ORIGINAL ARTICLE

Antioxidant properties of some selected Indian medicinal plants: Their correlation with total phenolic contents

Rayasandra Umesh Abhishek, Devihalli Chikkaiah Mohana, Sreerangegowda Thippeswamy, Kiragandur Manjunath
Department of Microbiology and Biotechnology, Jnanabharathi Campus, Bangalore University, Bangalore, Karnataka, India

Background: Antioxidants play a major role in protecting the biological systems against oxidative stress, which is associated with development of many chronic diseases and disorders. Plants are the main sources of natural antioxidants in the form of phenolic compounds, which help human beings to deal with oxidative stress, caused by free radical damage. **Aim:** The present study aimed at the evaluation of antioxidant properties of extracts of different medicinal plants and their correlation with the total phenolic contents (TPCs). **Materials and Methods:** The selected plant materials were sequentially extracted with solvents with increasing polarity and analysed for their phytochemical constituents and antioxidant properties. The free radical scavenging activity of solvent extracts was assessed by 2,2-diphenyl-1-picrylhydrazyl assay and TPCs by Folin-Ciocalteu method. **Results and Conclusions:** In preliminary assay, 35 plants were screened for antioxidant properties, among them four plants were selected for the study. The methanol extracts of *Acacia catechu* (AC-ME), *Adenanthera pavonia* (AP-ME), *Holopetelea integrifolia* (HI-ME) and ethanol extract of *Terminalia paniculata* (TP-EE) showed highest antioxidant activity with 50% inhibitory concentration (IC_{50}) value ranging from 7.5 to 350 µg/mL. The percentage radical scavenging activities of samples were compared to the standard butylated hydroxytoluene (BHT). The antioxidant activity of extracts and BHT in increasing order was HI-ME < AP-ME < TP-EE < BHT < AC-ME. A positive correlation was observed between antioxidant activity and TPCs. The results suggested that the phenolic compounds in extracts are most likely to be responsible for the observed antioxidant activity.

Key words: Antioxidant activity, correlativity, 2,2-diphenyl-1-picrylhydrazyl assay, medicinal plants, phytochemicals, total phenolic contents

INTRODUCTION

Reactive oxygen species (ROS) cause damage to lipids, proteins and nucleic acids through oxidation and thereby associated with various diseases, such as atherosclerosis, arthritis, neurodegenerative disorders and cancer.[1] Antioxidant supplements can counteract with harmful effects of excessive ROS by inducing endogenous defence system. Synthetic antioxidants play a major role in protecting biological systems against oxidative stress, which is associated with development of neurodegenerative disorders and chronic diseases.[2,3] However, application of synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole and propyl gallate has been reported for their remarkable side-effects.[4] In recent years, the use of natural antioxidants in the food processing and pharmaceutical industry has gained interest due to their presumed safety, nutritional and therapeutic values.

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.116388

Several plants that grow in India are well-known for various therapeutic values that could be due to their antioxidant properties.[5] Plants are the main source of natural antioxidants in the form of phenolic compounds (phenolic acids, flavonoids and polyphenols). Most of the anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective drugs derived from natural origin have been reported to have antioxidant/radical scavenging mechanism as part of their activity.[6] The ingestion of natural antioxidants has been associated with the reduced risk of cancer, cardiovascular disease, diabetes and other diseases associated with ageing.[7] Hence, interest has been increased for finding antioxidants of plant source, which are safe and suitable for use in food and/or medicine. As a part of our project, we have screened more than 35 plants, among them four plants viz., *Acacia catechu*, *Adenanthera pavonia*, *Holopetelea integrifolia* and *Terminalia paniculata*, were selected for their significant antioxidant activity. These are the large deciduous trees distributed throughout the greater part of India. The present study

Address for correspondence: Dr. Devihalli Chikkaiah Mohana, Department of Microbiology and Biotechnology, Jnanabharathi Campus, Bangalore University, Bangalore - 560 056, Karnataka, India. E-mail: mohanadc@gmail.com
Received: 19-02-2013; **Accepted:** 03-04-2013

was undertaken to evaluate the above selected medicinal plants for major phytoconstituents and their relationship with the antioxidant properties.

MATERIALS AND METHODS

Chemicals

All the chemicals, solvents and standard drugs were procured from SRL, Mumbai, India. The thin-layer chromatography (TLC, Silica gel 60) plates were purchased from Merck (Dermhadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich, Steinheim, Germany. All chemicals and solvents used were of analytical grade.

Collection of Plant Materials

The plant materials of 35 plants were collected from the different parts of Southern Karnataka [Table 1]. The plant materials were washed thoroughly with distilled water and shade-dried at room temperature. The dried leaves were ground well and stored in airtight containers. Authenticated voucher specimens of the selected plants were deposited in the Herbarium of Department of Microbiology and Biotechnology, Bangalore University, Bangalore (BUB: MB and BT: DCM: Voucher No. 37 to 71).

Preparation of Aqueous Extracts

All the plant materials (50 g each) were separately macerated with 100 mL sterile distilled water in a warring blender, filtered through muslin cloth and then centrifuged at 4000 g for 30 min. The supernatant was filtered through Whatman No. 1 filter paper[8] and subjected to antioxidant activity assay following the procedure of Dureja *et al*.[9] The plants, which showed the highest activity were selected for solvent extraction.

Preparation of Solvent Extracts

The selected plant materials of *A. catechu* (leaves), *A. pavonia* (leaves), *H. integrifolia* (leaves) and *T. paniculata* (fruits) were subjected to solvent extraction by Soxhletion method.[10] Fifty grams of the powdered plant sample was successively extracted with 200 mL of petroleum ether, toluene, chloroform, methanol and ethanol with their increasing polarity. All the solvent extracts were concentrated separately under reduced pressure using a rotary flash evaporator and subjected to phytochemical analysis and antioxidant activity following the standard methods.[11,12]

Determination of Total Phenolic Contents

Each extract of all the four plants was evaluated quantitatively for their phenolic contents following procedure of Li *et al*., with minor modifications.[13] Briefly, 1 mL of each extracts (1 mg/mL) were added to 0.1 mL of Folin-Ciocalteu reagent and kept for 5 min at room temperature. After, 2 mL of 15% sodium carbonate was added and diluted to 10 mL by adding distilled water, then incubated at room temperature for 90 min in dark condition. Absorbance was measured at 720 nm using the double beam ultraviolet-visible (UV-VIS) spectrophotometer (Elico, India). A standard calibration curve was plotted using the gallic acid (0.0 to 1.0 mg/mL). TPC was expressed as gallic acid equivalent (mg of gallic acid/mg of dry weight of extract) based on the calibration curve.

Table 1: Total number of plants screened preliminarily for their antioxidant properties

Plant name	Part used	Family	Antioxidant activity
Acacia catechu	L	*Mimosaceae*	+++
Acacia chundra	L	*Fabaceae*	–
Adenanthera pavonia	L	*Mimosaceae*	+++
Asperagus racemosus	L	*Liliaceae*	++
Bauhinia acuminata	L	*Caesalpiniaceae*	–
Breynia vitisidaea	L	*Euphorbiaceae*	–
Calotropis gigantea	L	*Apocyanceae*	++
Carissa carandas	L	*Apocyanceae*	+
Cassia alata	L	*Fabaceae*	++
Cassia siamea	L	*Fabaceae*	++
Cassia tora	L	*Fabaceae*	++
Coleus amboinicus	L	*Lamiaceae*	–
Couroupita guianensis	L	*Lecythidaceae*	++
Delonix regia	L	*Fabaceae*	–
Dodonaea viscosa	L	*Sapindaceae*	++
Euphorbia tirucalli	L	*Euphorbiaceae*	–
Ficus bengalensis	L	*Moraceae*	+
Gliricidia sepium	L	*Fabaceae*	+
Holoptelea integrifolia	L	*Ulmaceae*	+++
Lagersteromia flosregenea	L	*Lythraceae*	–
Millingtonia hortensis	L	*Bignoniaceae*	–
Peltophorum pterocarpum	L	*Fabaceae*	++
Ricinus communis	L	*Euphorbiaceae*	+
Saccharum spontaneum	L	*Poaceae*	–
Salacia oblonga	L	*Celastraceae*	–
Sesbania grandiflora	L	*Fabaceae*	+
Solanum indicum	L	*Solanaceae*	–
Spathodea campanulata	L	*Bignoniaceae*	++
Spilanthes paniculata	L	*Asteraceae*	+
Tabebuia argentea	L	*Bignoniaceae*	+
Terminalia paniculata	L and F	*Combretaceae*	+++
Thespesia populnea	L	*Malvaceae*	+
Tylophora indica	L	*Asclepiadaceae*	–
Vitex negundo	L	*Lamiaceae*	+
Ziziphus mucronata	L	*Rhamnaceae*	+

L – Leaves; F – Fruits; – – No activity; + – Low activity; ++ – Moderate activity; +++ – Significant activity

Free Radical Scavenging Activity

Qualitative evaluation by TLC method

The qualitative evaluation of the radical scavenging activity of solvent extracts were determined by TLC method.[9]

Briefly, all the solvent extracts were dissolved in methanol and spotted on TLC plates (100 µg/spot), air dried and then sprayed with a methanolic solution of 0.1 mM DPPH. The sprayed plate was observed to change in colour from purple to pale yellow. The solvent extracts which showed positive results were quantitatively evaluated.

Quantitative evaluation by spectrophotometric method

The DPPH radical scavenging activity of plant extracts and BHT was determined by using the method described by Ebrahimabadi *et al*.[14] Briefly, plant extract of different concentrations ranging from 15 to 500 µg/mL were prepared by using two-fold dilution method. A solution of 0.1 mM DPPH was prepared in the methanol. The reaction was containing 1 mL of sample and 3 mL of 0.1 mM DPPH, allowed for incubation in dark condition for 30 min (28°C). Methanol was used as negative control and BHT as standard. The absorbance of the reaction mixture was measured at 517 nm by using the double beam UV-VIS spectrophotometer (Elico, India). The ability of the sample to scavenge DPPH was calculated as:

$$\text{Radical scavenging activity (\%)} = A_{control} - A_{sample}/A_{control} \times 100$$

where $A_{control}$ is the absorbance of the control (DPPH + methanol) and A_{sample} is the absorbance of the sample (DPPH + methanol + sample).

Determination of IC_{50} values

IC_{50} values were calculated from the linear regression of the percentage antioxidant activity against concentrations of extracts used.[4] IC_{50} values were defined as the concentrations of samples required for the conversion of the half of the DPPH radicals to their more stable molecular counterparts 2,2-diphenyl-1-picrylhydrazines.[14]

Statistical Analysis

Data given were analysed by the Statistical Package for the Social Sciences (SPSS) 20 (SPSS Inc. IBM, USA). Differences between means were determined using Tukey univariate comparisons ($P \leq 0.05$).

RESULTS

The therapeutic values of various medicinal plants are associated with their antioxidant properties. Antioxidants have become an essential part of food preservation technology and contemporary health-care. In order to evaluate the antioxidant activity of natural products, it is necessary to investigate the major phytoconstituents, which are involved in such activities. In this context, we screened aqueous extracts of 35 plants for their antioxidant properties using the DPPH radical scavenging assay. Among 35 plants, 23 showed varying degree of activities [Table 1], of which four plants viz., *A. catechu*, *A. pavonia*, *H. integrifolia* and *T. paniculata* showed the highest activity were subjected to successive solvent extraction. The preliminary phytochemical investigation of successive solvent extracts revealed the presence of various chemical constituents is represented in Table 2. The phenolic compounds were found to be present in the methanol extracts of *A. catechu* (AC-ME), *A. pavonia* (AP-ME) and *H. integrifolia* (HI-ME) and ethanol extract of *T. paniculata* (TP-EE).

The TPCs of plant extracts were quantified spectrophotometrically based on the reduction of Folin-Ciocalteu reagent. The TPCs of AC-ME, AP-ME, HI-ME and TP-EE were estimated as 226 mg gallic acid equivalent (GAE)/g, 34 mg GAE/g, 20 mg GAE/g and 172 mg GAE/g of their dry extracts, respectively [Table 3]. These values indicate that each milligram of the plant extracts contain phenolic compounds equivalent to the pure gallic acid. The increasing order of TPCs of test extracts was HI-ME < AP-ME < TP-EE < AC-ME.

The antioxidant activity of AC-ME, AP-ME, HI-ME and TP-EE were evaluated quantitatively by spectrophotometric method. The DPPH radical scavenging activity of AC-ME, AP-ME, HI-ME and TP-EE were 82.78% (IC_{50} 7.5 µg/mL),

Table 2: Phytochemical analysis of the selected medicinal plants

Phytoconstituents	AC	AP	HI	TP
Alkaloids	+	+	+	+
Carbohydrates	–	–	–	–
Cardiac glycosides	+	+	–	–
Flavonoids	–	–	–	–
Glycosides	+	+	+	+
Phenolic compounds	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	–
Tannins	+	+	+	+
Terpenoids	+	+	–	–

AC – *Acacia catechu*; AP – *Adenanthera pavonia*; HI – *Holopetelea integrifolia*; TP – *Terminalia paniculata*; + – Present; – – Absent

Table 3: Linear correlation between the radical scavenging activity and TPCs of selected plant extracts

Samples	Antioxidant activity		TPCs (mg GAE/g of extract)
	Percentage inhibition (%)	IC_{50} (µg/mL)	
AC-CE	82.01±0.53	7.5±0.12	226±3.06
AP-ME	68.21±0.15	99.2±0.20	34±1.15
HI-ME	63.83±0.25	350±8.66	20±0.87
TP-EE	79.53±0.32	21.4±0.46	172±2.31
BHT	92.08±0.35	15.0±1.15	NA

NA – Not applicable; TPCs – Total phenolic contents; GAE – Gallic acid equivalent; AC-ME – Methanol extract of *Acacia catechu*; AP-ME – Methanol extract of *Adenanthera pavonia*; HI-ME – Methanol extract of *Holopetelea integrifolia*; TP-EE – Ethanol extract of *Terminalia paniculata*; BHT – Butylated hydroxytoluene; SEM – Standard error of mean, Values are represented as mean±SEM (n=3, P≤0.05)

68.78% (IC_{50} 99.2 µg/mL), 64.39% (IC_{50} 350 µg/mL) and 79.48% (IC_{50} 21.4 µg/mL), respectively. AC-ME showed the highest activity 82.78% with least IC_{50} (7.5 µg/mL), while the HI-ME showed lower activity 64.39% with higher IC_{50} (350 µg/mL) value [Figure 1]. A high percentage of radical scavenging indicated a strong antioxidant activity. The IC_{50} values corresponding to the amount/concentration of extract required to scavenge 50% of DPPH radicals present in the reaction mixture. When compared to the positive control BHT (IC_{50} 15 µg/mL), AC-ME showed significantly high activity with lower IC_{50} values (IC_{50} 7.5 µg/mL) and even TP-EE showed significant activity with IC_{50} 21.4 µg/mL. AC-ME and TP-EE showed lower IC_{50} values with significantly high antioxidant activities, whereas AP-ME and HI-ME extracts showed higher IC_{50} values with consequently low antioxidant activities [Table 3]. The IC_{50} values of test extracts and standard (BHT) in increasing order was HI-ME < AP-ME < TP-EE < BHT < AC-ME. Table 3 represents the correlation of antioxidant activity with the amount of TPCs of plant extracts. These results demonstrate a positive correlation between antioxidant activity and TPCs.

DISCUSSION

DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods and to quantify antioxidants in complex biological systems.[15] The antioxidant activity of the phenolic compounds is mainly due to their redox properties, which play a key role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.[16]

It is well-reported that the presence of different hydroxyl containing and phenolic compounds such as polyphenols, tannins, phenolic acids and flavonoids in the plants. These compounds are commonly water soluble and their presence in the polar extracts such as methanol and ethanol extracts may be the main cause of its considerable radical-scavenging activity.[14,17] Methanol is the most suitable solvent for the extraction of polyphenolic compounds from plant materials, due to its ability to inhibit the oxidation of polyphenols and its ease of evaporation compared to water.[18]

Among the four plants screened, the most potent radical scavenger was AC-ME followed by TP-EE, the low concentration and high inhibitory activity reflects a highly significant antioxidant activity. It can be predicted that the consequently low antioxidant activities of AP-ME and HI-ME might be due to their lower phenolic contents. The selected plants were reported in Ayurveda for their various medicinal values, in this respect, these plants could be good sources of antioxidants in the food industry in the form of food additives with no toxicity.

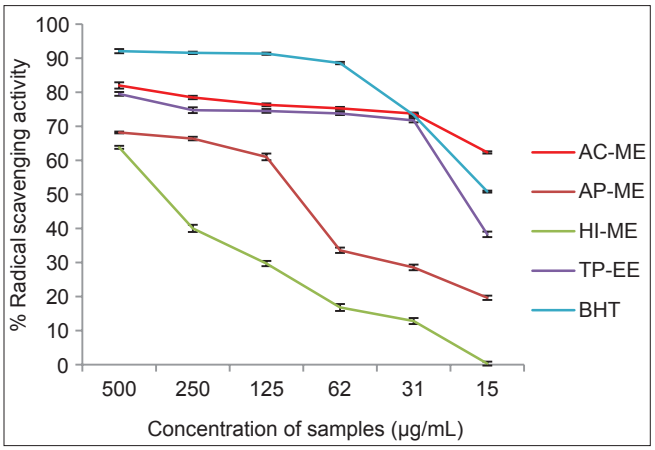

Figure 1: Percentage scavenging of 2,2-diphenyl-1-picrylhydrazyl radicals at different concentrations of plant extracts and butylated hydroxytoluene (values are represented as mean ± SEM ($n = 3$, $P \leq 0.05$))

CONCLUSION

In this study, a strong correlation between antioxidant activities and their TPCs indicated that phenolic compounds were a major contributor of antioxidant activity of these plants. Thus, these plants could serve as potential sources of natural antioxidants against oxidative stress, which is associated with neurodegenerative diseases and biological damage in living tissues. It can be concluded that these plants could serve as a natural source of antioxidants in the food industry and with their other pharmacological properties. Hence, further investigation is required to isolate and elucidate the active principles, and to evaluate pharmacological properties using animal models.

REFERENCES

1. Zeng LB, Zhang ZR, Luo ZH, Zhu JX. Antioxidant activity and chemical constituents of essential oil and extracts of Rhizoma *Homalomenae*. Food Chem 2011;125:456-3.
2. Bamoniri A, Ebrahimabadi AH, Mazoochi A, Behpour M, Kashi FJ, Batooli H. Antioxidant and antimicrobial activity evaluation and essential oil analysis of *Semenovia tragioides* Boiss. from Iran. Food Chem 2010;122:553-8.
3. Trigui M, Hsouna AB, Tounsi S, Jaoua S. Chemical composition and evaluation of antioxidant and antimicrobial activities of Tunisian *Thymelaea hirsuta* with special reference to its mode of action. Ind Crops Prod 2013;41:150-7.
4. Ebrahimabadi AH, Ebrahimabadi EH, Djafari-Bidgoli Z, Kashi FJ, Mazoochi A, Batooli H. Composition and antioxidant and antimicrobial activity of the essential oil and extracts of *Stachys inflata* Benth from Iran. Food Chem 2010;119:452-8.
5. Tippani R, Porika M, Allenki V, Anreddy RN, Yellu NR, Krishna DR, *et al*. Antioxidant and analgesic activities of *Pterocarpus marsupium* Roxb. J Herbs Spices Med Plants 2010;16:63-8.
6. Priya OS, Viswanathan MB, Balakrishna K, Venkatesan M. Chemical constituents and *in vitro* antioxidant activity of *Phyllanthus wightianus*. Nat Prod Res 2011;25:949-58.
7. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complement Altern Med 2010;10:21.

8. Thippeswamy S, Praveen P, Mohana DC, Manjunath K. Antimicrobial evaluation and phytochemical analysis of a known medicinal plant *Samanea saman* (Jacq) Merr against some human and plant pathogenic bacteria and fungi. Int J Pharm Bio Sci 2011;2:443-52.
9. Dureja AG, Dhiman K. Free radical scavenging potential and total phenolic and flavonoids content of *Ziziphus mauritiana* and *Ziziphus nummularia* fruit extracts. Int J Green Pharm 2012;6:187-92.
10. Mohana DC, Prasad P, Vijaykumar V, Raveesha KA. Plant extract effect on seed-borne pathogenic fungi from seeds of paddy grown in Southern India. J Plant Prot Res 2011;5:101-6.
11. Sofowara A. Medicinal Plants and Traditional Medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd.; 1993. p. 289-91.
12. Rojas R, Bustamante B, Bauer J, Fernández I, Albán J, Lock O. Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol 2003;88:199-204.
13. Li HB, Wong CC, Cheng KW, Chen F. Antioxidant properties *in vitro* and total phenolic contents in methanol extracts from medicinal plants. LWT-Food Sci Technol 2008;41:385-90.
14. Ebrahimabadi AH, Mazoochi A, Kashi FJ, Djafari-Bidgoli Z, Batooli H. Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of *Salvia eremophila* Boiss. from Iran. Food Chem Toxicol 2010; 48:1371-6.
15. Esmaeili MA, Sonboli A. Antioxidant, free radical scavenging activities of *Salvia brachyantha* and its protective effect against oxidative cardiac cell injury. Food Chem Toxicol 2010;48: 846-53.
16. Kilani-Jaziri S, Bhouri W, Skandrani I, Limem I, Chekir-Ghedira L, Ghedira K. Phytochemical, antimicrobial, antioxidant and antigenotoxic potentials of *Cyperus rotundus* extracts. S Afr J Bot 2011;77:767-76.
17. Castilho PC, Savluchinske-Feio S, Weinhold TS, Gouveia SC. Evaluation of the antimicrobial and antioxidant activities of essential oils, extracts and their main components from oregano from Madeira Island, Portugal. Food Control 2012;23:552-8.
18. Lim YY, Quah EP. Antioxidant properties of different cultivars of *Portulaca oleracea.* Food Chem 2007;103:734-40.

How to cite this article: Abhishek RU, Mohana DC, Thippeswamy S, Manjunath K. Antioxidant properties of some selected Indian medicinal plants: Their correlation with total phenolic contents. Int J Green Pharm 2013;7:117-21.

Source of Support: Department of Science and Technology (No.SR/FT/LS-141/2008), Government of India, New Delhi and University of Grant Commission (F.No.37-82/2009 (SR)), New Delhi, **Conflict of Interest:** None declared.