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Research Article

In Vitro Free Radical Scavenging Activity of Methanol Extracts of Some Selected Medicinal Plants of Nepal

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Abstract

Antioxidant potential of phenolic content from methanol extracts of fifteen selected medicinal plants of Nepal were analyzed by using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) scavenging assay, reducing power and total antioxidant capacity. Out of them *Acacia catechu*, *Berberis aristata*, *Bauhinia variegata*, *Cleistocalyx operculatus*, *Shorea robusta, Bergenia ciliata, Phyllanthus emblica, Bombax ceiba, Lyonia ovalifolia, Bridelia retusa, Drymaria diandra, Euphorbia hirta, Callicarpa sp*., *Ziziphus mauritiana,* and *Scoparia dulcis* showed potent antioxidant activity as compared with the IC_{50} value of the reference standard ascorbic acid. The total phenolic content was measured to be 95.80±3.6 mg GAE/gm in *Ziziphus mauritiana* and 169.35±0.2 mg GAE/gm in *Acacia catechu.* The total flavonoid content was measured to be 10.70±0.0 mg QE/gm in *Callicarpa sp.* and 18.6±0.3 mg QE/gm in *Acacia catechu.* This study indicated medicinal plants in Nepal have strong antioxidant potential with high total phenolic and flavonoids content. The methanol extract of these plants may be a potent source of natural antioxidant and its use in the management of diseases associated with oxidative stress is justified. This study may provide the scientific basis for traditional use of those plants and may provide valuable information for further research.

Keywords: Medicinal plants; Antioxidant; DPPH; Plant extract free radical

Introduction

Nepal has significantly diverse ecosystems producing a wide range of unique and valuable medicinal plant resources. The Ayurvedic health care system depends solely on the use of these highly valued native medicinal plants [1]. The majority of Nepalese peoples are dependent on indigenous use of medicinal plants. Medicinal and aromatic plants play vital role in Nepalese livelihood health and socio-economic prospects. The majority of Nepal's population, especially tribal, ethnic groups and mountain people relies on traditional medical practices. In many cases this practice is transmitted orally from generation to generation and confined to them [2,3]. Very few systematic studies have been done on the medicinal plants of Nepal for their antioxidant and anticancer activities. Thus, in the present study, we collected and screened the Nepalese medicinal plants from different ecological regions of Nepal for the antioxidant activity leading to the isolation of active compounds. Oxidation induced by Reactive Oxygen Species (ROS) result in cell membrane disintegration, membrane protein damage and DNA mutation. The effect can further initiate or propagate the development of many diseases, such as cancer, liver injury, cardiovascular disease, tumour inflammation, hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal, ulcerogenesis, asthma, rheumatism and neurodegenerative diseases [4-6]. Although the body possesses such defense mechanism, as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS, continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage [7,8]. Oxidants are capable of stimulating cell division, which is a critical factor in mutagenesis when a cell with a damaged DNA stand divides, cell metabolism and duplication becomes deranged [9]. Thus, mutation can arise which in turn is an important factor in carcinogenesis. Cigarette smoking and chronic inflammation are of the major causes of cancer which have strong free radical components in their mechanism of action [10-12]. Some reports have indicated that people who smoke tend to have lower antioxidant levels than non-smokers and these smokers are at an increased risk for both cancer and cardiovascular disease [13]. It is generally assumed that frequent consumption of plant derived phytochemicals from vegetables; fruits, tea and herbs may contribute to shift the balance toward an adequate antioxidant status [14-16]. In this study we selected fifteen medicinal plants from different ecological regions of Nepal based on ethnobotanical applications. We found that these medicinal plants are the potent sources to isolate the pure antioxidant compound that could be used for management of different chronic illnesses.

Materials and Method

Plant materials

The plant samples were collected from different ecological regions of Nepal based on their ethnobotanical uses as shown in Figure 1 and Table 1. Dr. Rita Chhetry, Research Officer, National Herbarium and Plant Resources, Ministry of Forests and Soil Conservation, Godawari, Nepal identified the plants.

Extraction

The collected plant samples was rinsed in clean water and dried at room temperature for 2 weeks. Thereafter the dried plants samples were ground into powder using a mortar and pestle; the powder obtained was then used to prepare the extracts. A total of 50 gram

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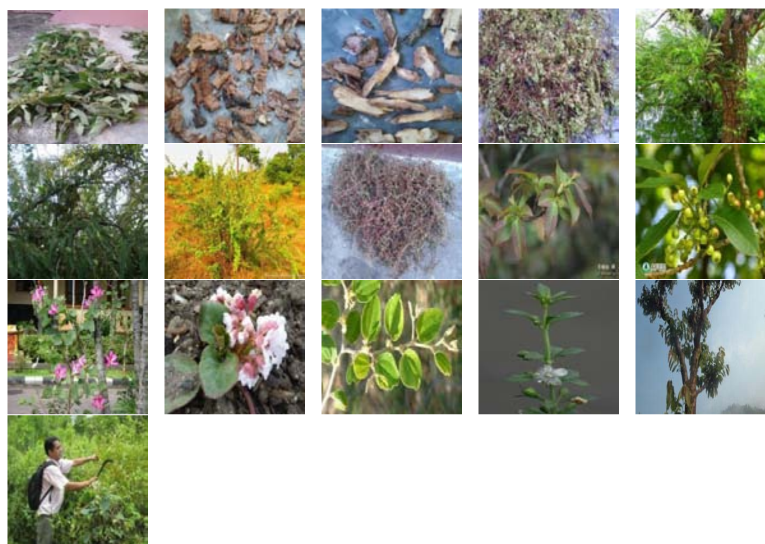

Figure 1: Plant material used for the extraction analysis, from top left, *Callicarpa sp., Shorea robusta, Bombax ceiba, Drymaria diandra, Acacia catechu, Phyllanthus emblica, Berberris aristata, Euphorbia hirta, Lyonia ovalifolia, Cleistocalyx operculatus, Bauhinia variegata, Bergenia ciliata, Ziziphus mauritiana, Scoparia dulcis, Bridelia retusa* and plant material collected from the site.

Table 1: List of plants used in the study with their traditional uses.

S.No.	Name of the plants	Family	Local Name	Locality	Extract %	Part used	Therapeutic uses
1	*Acacia catechu*	Fabaceae	Khayar	Chitwan	30	Bark	Antidiuretics, coolant
2	*Shorea robusta*	Dipterocarpaceae	Sal	Chitwan	14.6	Bark	Analgesics, antibacterial
3	*Phyllanthus emblica*	Phyllanthaceae	Amala	Chitwan	36	Leaf	Diabetes
4	*Berberis aristata*	Berberidaceae	Chutro	Kathmandu	28	Leaf	Antifungal, antibacterial
5	*Euphorbia hirta*	Euphorbiaceae	Dudhe jhar	Chitwan	10.8	Whole plant	Antimicrobial
6	*Lyonia ovalifolia*	Ericaceae	A anger	Syangja	12	Leaf	Skin diseases, antiparasite.
7	*Bridelia retusa*	Euphorbiaceae	Gayo	Syangja	42.2	Bark	Antirheumatic, antifungal
8	*Cleistocalyx operculatus*	Myrtaceae	Kyamuno	Syangja	31	Bark	Muscular swelling
9	*Bauhinia Variegata*	Fabaceae	Koiralo	Syangja	55.2	Bark	Asthma and ulcer
10	*Bergenia ciliata*	Saxifragaceae	Pakhanvedh	Manang	37.2	Root	Stone in the body
11	*Bombax ceiba*	Bombaceae	Simal	Chitwan	48.4	Bark	Oxylosis activity
12	*Callicarpa sp.*	Labiatae	Dhaichamle	Chitwan	10.4	Aerial parts	Antifungal
13	*Ziziphus mauritiana*	Rhamnaceae	Bayar	Chitwan	24.8	Leaf	Gastrointestinal, antivirus
14	*Drymaria diandra*	Caryophyllaceae	Abhijhalo	Chitwan	6.8	Whole plant	Anti HIV, sinusitis
15	*Scoparia dulcis*	Scrophulariaceae	Chini jhar	Chitwan	12.91	Whole plant	Antidiabetic, anti-inflammatory

of each of the ground powder was weight into conical flasks and 500 ml methanol poured into each of the flasks and soaked for 72hrs and filtered using Whatman No 1.filter paper. The filtrate obtained was concentrated under reduced pressure in a rotatory evaporator to obtain the crude extract. These crude extracts were used for further investigation of total polyphenol content, flavonoid content and antioxidant properties.

Total polyphenol content determination

The total phenolic content was determined using the Folin–Ciocalteu phenol reagent. 0.1 mL of each extract (2.5 mg/mL) was separately mixed with 1 mL of Folin–Ciocalteu phenol reagent and 0.5 mL of aqueous 1 M Na_2CO_3 solution. The reaction mixture was allowed to stand for about 15 minutes and the absorbance of the reactants was measured at 765 nm using the UV- visible spectrophotometer. The calibration curve was obtained using the solution of gallic acid as standard in methanol using the concentration ranging from 25-250 μg/mL. Based on this standard graph of gallic acid, the concentrations of the individual samples were calculated. The total polyphenol content was expressed in terms of the milligrams of the gallic acid equivalent per gram of the dry mass (mg GAE g^{-1}) [16].

Total flavonoid content determination

The total flavonoid content in the fifteen selected medicinal plant extracts was estimated by using the Aluminium chloride ($AlCl_3$) colorimetric method. In this method, 0.25 mL extract (10 mg/mL) was separately mixed with the 0.75 mL of methanol, 0.05 ml of the 10% aluminum chloride, 0.05 ml of the 1 M potassium acetate

Table 2: Phytochemical analysis of the plants.

S.No.	Name of the plants	Poly.p	Ster.	Flav.	Alk.	Gly.	Red. S.	Tann.	Car. G.	Ant. Q.	Caro.	Sap.
1	*Acacia catechu*	++	--	+	--	+	+	-	+	+	-	+
2	*Shorea robusta*	++	--	+	--	-	-	+	+	+	-	-
3	*Phyllanthus emblica*	+++	++	++	--	-	-	-	+	+	-	-
4	*Berberis aristata*	++	++	+	++	-	-	-	-	+	-	-
5	*Euphorbia hirta*	+	++	+	++	-	-	-	-	+	+	-
6	*Lyonia ovalifolia*	++	-	++	--	-	-	+	+	+	-	+
7	*Bridelia retusa*	++	--	--	+	+	+	+	+	+	+	-
8	*Cleistocalyx operculatus*	+++	--	--	+	+	+	+	-	+	+	-
9	*Bauhinia Variegata*	+	--	--	+	-	-	+	+	+	+	+
10	*Bergenia ciliata*	++	--	--	++	+	+	+	-	+	+	-
11	*Bombax ceiba*	+	--	-	++	+	+	+	+	+	+	+
12	*Callicarpa sp.*	++	++	++	+	-	-	+	-	+	+	-
13	*Ziziphus mauritiana*	++	--	+	++	-	-	+	+	+	+	+
14	*Drymaria diandra*	+	+	+	-	-	-	+	-	-	+	-
15	*Scoparia dulcis*	+	+	+	-	+	+	-	+	+	+	+

Poly. P. = Polyphenols, Ster. = Steroids, Flav. = Flavonoids, Alk. = Alkaloids, Gly = Glycosides, Red. S.= Reducing sugars, Tann. = Tannins, Car. G. = Cardiac glycosides, Ant. Q. = Anthraquinone, Caro. = Carotenoids, Sap. = Saponins.

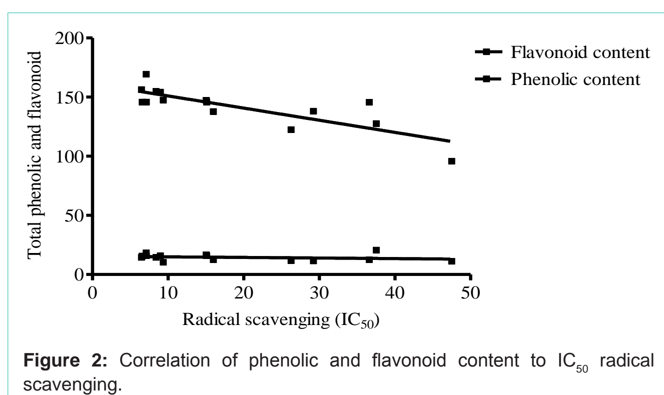

Figure 2: Correlation of phenolic and flavonoid content to IC_{50} radical scavenging.

(CH_3COOK) and 1.4 mL of the distilled water. The reaction mixture was allowed to stand for about 30 minutes in room temperature. The absorbance of the mixture was measured at 415 nm using the UV visible spectrophotometer. The calibration curve was constructed with the help of standard quercetin solutions in methanol with the concentration ranging from the 10-100µg /mL. The total flavonoid content was expressed in terms of the milligram of quercetin equivalent per gram of the dry mass (mg QE/gm) [17].

DPPH radical scavenging assay

The free radical scavenging activity was measured by using DPPH assay [18-20]. Different concentration of test samples (5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μg/mL) was prepared while the concentration of DPPH was 0.2mM in the reaction mixture. These reaction mixtures were taken in tubes and incubation at 37 ^{0}C for 30 min. Discolorations were measured at 517 nm using a UV-Visible Spectrophotometer. Percent radical scavenging activity by sample treatment was determined by comparison with methanol treated control group; ascorbic acid was used as positive control. Measurement was performed at least in triplicate. The percentage scavenging of the DPPH free radical was calculated using the following equation.

% Scavenging = AO-AT/AO*100 (where AO=Absorbance of DPPH Solution, AT= Absorbance of Test or Reference)

The inhibition curve was plotted for the triplicate experiments and represented as percentage of mean inhibition ± standard deviation and the IC_{50} values were obtained.

Statistical analysis

Antioxidant activity, total phenolic content, and flavonoid content reported as the mean ± Standard Deviation (SD). Significant differences for multiple comparisons were determined using one-way Analysis Of Variance (ANOVA). Duncan's multiple range tests was used to assess the significant differences with the SPSS statistical analysis package (version 15.0; SPSS Inc., Chicago, IL, USA). Difference at $P<0.05$ were considered statistically significant.

Results and Discussion

Total phenolic and flavonoid content

The total phenolic content was found to be 95.80±3.6 mg GAE/gm in *Ziziphus mauritiana* and 169.35±0.2 mg GAE/mg in *Acacia catechu*. The total flavonoid content was found to be 10.70±0.0 mg QE/gm in *Callicarpa sp.* and 18.6±0.3 mg QE/gm in *Acacia catechu*. The extracts of *Acacia catechu, Bauhinia variegata, Cleistocalyx operculatus, Phyllanthus emblica, Shorea robusta, Berberis aristata* have high value of phenolic and flavonoid content exhibited the greatest antioxidant activity. The correlation between the total flavonoid and phenolic content with free radical scavenging (IC_{50}) values showed that higher the phenolic and flavonoid content lower the IC_{50} values and higher the antioxidant activity as shown in Figure 1 and Table 3.

The total phenolics content of these plant extracts are compared to the plant extracts of some previously studied plants [18-20]. Total phenolics of some previously studied plant extracts was found as *Origanum dictamnus* (8.2±0.3 mg GA/gm), *Eucalyptus globules*

Table 3: Total phenolic content, flavonoid content and free radical scavenging (IC_{50}).

Plant extracts	Free radical scavenging (IC_{50})	Total phenolic mg GAE/gm	Total flavonoid mg QE/gm
Drymaria diandra	26.27±0.19	122.45±0.96	11.51±0.30
Euphorbia hirta	29.23±0.21	138.10±4.90	11.54±0.00
Shorea robusta	6.58±0.16	145.80±5.00	14.88±0.80
Acacia catechu	7.11±0.02	169.35±0.25	18.63±0.30
Lyonia ovalifolia	15.99±0.13	137.75±1.55	12.56±0.00
Phyllanthus emblica	8.99±0.09	154.15±0.85	15.60±0.20
Berberis aristata	7.14±0.08	145.75±0.05	18.32±2.40
Bridelia retusa	15.07±0.02	147.20±1.50	16.64±0.00
Cleistocalyx operculatus	8.41±0.25	154.75±2.85	13.83±0.60
Bauhinia variegata	6.48±0.08	156.30±0.30	16.04±1.40
Bergenia ciliata	15.10±0.07	145.85±0.15	15.71±0.10
Bombax ceiba	9.37±0.12	147.45±0.85	12.54±0.10
Callicarpa sp.	37.54±0.64	127.60±0.90	10.70±0.09
Ziziphus mauritiana	47.50±0.21	95.80±3.60	11.16±3.60
Scoparia dulcis	36.60±1.20	145.75±0.05	12.54±0.10

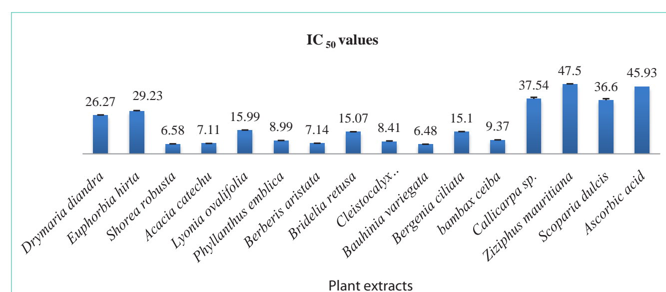

Figure 3: DPPH radical scavenging activity of the methanol extract. Values are the average of triplicate experiments and represented as mean± standard deviation (IC_{50}).

(10.5±0.3), *Sideritis cretica* (8.6±0.2), *Thymus vulgaris* (8.0±0.1), *Satureja thymbra* (9.2±0.1), *Lavandula vera* (4.9±0.1), *Lippa triphylla* (7.7±0.1) and *Matricaria chamomilla* (6.1±0.1) [17] as shown in table 3. The result showed that the plant extracts studied in this work showed the potent sources of secondary metabolites and could be used as the sources to isolate the active ingredient.

DPPH scavenging assay

In DPPH scavenging assay the IC_{50}value of *Acacia catechu* was found to be 7.11±0.02 μg/mL, *Berberis aristata* 7.14±0.08 μg/mL, *Bauhinia variegata* 6.48±0.08 μg/mL, *Cleistocalyx operculatus* 8.41±0.25 μg/mL, *Shorea robusta* 6.58±0.16 μg/mL, *Bergenia ciliata* 15.10±0.07 μg/mL, *Phyllanthus emblica* 8.99±0.09 μg/mL, *Bombax ceiba* 9.37±0.12 μg/mL, *Lyonia ovalifolia* 15.99±0.13 μg/mL, *Bridelia retusa* 15.07±0.02 μg/mL, *Drymaria diandra* 26.27±0.19 μg/mL, *Euphorbia hirta* 29.23±0.21 μg/mL, *Callicarpa sp.* 37.54±1.20 μg/mL, *Ziziphus mauritiana* 47.50±0.21 μg/mL and *Scoparia dulcis* 36.60±1.20 μg/mL shows potent antioxidant activity while the IC_{50} value of the reference standard ascorbic acid was 45.93 μg/mL as shown in Figure 3 and Table 3. Total antioxidant activity was also found to increase in a dose dependent manner.

Bioactivity studies of two species of mimosa species showed prominent antioxidant activity [21], methanol extract of the bark of *Machilusass odoratissima* exhibited high free radicals scavenging activity [20]. In our study, the antioxidant capacity of these medicinal plants extract could be compared with the results of previously studied plant extract. The antioxidant capacities in mg ascorbic acid per gram for plants were *Thymus vulgaris* (0.6±0.3), *Lavandula vera* (0.6±0.4), *Rosmarinus officinalis* (0.5±0.1), *Origanum dictamnus* (0.2±0.2), *Sideritis cretica* (0.8±0.1), *Salvia officinalis* (0.4±0.1) and *Origanum vulgare* (0.3±0.1) [17]. The result suggested that the plants extract studied in this work are the potent sources of antioxidants in comparison to these plant extracts as shown in Figure 3 and 4 as well as in Table 2.

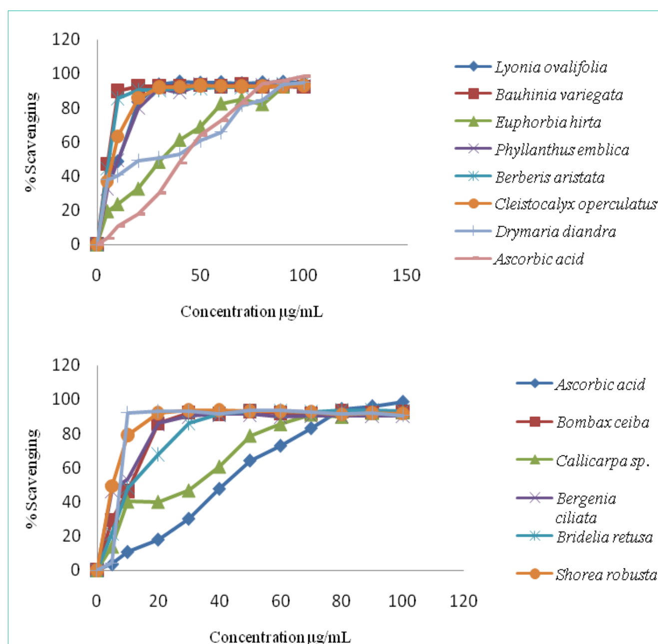

Figure 4: Percentage scavenging of DPPH free radical.

Conclusion

Free radical scavenging activity of the plant extract through the annihilation of the DPPH radical showed that *Acacia catechu*, *Bauhinia variegata, Shorea robusta* and *Phyllanthus emblica* are potent source of antioxidant with the strongest DPPH radical scavenging activity. The possible mechanism of antioxidant activity is due to the presence of phytoconstituents such as flavonoids and polyphenols present in the methanolic extract of plants. Plant extract of *Acacia catechu*, *Bauhinia variegata*, *Shorea robusta* and *Phyllanthus emblica* demonstrated the highest phenolic content and flavonoid content. The results indicated that the plants in this study are the potent antioxidant sources. These plant extracts could be used for isolating the active compound and it could be used for discovery of new drug in future. Total phenolic and flavonoid content validated the idea behind use of traditional medicinal plants to treat different diseases and could be used sources of active compounds in future study.

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