ANTIOXIDANT ACTIVITY OF SELECTED NATURAL MEDICINES USED IN NEPAL

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ABSTRACT

Diminished antioxidant defence or increased production of reactive oxygen and nitrogen species in the biological system can result into oxidative stress which can cause damage to deoxyribonucleic acid (DNA), proteins, lipids and as a result different disease states arise like cancer, neurodegenerative diseases, rheumatoid arthritis. Antioxidants from different plant resources can significantly delay or prevent oxidation of the substrate and hence prevents from various diseases. Therefore, present research was focused in search of potent natural antioxidants. For the study, methanolic extracts of twenty-five common natural medicines, mostly spices were screened using 1,1–diphenyl–2–picrylhydrazyl (DPPH) radical for their antioxidative activities. Among them, extracts of Chebulae Fructus, Terminalia Billericae Fructus, Phyllanthi Fructus, Cinnamomi Cortex, Arecae Semen, Pericarpium Punicae Granati, Syzygiae Fructus, Rhei Rhizoma, Pterocarpi Lignum and Santali Lignum Albi showed potent antioxidative activity with EC50 values being 1.5, 2.1, 1.4, 2, 1.5, 1.45, 2.7, 2.9, 3, 3.8 µg/mL, respectively. Ascorbic acid (EC50: 2.6 µg/mL) was used as positive control. Therefore, consumers can increase their intake of foods rich in antioxidant compounds that can lower the risk of chronic health problems.

Key Words: *Free radicals, ROS, RNS, antioxidants, DPPH & natural medicines.*

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the collective terms often used by scientists to include not only the oxygen and nitrogen radicals such as superoxide(O_2^-) hydroxyl (OH) nitric oxide (NO) nitrogen dioxide (NO_2) but also some non-radical derivatives of oxygen and nitrogen like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), peroxynitrite ($ONOO^-$) nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3).[1] Free radicals are generated during oxidative metabolism and energy production in the biological system.[2] Rapid production of free radicals can lead to oxidative damage to biomolecules and may cause disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative diseases, and premature aging.[3]

The oxidative stress may take place at different sites, different times, and by different mechanisms, and hence various antioxidants with different functions constituting a defence system against oxidative stress in vivo must be required.[4] Antioxidant defences comprise enzymes like superoxide dismutase, catalase, peroxidase; proteins like transferrins, haptoglobins, haemopexin and metallothionein; low-molecular-mass agents like glutathione, bilirubin and uric acid; and antioxidants from diet like ascorbic acid and α-tocopherol.1 In recent years, the antioxidative property of food constituents has been seriously noted by medical and nutritional experts.[5] Natural antioxidants from natural foods such as herbs, vegetables, fruits, oilseeds, spices, green tea, and cereals have been studied.[6]

Antioxidant-based drug formulations are being used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases and aging.[7] Spices and other medicinal herbs are regularly consumed by people especially in Asian countries like Nepal and India. So, the present study attempted to screen the antioxidative activity of commonly used spices and some other herbs used in Nepal.

MATERIALS AND METHODS

Chemicals and Equipments

All organic solvents and chemicals used in the study were of analytical grades. 1,1-diphenyl-2-picryl hydrazyl (DPPH) was purchased from Wako Pure Chemicals Co. Ltd., Osaka, Japan. HPLC grade methanol used for the DPPH assay was from Merck Limited, India. Ascorbic acid was the product of Qualigens Fine Chemicals, India. Silica gel for column chromatography was of 60-120 mesh from Qualigens Fine Chemicals, India. Silica gel for Thin Layer Chromatography (TLC) was Silica gel G from Merck Company Limited, India. UV-2101 Spectrophotometer was manufactured by Auxylab, S.L., India.

Collection of Natural Medicines

All the natural medicines were collected from the crude drug market at Bindhyabasini Tole, Pokhara. The authentic samples of these natural medicines were preserved in the Museum of Materia Medica, School of Health and Allied Sciences, Pokhara University. The list of the natural medicines is given in Table 1.

Table 1: List of the natural medicines included in the study

SN	Natural medicine	Local name	Biological source	Family	Crude drug voucher no.
1	Cinnamomi Cortex	Dalchini	*Cinnamomum zeylanicum* Nees	Lauraceae	361
2	Syzygiae Fructus	Lwang	*Syzygium aromaticum* Merril et Peery	Myrtaceae	362
3	Foeniculi Fructus	Saunf	*Foeniculum vulgare* Mill	Umbelliferae	363
4	Glycyrrhizae Radix	Jethimadhu	*Glycyrrhizae glabra* Linn.	Fabaceae	364
5	Picrorhizae Rhizoma	Kutki	*Picrorhizae scrophulariiflora* Pennell	Scrophulariaceae	365
6	Zingiberis Rhizoma	Sutho	*Zingiber officinalis* Willd. Rosc.	Zingiberaceae	366
7	Rhei Rhizoma	Padamchal	*Rheum palmatum* Linn.	Polygonaceae	367
8	Chebulae Fructus	Harro	*Terminalia chebula* Retz.	Combretaceae	368
9	Terminaliae Billericae Fructus	Barro	Terminalia bellerica (Gaetrn) Roxb.	Combretaceae	369
10	Phyllanthi Fructus	Amala	Phyllanthus embellica Linn.	Euphorbiaceae	370
11	Capsici Fructus	Khursani	*Capsicum annum* Linn.	Solanaceae	371
12	Curcumae Rhizoma	Besar	*Curcuma longa* Linn.	Zingiberaceae	372
13	Arecae Semen	Supari	*Areca catechu* L.	Palmae	373
14	Amomi Semen	Alainchi	*Amomum subulatum* Roxb.	Zingiberaceae	374
15	Zanthoxyli Fructus	Timur	*Zanthoxylum armatum* DC	Rutaceae	375
16	Piperis Fructus	Marich	*Piper nigrum* L.	Piperaceae	376
17	Trigonellae Semen	Methi	*Trigonella foenum-graecum* L.	Leguminosae	377
18	Pericarpium Punicae Granati	Anar	*Punica granatum* L.	Punicaceae	378
19	Withaniae Radix	Ashvagandha	*Withania somnifera* Dunal	Solanaceae	379
20	Pterocarpi Lignum	Raktachandan	*Pterocarpus santalinus* L.f.	Leguminosae	380
21	Santali Lignum Albi	Srikhanda	*Santalum album* L.	Santalaceae	381
22	Cumini Fructus	Jira	*Cuminum cyminum* L.	Umbelliferae	382
23	Coriandri Fructus	Dhaniya	*Coriandrum sativum* L.	Umbelliferae	383
24	Dactylorhizae Rhizoma	Panchaunle	*Dactylorhiza hatagirea* (D. Don) Soo	Orchidaceae	384
25	Brassicae Semen	Sursyu	*Brassica alba* L.	Cruciferae	385

Preparation of Extracts for DPPH Radical Scavenging Activity

Ground dried samples of each natural medicine (30 g) were extracted with methanol (2x180 mL) at room temperature for 24 hours. The extracts were concentrated to dryness under rotary evaporator.

Determination of DPPH Radical Scavenging Capacity

DPPH radical scavenging activity was measured according to the method of Hegazi et al.[8] with slight modifications. One mL of methanolic solution of each extract at various concentrations (10 µg/mL, 50 µg/mL and 100 µg/mL) was mixed with 1 mL of methanolic solution of DPPH (approx. 60 µM). The reaction mixture was shaken vigorously and left for 30 minutes at room temperature. The radical scavenging (antioxidative) activity of extracts corresponding to the scavenging of DPPH radical was measured at 520 nm by absorbance of UV spectrophotometer.

Radical scavenging activity (%) = (control absorbance - extract absorbance) X 100 %/ control absorbance

where, control is the test solution without extract.

Ascorbic acid was used as the positive control (distilled water was used as the solvent).

Those natural medicines which showed potent radical scavenging activity were again analysed for the DPPH radical scavenging at concentration 1 µg/mL, 2 µg/mL, 5 µg/mL, 10 µg/mL, 15 µg/mL and 20 µg/mL. From these data, calibration curve was plotted and Effective Concentration (EC_{50}) value was calculated. The antioxidative activity was expressed by EC_{50}. The EC_{50} value is defined as the concentration (µg/mL) of the extract required for 50% reduction of the DPPH radical absorbance.

RESULTS AND DISCUSSION

Twenty-five commonly used natural medicines listed in Table 1 were analysed for their antioxidative activity. Methanolic extracts of these natural medicines were prepared in various concentrations (10 µg/mL, 50 µg/mL and 100 µg/mL) and DPPH radical scavenging activity was determined. The DPPH radical scavenging activity of these natural medicines was expressed as % DPPH radical scavenged. Ascorbic acid was used as the positive control.

Methanolic extracts of Cinnamomi Cortex, Syzygiae Fructus, Rhei Rhizoma, Chebulae Fructus, Terminalia Billericae Fructus, Phyllanthi Fructus, Arecae Semen, Pericarpium Punicae Granati, Pterocarpi Lignum, and Santali Lignum Albi showed potent antioxidative activity (Figure 1). These extracts were further analysed to calculate the EC_{50} value.

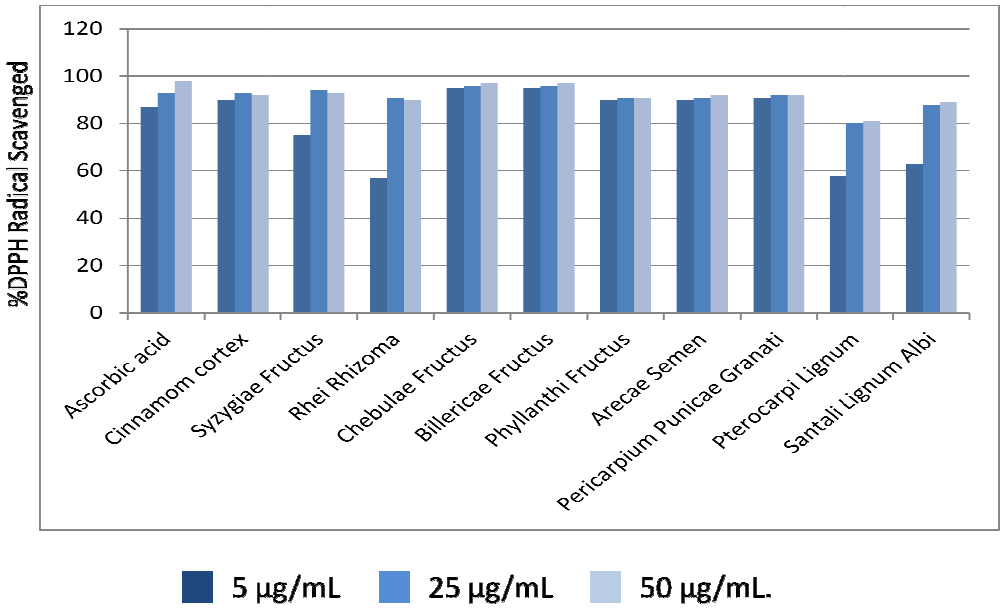

Figure 1: DPPH radical scavenging activity of natural medicines that showed potent antioxidative activity

Methanolic extracts of Foeniculi Fructus, Glycyrrhizae Radix, Picrorhizae Rhizoma, Zingiberis Rhizoma, Capsici Fructus, Curcumae Rhizoma, Amomi Semen, Zanthoxyli Fructus, Piperis Fructus, Trigonellae Semen, Withaniae Radix, Cumini Fructus, Coriandri Fructus, Dactylorhizae Rhizoma, and Brassicae Semen showed relatively less potent radical scavenging activity (Figure 2).

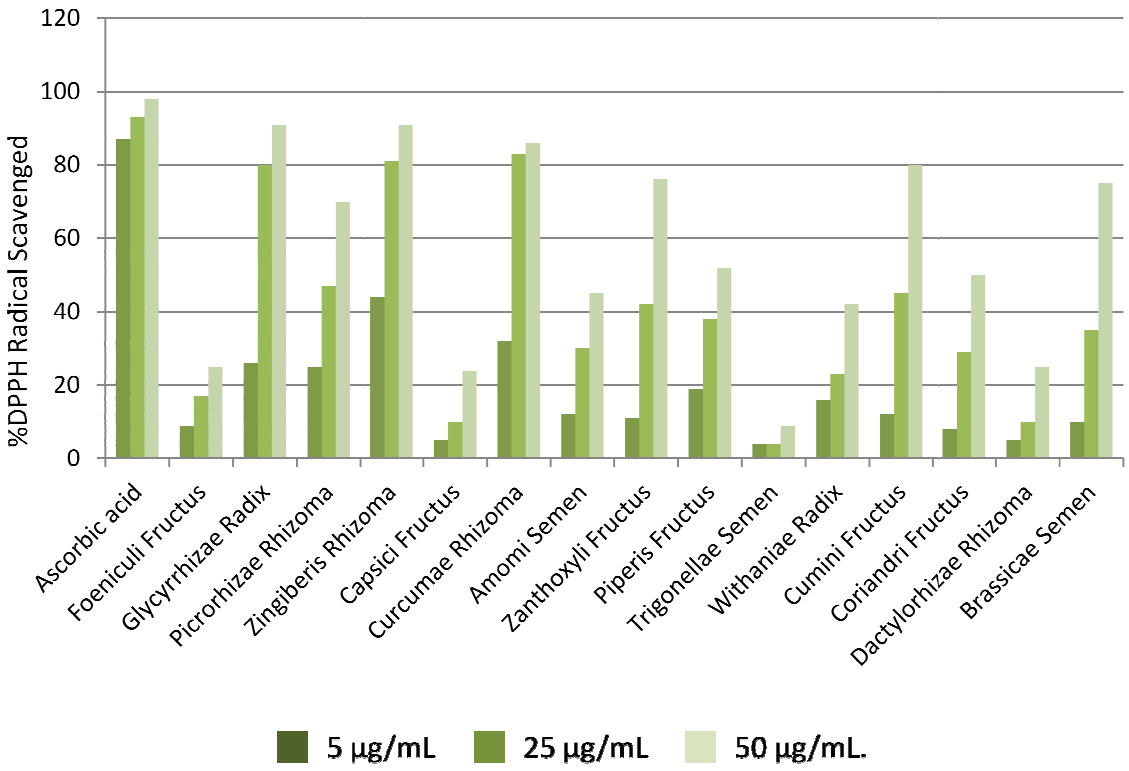

Figure 2: DPPH radical scavenging activity of natural medicines that showed less potent antioxidative activity

For those extracts which showed potent free radical scavenging activity, the concentration of the extract solutions were prepared as 1, 2, 5, 10,15, and 20 µg/mL and EC_{50} values were calculated from calibration curve. Chebulae Fructus (EC_{50} 1.5 µg/mL), Terminalia Billericae Fructus (EC_{50} 2.1 µg/mL), Phyllanthi Fructus (EC_{50} 1.4 µg/mL), Cinnamomi Cortex (EC_{50} 2.0 µg/mL), Arecae Semen (EC_{50} 1.5 µg/mL), Pericarpium Punicae Granati (EC_{50} 1.45 µg/mL) were more potent than Ascorbic acid (EC_{50} 2.6 µg/mL). Syzygiae Fructus (EC_{50} 2.7 µg/mL), Rhei Rhizoma (EC_{50} 2.9 µg/mL), Pterocarpi Lignum (EC_{50} 3 µg/mL) and Santali Lignum Albi (EC_{50} 3.8 µg/mL) were relatively less potent than ascorbic acid (Figure 3). Phyllanthi Fructus extract was the most potent.

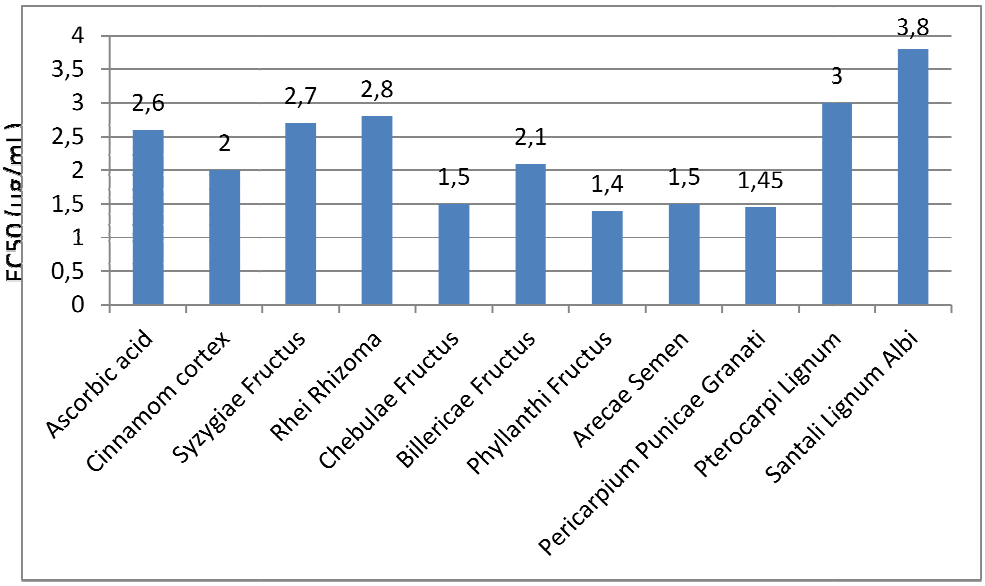

Figure 3: EC_{50} value of natural medicine extracts and ascorbic acid

In the particular study, DPPH free radical scavenging assay was used since it is a simple and acceptable method to evaluate antioxidant activity of plant extracts. In the presence of an antioxidant, DPPH radical form stable molecules by gaining one more electron or hydrogen atom and the absorbance decreases thus has applications in determination of free radical scavenging activity of natural products as well as synthetic compounds.[9] Lower IC50 value indicates higher antioxidant activity.[10]

So, our study proved the antioxidant activity of selected spices and herbs and it indicates that plant extracts showing higher antioxidant activity could be the significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses and its related disorders.

CONCLUSION

Methanolic extracts of twenty-five commonly used Nepalese natural medicines were screened for their antioxidative activity by DPPH method. From the study it was found that six of the natural medicines Chebulae Fructus, Terminalia Billericae Fructus, Phyllanthi Fructus, Cinnamomi Cortex, Arecae Semen, and Pericarpium Punicae Granati were even more potent than the positive control, ascorbic acid. So these natural sources of antioxidants can be used to prevent or treat different types of diseases which might be cheaper and safer than the other synthetic antioxidants.

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