jalapa    | + + + + - + + -     |
| Drymaria diandra    | + - + - - - - - -   |

(+) indicates present and (-) indicates absent
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.
The IC\textsubscript{50} values were calculated from the graph plotted between percentages of radical scavenging activity against extracts concentration. The results of the IC\textsubscript{50} values are presented in Table 4.

Table 4: IC\textsubscript{50} values of different plant extracts and ascorbic acid

| S.N. | Code | Sample                      | IC\textsubscript{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 azedarach (barks)     | 85.07± 2.7                        |
| 6    | M7   | Woodfordia fruticosa (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\textsubscript{50} values of extracts of barks of *Artocarpus lakoocha* (41.42±3.1 µg/mL) and flowers of *Woodfordia fruticosa* (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\textsubscript{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 azedarach* (IC\textsubscript{50} value 85.07±2.7 µg/mL) and rhizomes of *Cissampelos pareira* (IC\textsubscript{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 fruticosa* 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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