Antioxidant Activity of Selected Medicinal Plants of Nepal

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ABSTRACT

Method
Collected plant species were subjected to maceration in methanol for 72 hrs. Antioxidant activity of plant extracts was assessed by using DPPH free radical scavenging method in different concentrations (1 µg/ml, 3 µg/ml, 5 µg/ml, 7 µg/ml and 10 µg/ml) and percentage inhibition and effective concentration (EC50) was calculated.

Result
Result indicates that EC50 of D. boryanum (3.75 µg/ml) and P. guajava (3.89 µg/ml) was less, EC50 of R. nepalensis (5.03 µg/ml) and S. japonica (6.75 µg/ml) was comparable and EC50 of M. macrophylla (7.86 µg/ml), B. asiatica (9.14 µg/ml), E. adenophorum (7.78 µg/ml), E. crassipes (8.21 µg/ml) and N. arbortritis (8.16 µg/ml) was higher than ascorbic acid (4.73 µg/ml).

Conclusion
Our result shows that D. boryanum and P. guajava possess higher antioxidant activity than the ascorbic acid implying that, they could be potential free radical scavenging agents and could be developed as pharmaceutical agents.

Keywords: Antioxidant, DPPH, Maceration, Methanol

INTRODUCTION

Many natural products are the source of therapeutic agents having potential pharmacological activity. Traditional medicinal practices have long history for serving human kind [1]. The knowledge of ethnobotany offers diverse natural products that have potential for therapeutic use. More than fifty percent of modern drugs are from natural products [2]. Nepal is a Himalayan country rich in plant diversity. The climatic zones, ranging from sub tropic to arctic region, have supported the variation in plant species [3, 4]. Nepal is home to more than 1600 species of medicinal and aromatic plants including 1515 species of angiosperms, 19 species of gymnosperms, 56 species of pteridophytes, 5 species of bryophytes, 18 species of lichens and 1 species of fungi [5]. Free radicals, also known as reactive oxygen species (ROS), are basic to biological process such as aerobic metabolism [6]. When generation of ROS is more than the detoxification ability of cells, undue ROS causes harm to DNA, enzymes, proteins, lipids and may become mediator of inflammation, cancer and cardiovascular diseases [7]. To tackle such disorder, antioxidants have been in use. Antioxidants impede oxidative course by countering with

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free radicals, chelating metal ions and behaving as oxygen scavengers [8]. Natural antioxidants have gained a lot of curiosity in modern age due to good antioxidant activity, less side effects and low production cost [9]. Various dietary antioxidants like ascorbic acid, vitamin E, carotenoids have been isolated from natural source to defend cells damage due to oxidative stress [10]. There is an inverse relationship between morbidity and mortality from oxidative stress and intake of natural antioxidants [11, 12, 13]. The exploration of antioxidants is increasing every year, however, the understanding of main component from medicinal plants is vague that are related in dropping the risk of chronic illness, diabetes, cancer and cardiovascular disease [9, 13, 15]. The present study was designed to collect, identify, prepare herbarium and to evaluate the antioxidant activity of selected medicinal plants.

Table 1: List of collected medicinal plants

| S.N. | Scientific name/ Family | Local name (Parts used) | Pharmacological use | Isolated Compounds |
|------|--------------------------|------------------------|---------------------|--------------------|
| 1    | Mussaenda macrophylla/ Rubiaceae | Dhobini (Roots) | It is used in Snake bite and active against oral pathogen. It shows antibacterial, anticoagulant, anti-inflammatory and hepatoprotective activity [3, 16]. | 3-O acetyloleandic acid, 3-O acetylduradical, rotundic acid and 16 α-hydroxyprotobassic acid [17]. |
| 2    | Rumex nepalensis/ Polygonaceae | Halhale (Roots) | It is used in body ache, headache, wound, diarrhea, dysentery, scabies, cold and cough [18]. | Torachrysone, epicatechin gallate, orcinol glucoside, aloesin, epicatechin and lyoniresinol 3 α-O-β-D-glucopyranoside [19]. |
| 3    | Dryoathyrium boryanum/ Aspidiaceae | Kalo neuro (Roots) | It is used as laxative, demulcent and stomachic [20]. | 3-hydroxyphloretin 6-O-hexoside, quercetin-7-hexoside, apigenin7-O-glucoside, luteolin 7-O-glucoside, apigenin 7-O-galactoside, 3-hydroxyphloretin 6-O-hexoside, luteolin-6-C-glucoside [21]. |
| No. | Plant Name                          | Part Used | Uses                                                                 | Identified Compounds                                                                 |
|-----|------------------------------------|-----------|----------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| 4   | *Berberis asiatica* / Berberidaceae| Chutro (Leaves) | It is used in eye and skin disease, jaundice, rheumatism and diabetes [22, 23]. | Berberine, palmmitine, jatrorrhizine, columbamine, tetrahydropalmmitine, berbamine, oxyberberine and oxyacanthine were identified from *B. Asiatica* [24]. |
| 5   | *Psidium guajava* / Mystraeace     | Amba (Fruits) | It is used in wounds, gastrointestinal disorder, lesions, ulcers, diarrhea, cholera, hypertension, obesity and diabetes mellitus [25]. | Myricetin, quercetin, nerolidiol, aromadendrene, 1,8-cineol, oleanic acid, ursolic acid, catecolic acid, guayavolic acid, maslinic acid, ellagic acid and β-sitosterol [26, 27]. |
| 6   | *Eupatorium adenophorum* / Asteraceae| Banmara (Leaves) | It is used in dysentery and stomachache. Decoction of this plant is used in jaundice. It also possesses pneumotoxic and hepatotoxic effects [28]. | Coumarin, 5-exo-hydroxy-borneol, O-hydroxyl cinnamic acid, 9β-hydroxy-ageraphorone and 9-oxo-10, 11-dehydroageraphorone [29]. |
| 7   | *Eichhornia crassipes* / Pontederiaceae | Jalkumbi (Leaves) | It possess antiinflammatory, anticancer and antibacterial activities. Anabolic steroid supports nitrogen maintenance in osteoporosis in animals with wasting illness [30, 31, 32]. | 2-methylresorcinol, catechol, pyrogallol, genitcic, salicylic acid, kaempferol, orientin quercetin, pipradrol [33]. |
| 8   | *Nyctanthes arbor-tristis* / Oleaceae | Parijat (Leaves) | It possess anti-inflammatory, antipyretic, anti-noiceptive, anti-leishmanial, immuno-stimulant, antimicrobial, antiviral activities [34]. | D- mannitol, sitosterone, astragaline, carotenoid, crocin-3, p-cymene [34]. |
| 9   | *Stephania japonica* / Menispermaceae | Batulepati (Leaves) | It is used in tuberculosis, cancer, fever, intestinal complaints, asthma, hyperglycemia and dysentery [35]. | Fangchinoline, tetrandrine [36]. |

**Preparation of extract**

Crude samples were washed with distilled water, cut into small pieces and shade dried. The dried sample were grounded and were allowed for cold maceration with methanol at room temperature for 72 h. 50 g of each plant material was macerated with 500 ml of methanol (in ratio of 1: 10) at room temperature for 24 h. The extract was filtered using Whatman No.1 filter paper to obtain methanolic extract. The residue left was again subjected to second and third successive maceration with 500 ml methanol for another 24 h under previous conditions. Methanolic extracts of sample were concentrated in rotary evaporator at 40°C and 250-175 mbar pressure at 90 rpm and 5°C chilling temperature. Further drying was done in
vacuum desiccator at pressure 60 mbar. Thus, obtained dried methanolic extract was stored at 4°C in refrigerator for further experiment.

Antioxidant activity
Antioxidant activity was tested by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay [37]. This method is simple, plain, replicable and economical. DPPH is deep violet in colour due to the delocalization of the auxiliary electrons. Antioxidant compounds donate hydrogen atom to the free radicals resulting to neutral colourless compound. Ascorbic acid was used as positive control which is standard antioxidant. DPPH solution without sample extract served as negative control.

Preparation of stock solution
The stock solution of 1 mg/ml of each sample extract in methanol was prepared. Ascorbic acid solution of the same concentration was prepared using methanol.

Preparation of plant samples
Different concentrations (10, 7, 5, 3 and 1 µg/ml) were prepared by serial dilution using methanol.

Preparation of ascorbic acid solution
Ascorbic acid was taken as standard. Different concentrations (10, 7, 5, 3 and 1 µg/ml) of ascorbic acid were prepared by serial dilution using methanol.

Preparation of DPPH Solution
A 100 µM DPPH solution was prepared by dissolving 19.7 mg of DPPH in 500 ml of methanol.

DPPH radical scavenging activity
DPPH was dissolved in 500 ml of methanol to prepare 100 µM DPPH solution. Extracted solution of 1 ml of different concentrations was mixed with 1 ml of DPPH solution. Then it was incubated for 30 minutes at room temperature and then the absorbance was measured in UV spectrophotometer at 517 nm. UV spectroscopy is based on the principle that molecules having π- electrons or nonbonding electrons can absorb energy in the form of ultraviolet or visible light and excite such electrons to higher orbital [38]. Each assay was performed in triplicates and radical scavenging activity was calculated by using following equation:

\[
\% \text{ Inhibition} = \frac{A - B}{A} \times 100\%
\]

Where, A is absorbance of DPPH solution (Negative Control), B is the absorbance of DPPH solution in the presence of test sample. The scavenging activity (%) was then plotted against concentration and from the graph.

STATISTICAL ANALYSIS:
Half maximal effective concentration (EC\textsubscript{50}) value was calculated by using linear regression analysis with Microsoft office excel 2007. All the values and data were expressed as Mean ± SEM, N=3, Where, SEM= Standard error of mean and N= Number.

RESULTS
The present study was done to explore antioxidant activity of medicinal plants of traditional practice having different phytochemicals [39]. Present study reveals dose dependent activity from 1 to 10 µg/ml for all selected plants. Result indicates that EC\textsubscript{50} of D. boryanum (3.75 µg/ml) and P. guajava (3.89 µg/ml) was less than ascorbic acid (4.73 µg/ml). Also, EC\textsubscript{50} of R. nepalensis (5.03 µg/ml) and S. japonica (6.75 µg/ml) was comparable and EC\textsubscript{50} M. macrophylla (7.86 µg/ml), B. asiatica (9.14 µg/ml), E. adenophorum (7.78 µg/ml), E. crassipes (8.21 µg/ml), and N. arbor-tristis (8.16 µg/ml) was slightly higher than that of ascorbic acid. All determinations (except EC\textsubscript{50}) were carried out in triplicate and the values are expressed as mean± SEM.
Table 2: Percentage inhibition and effective concentration (EC50) of medicinal plants and Ascorbic acid.

| Sample            | Percentage inhibition ± SEM | EC50  |
|-------------------|-------------------------------|-------|
|                   | 1 µg/ml | 3 µg/ml | 5 µg/ml | 7 µg/ml | 10 µg/ml |
| Ascorbic acid     | 24.68±1.23 | 39.64±0.35 | 52.67±0.34 | 65.87±2.17 | 81.87±0.21 | 4.73 |
| *M. macrophylla*  | 5.31±2.21 | 19.78±0.98 | 32.76±0.31 | 44.76±0.65 | 62.84±0.67 | 7.86 |
| *R. nepalensis*   | 25.17±0.34 | 35.87±2.32 | 49.65±0.45 | 61.98±0.76 | 82.84±0.22 | 5.03 |
| *D. boryanum*     | 35.52±0.45 | 46.64±1.23 | 57.34±1.22 | 65.89±0.34 | 80.63±0.54 | 3.75 |
| *B. asiatica*     | 18.66±0.87 | 25.67±0.34 | 33.54±2.39 | 40.65±1.17 | 54.11±0.32 | 9.14 |
| *P. guajava*      | 33.22±0.12 | 45.89±0.31 | 56.87±2.26 | 66.87±2.31 | 82.43±1.13 | 3.89 |
| *E. adenophorum*  | 23.37±2.12 | 32.45±1.12 | 39.98±1.11 | 46.76±1.25 | 58.21±2.21 | 7.78 |
| *E. crassipes*    | 19.61±0.34 | 27.76±1.76 | 37.54±1.91 | 45.65±0.11 | 56.66±0.76 | 8.21 |
| *N. arbor-tristis*| 7.58±0.56 | 17.89±2.29 | 29.65±0.67 | 42.76±0.13 | 61.87±0.14 | 8.16 |
| *S. japonica*     | 19.02±0.22 | 28.87±2.23 | 39.76±0.21 | 50.87±0.50 | 68.58±0.34 | 6.75 |

Figure 1: Percentage inhibition of medicinal plants and Ascorbic acid
DISCUSSION
ROS are highly unstable unit with one or more odd electrons. The movement of free radical can cause severe damage to the body. Various plants with antioxidant activity can defend themselves against ROS. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms [40]. Various spectrometric methods like DPPH assay, FRAP (ferric reducing antioxidant power) assay, PFRAP (potassium ferricyanide reducing power) assay, TRAP (total peroxyl radical trapping antioxidant parameter) assay, ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) are used to establish in vitro antioxidant activity[41]. Compounds having high in vitro antioxidant activity are likely to demonstrate high in vivo antioxidant activity [42]. In this research, antioxidant activity of nine plants was studied using DPPH free radical scavenging method. Result indicates that, extract of *D. boryanum* and *P. guajava* have good antioxidant activity than standard ascorbic acid. It implies that, plants constitute those compounds that can donate hydrogen atom to the odd electron which is responsible for radical’s reactivity [43]. The possible mechanism by which plant inhibit the free radicals would be attributed to the inhibitory effect of extract towards generation of free radicals in the *in vitro* reaction system [44].

CONCLUSION
The results in the study signify that the extract of *D. boryanum* and *P. guajava* reveals free radical scavenging activity against DPPH radical. The antioxidant activity of these extract might be due to their polyphenolic content and phytochemical constituents. This research suggested that of *D. boryanum* and *P. guajava* can be use as a source of natural antioxidants.
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AUTHORS’ CONTRIBUTIONS
Mr. GS was involved with the design of the study, and wrote the first draft of the manuscript. Mr. GS, Mr. BS, Mr. MA and Mr. GL collected data and involved with data analysis. Mr. GS performed statistical analysis. Mr. GS and Ms. PK revised the manuscript for important intellectual content. All authors read, edited and approved the final manuscript.

COMPETING INTERESTS
The authors declare that they have no competing interests.

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