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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 (EC_{50}) was calculated.

Result

Result indicates that EC_{50} of *D. boryanum* (3.75 µg/ml) and *P. guajava* (3.89 µg/ml) was less, EC_{50} of *R. nepalensis* (5.03 µg/ml) and *S. japonica* (6.75 µg/ml) was comparable and EC_{50} 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. gvajava* 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 theurapeutic 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.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and solvents used during the experiment were of analytical grade. Methanol, which was used as principle solvent for maceration, was obtained from Fisher Scientific, India. Chemicals like ascorbic acid and DPPH were purchased from Qualigens fine chemicals, India and Wako Pure Chemicals, Japan respectively. Instruments like UV-Vis spectrophotometer and Rotary Evaporator were acquired from Shimadzu, Japan and Buchi Labortechnik, Switzerland respectively.

Plant materials

Selected medicinal plants were collected from the different region of the Nepal. Identification of plants was done at National Herbarium and Plant Laboratories, Godawari, Lalitpur Provience No. 3 Nepal.

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 activitiy [3, 16].	3-O acetyloleanolic acid, 3-O acetyldaturadiol, 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, epicatechein gallate, orcinol glucoside, aloesin, epicatechein 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-hydroxy phloretin 6-O-hexoside, luteolin-6-C-glucoside [21].

4	*Berberis asiatica*/ Berberidaceae	Chutro (Leaves)	It is used in eye and skin disease, jaundice, rheumatism and diabetes [22, 23].	Berberine, palmitine, jatrorrhizine, columbamine, tetrahydropalmitine, berbamine, oxyberberine and oxyacanthine were identified from *B. Asiatica* [24].
5	*Psidium guajava*/ Mystraece	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, 9*β*-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, genetisic, salicylic acid, kaempferol, orientin quercetin, pipradrol [33].
8	*Nyctanthes arbor-tristis*/ Oleaceae	Parijat (Leaves)	It possess anti-inflammatory, antipyretic, anti-nociceptive, anti-leishmanial, immuno-stimulant, antimicrobial, antiviral activities [34].	D- mannitol, sitosterole, 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 Whattman 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 40ºC and 250-175 mbar pressure at 90 rpm and 5ºC 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:

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

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.

STASTISTICAL ANALYSIS:

Half maximal effective concentration (EC_{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_{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_{50} of *R. nepalensis* (5.03 µg/ml) and *S. japonica* (6.75 µg/ml) was comparable and EC_{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_{50}) were carried out in triplicate and the values are expressed as mean± SEM.

Table 2: Percentage inhibition and effective concentration (EC_{50}) of medicinal plants and Ascorbic acid.

Sample	Percentage inhibition ± SEM					EC $_{50}$
	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±0.56	68.58±0.34	6.75

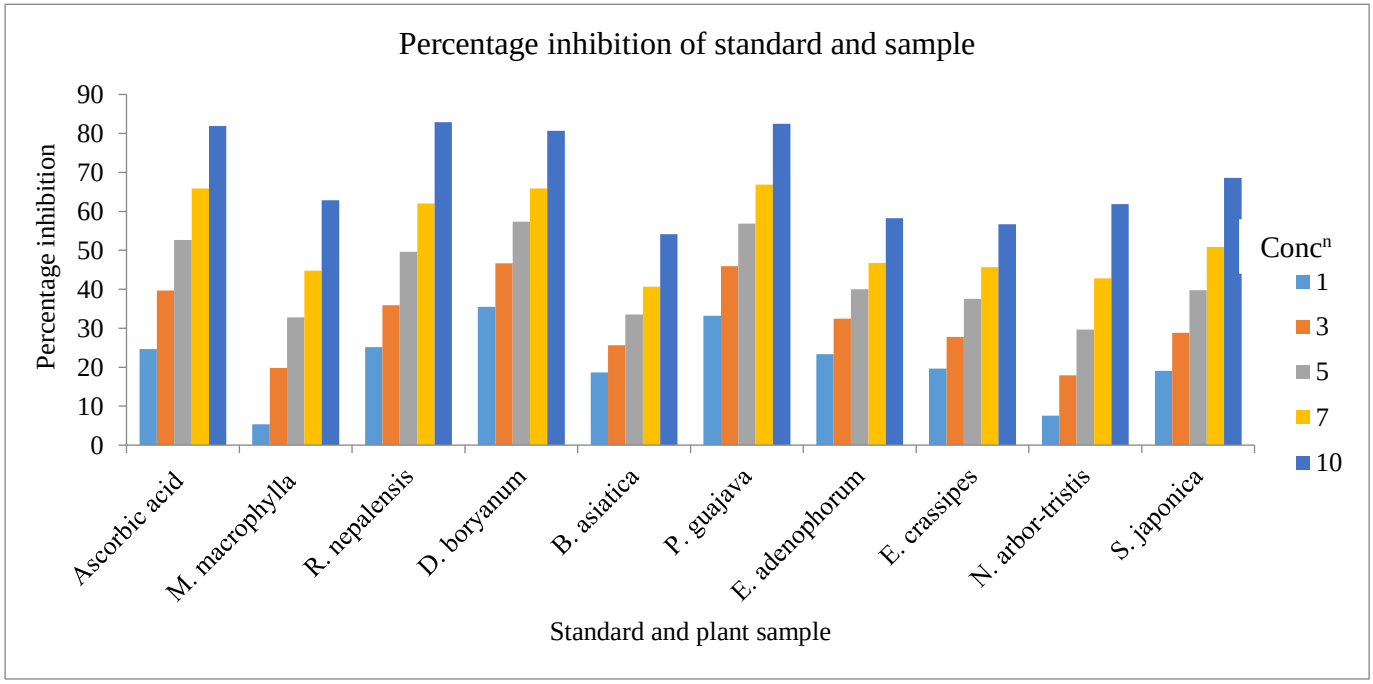

Figure 1: Percentage inhibition of medicinal plants and Ascorbic acid

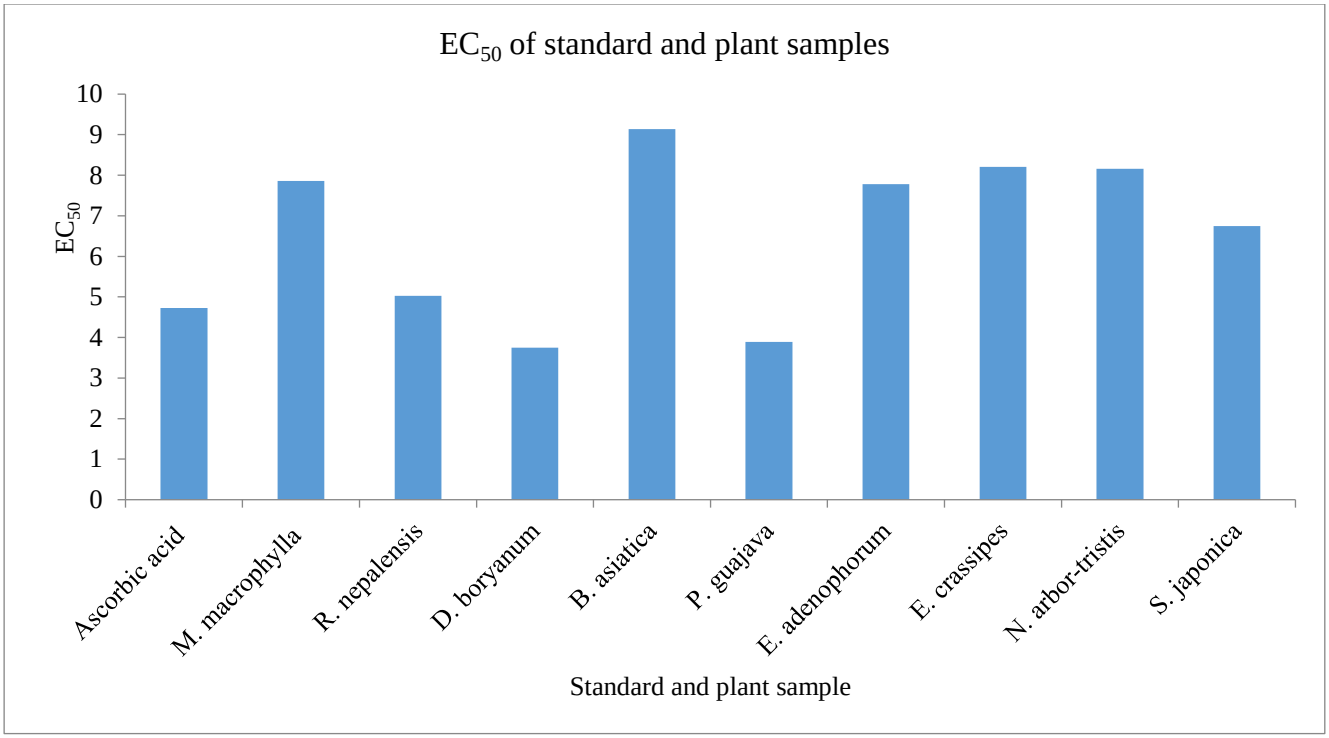

Figure 2: Effective concentration of selected medicinal plants

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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