Original Research Article

Antioxidant Activity of Selected Medicinal Plants of Nepal.

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ABSTRACT

Objective
The objective of the study was to investigate the antioxidant activity of selected medicinal plants.

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) values were 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 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 slightly 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 good free radical scavenging agents and should be urbanized as a future pharmaceutical agent.

Keywords: Antioxidant, DPPH, Maceration, Methanol

INTRODUCTION

Nature is source of therapeutic agents having potential pharmacological activity. Traditional medicinal practices have long history for serving human kind. The knowledge of ethnobotany offers the diverse natural products which possess the foundation for research and development of lead molecule. Also, more than fifty percent of modern drugs are from natural products [1]. Nepal is a Himalayan country rich in plant diversity, where there are more than 1600 species of medicinal and aromatic plants are found 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 [2]. The climatic zone, ranging from sub tropic to arctic region, has also supported the variation in plant species [3, 4].

Free radicals, also known as reactive oxygen species (ROS), are basic to biological process and correspond to a vital part of aerobic metabolism. When generation of ROS is higher than detoxification ability of cell, undue ROS causes harm to DNA, enzymes, proteins, lipids and serves as mediator of inflammation and cancer. Such circumstances are believed to be vital causal aspect in the progress of ailment such as diabetes, arteriosclerosis, cancer and cardiovascular diseases [5]. In the management of such disorder, antioxidants have achieved a huge significance. These free radicals scavengers impede with the oxidative course by countering with free radicals, chelating metal ions and behaving as oxygen scavengers [6]. Due to their immense antioxidant activity, less side effects and low cost, the physiological effects of natural antioxidants have produced lots of curiosity in the modern age [7]. Various dietary antioxidants like ascorbic acid,

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vitamin E, carotenoids have been tried to isolate from natural source by researchers as they can defend the cell from damage provoked by oxidative stress. There is an inverse relation between morbidity and mortality from oxidative stress and intake of natural antioxidants [8]. There is an increase in exploration for antioxidants from natural sources ever since. It is not known which component of medicinal plants are related in dropping the risk of chronic illness, but antioxidants are supposed to have a main function in the preventive nature of plant medicine [9]. 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 were of analytical grade. Equipments, glasswares and other requirements were provided by School of Health and Allied Sciences, Pokhara University. 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, Nepal. Crude drug samples and herbarium specimens are preserved in museum of School of Health and Allied Science, Pokhara University.

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, 10].	3-O acetyloleanolic acid, 3-O acetyldaturadiol, rotundic acid and 16 α-hydroxyprotobassic acid [11].
2	*Rumex nepalensis*/ Polygonaceae	Halhale (Roots)	It is used in body ache, headache, wound, diarrhea, dysentery, scabies, cold and cough [12].	Torachrysone, epicatechein gallate, orcinol glucoside, aloesin, epicatechein and lyoniresinol 3 α–O-β-D-glucopyranoside [13].
3	*Dryoathyrium boryanum*/ Aspidiaceae	Kalo neuro (Roots)	It is used as laxative, demulcent and stomachic [14].	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 [15].
4	*Berberis asiatica*/ Berberidaceae	Chutro (Leaves)	It is used in eye and skin disease, jaundice, rheumatism and diabetes [16, 17].	Berberine, palmitine, jatrorrhizine, columbamine, tetrahydropalmitine, berbamine, oxyberberine and oxyacanthine were identified from *B. Asiatica* [18].
5	*Psidium guajava*/ Mystraece	Amba (Fruits)	It is used in wounds, gastrointestinal disorder, lesions, ulcers, diarrhea, cholera, hypertension, obesity and diabetes mellitus [19].	Myricetin, quercetin, nerolidiol, aromadendrene, 1,8-cineol, oleanic acid, ursolic acid, catecolic acid, guayavolic acid, maslinic acid, ellagic acid and β-sitosterol [20, 21].
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 [22].	Coumarin, 5-exo-hydroxy-borneol, *O*-hydroxyl cinnamic acid, 9β-hydroxy-ageraphorone and 9-oxo-10, 11-dehydroageraphorone [23].
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 [24, 25, 26].	2-methylresorcinol, catechol, pyrogallol, genetisic, salicylic acid, kaempferol, orientin quercetin, pipradrol [27].

8	*Nyctanthes arbor-tristis*/ Oleaceae	Parijat (Leaves)	It possess antiinflammatory, antipyretic, antinociceptive, antileishmanial, immunostimulant, antimicrobial, antiviral activities [28].	D- mannitol, sitosterole, astragaline, carotenoid, crocin-3, *p*-cymene [28].
9	*Stephania japonica*/ Menispermaceae	Batulepati (Leaves)	It is used in tuberculosis, cancer, fever, intestinal complaints, asthma, hyperglycemia and dysentery [29].	Fangchinoline, tetrandrine [30].

Preparation of extract

Crude samples were washed with distilled water, cut into small pieces and shade dried. The dried and grounded crude drugs 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 all the plant parts were concentrated in rotary evaporator at 40 ^{o}C and 250-175 mbar pressure at 90 rpm and 5 ^{o}C chilling temperature. Further drying was done in vacuum desiccator at pressure 60 mbar. Thus, obtained dried methanolic extract was stored at 4 ^{o}C in refrigerator for further experiment.

Antioxidant activity

Antioxidant activity was tested by DPPH free radical scavenging assay [31]. This method is simple, plain, replicable and economical. Due to the delocalization of the auxiliary electrons, DPPH is stable free radical which is signature for the deep violet colour of DPPH. Antioxidant compounds offer hydrogen atom to the free radicals resulting to neutral compounds with loss of colour. A standard antioxidant ascorbic acid was used as positive control. DPPH solution without extract or ascorbic acid served as negative control.

Preparation of stock solution

The stock solution of 1 mg/ml of each plant 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. Extract 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 the absorbance was measured at 517 nm in UV spectrophotometer. UV follows absorbance spectroscopy and works on the principle of molecules having π- electrons can absorb energy in the form of ultraviolet or visible light and excite these π- electrons to higher antibonding orbit [32]. Each assay was performed in triplicates. 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.

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 [33]. 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 which implies their potency. 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

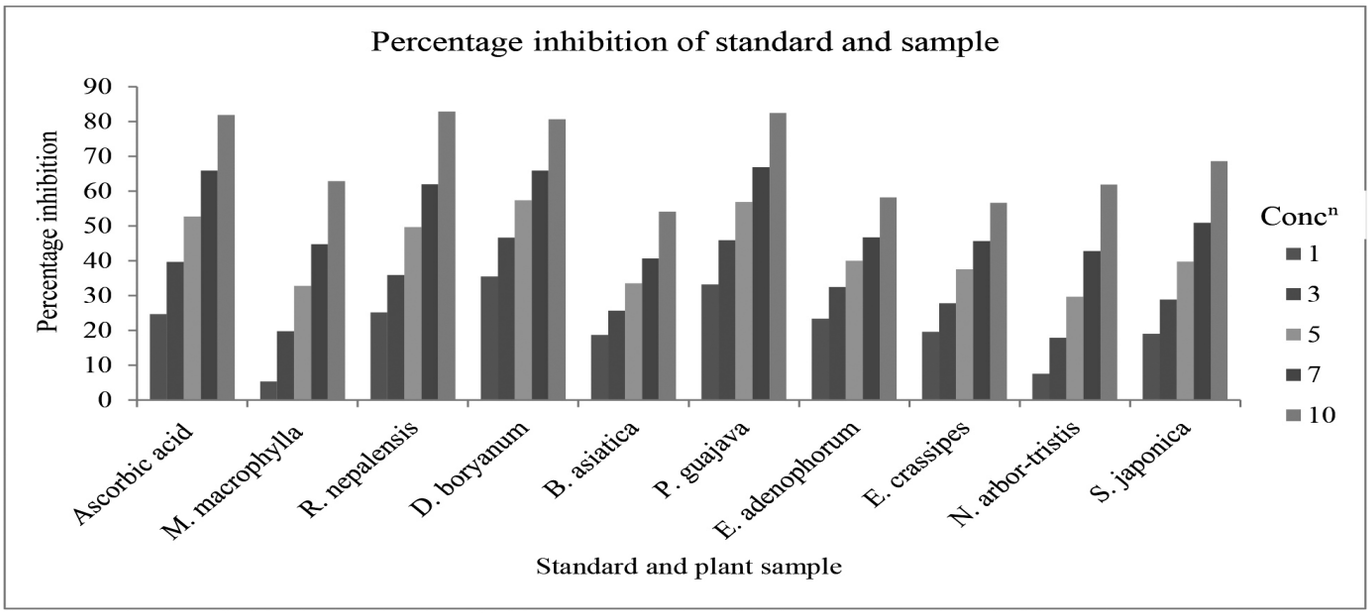

Figure 1: Percentage inhibition of medicinal plants and Ascorbic acid

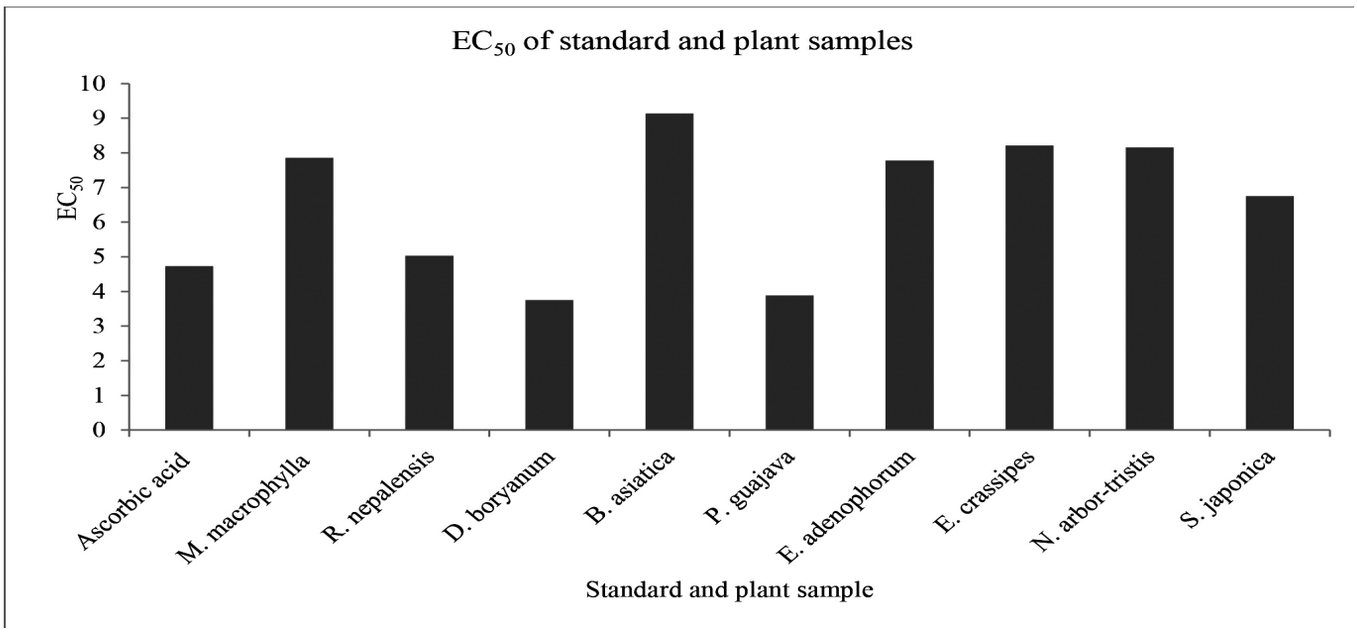

Figure 2: Effective concentration of selected medicinal plants

DISCUSSION

Free radicals are highly unstable unit which can subsist with one or more odd electrons. The movement of free radical can cause severe damage to the body cell and tissue. Various floras with radical scavenging activity can defend themselves against these unstable units. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms [34]. Various methods have been used to establish the antioxidant activity *in vitro* to permit the quick screening of compounds as compounds having low antioxidant activity *in vitro*, would likely to demonstrate less potency *in vivo* [35]. In this research, antioxidant activity of nine plants was studied using DPPH free radical scavenging method. While, the potency of extract to scavenge these free radicals rely upon the mechanism of antioxidant activity and methodology applied in this research [5]. The result indicates that, extract of *D. boryanum* and *P. guajava* have good antioxidant activity. It implies that, plants constitute those compounds that can give hydrogen atom to the odd electron which is responsible for radical's reactivity [36]. The radical scavenging potential of the plants were compared against the standard ascorbic acid signifying that the plants can be effective scavengers of free radicals. 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 [37].

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* could be a probable source of natural antioxidant which will possess huge value as curative agents in slowing the progress of reactive oxygen species and associated oxidative stress related degenerative disease.

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