

Arid zone plants as natural antioxidant agents

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Manuscript received 12 May 2008, accepted 16 September 2008

Abstract : Methanol and dichloromethane extracts of flowers for total phenolic content along with antioxidant potential were examined using 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) solution-based chemical assay of 15 medicinally important arid zone plants. The results showed that of all these extracts, highest total phenolic content was observed in *Acacia nilotica* (58.60 ± 0.40 GAE/100 g FW) followed by *Tephrosia hamiltonii* (52.00 ± 0.41 GAE/100 g FW). Likewise, each studied extract demonstrated potential antioxidant activity but the highest activity was recorded in dichloromethane extract of *T. hamiltonii* ($RC_{50} = 5.5$ μ g/ml) and in methanol extract of *Calotropis procera* ($RC_{50} = 4.5$ μ g/ml). All the arid zone plants selected in this study exhibited stronger antioxidant activity and significantly higher levels of total phenolics, which may prove to be potential natural sources of potent antioxidant and beneficial chemopreventive agents.

Keywords : Arid zone plants, total phenolics, antioxidant agents.

Introduction

Plants and plant products are part of the vegetarian diet and a number of them exhibit medicinal properties. Recent reports indicated that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases^{1,2}. Hence, search for new synthetic and natural antioxidants is essentially important. Although, initial research on antioxidants was mostly on isolated pure compounds, recent focus is more on natural formulations^{3,4}. It has been found that compounds in their natural formulations are more active than their isolated form⁵. Oxygen reactive species are now recognized to be involved in great variety of diseases and even in normal aging. Thus, free radicals appear to constitute a particularly promising target for improving drug treatment of various pathological conditions. Plants synthesize numerous common phenolic metabolites with free radical scavenging properties. The reduction of DPPH radical proved to be well suited for the detection of radical scavengers in crude plant extracts. This radical has been used in particular for the screening of plants^{6,7} and for the detection of radical scavengers in marine bacteria⁸. Hence, as part of our studies on the phytochemistry of plants, we investigated 15 arid zone plants for their total phenolic and antioxidant potentials.

Results and discussion

The amount of total phenolic content of 15 arid zone plants exhibited significant variation ranging from 41.00 ± 0.50 to 58.60 ± 0.40 mg GAE/100 g FW in methanol extracts of different plant species (Table 1). Out of these, *A. nilotica* contained the maximum phenolic compounds (58.60 ± 0.40 mg GAE/100 g FW) followed by *T. hamiltonii* (52.00 ± 0.41 mg GAE/100 g FW) and *Cassia occidentalis* (50.80 ± 0.88 mg GAE/100 g FW). Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids⁹. Phenolic acids are widely distributed in plant kingdom¹⁰ and have repeatedly been implicated as natural antioxidants in fruits, vegetables and other plants, e.g. caffeic acid, ferulic acid and vanillic acid. The results suggested that the phenolic compounds contributed significantly to the antioxidant capacity of the arid zone plants.

Both, dichloromethane and methanol extracts gave increased antioxidant activity with the increased concentration of extracts, and thus, exhibited dose-dependency. Nevertheless, the most effective antioxidant activity was shown by *T. hamiltonii* ($RC_{50} = 5.5$ μ g/ml) followed by *Thevetia peruviana* ($RC_{50} = 6$ μ g/ml) in the dichloromethane extracts. Similarly, in methanol extracts

Table 1. Yield, total phenolic content, antioxidant activity and % inhibition of DPPH of arid zone plants

Plant name	Yield (%)		RC ₅₀ (µg/ml)		Total phenolic (mg GAE/100 g FW)		% Inhibition of DPPH (µg/ml)							
							10		20		40		60	
	DCM	MeOH	DCM	MeOH	DCM	MeOH	DCM	MeOH	DCM	MeOH	DCM	MeOH	DCM	MeOH
<i>Acacia nilotica</i>	1.75	1.18	NT	5.5	NT	5.5	–	93.20	–	94.72	–	95.07	–	95.57
<i>A. senegal</i>	6.15	5.53	8	7	61.30	46.50 ± 0.00	61.30	67.52	68.25	71.22	70.67	74.87	73.05	77.97
<i>Calotropis procera</i>	0.17	3.77	6.5	4.5	72.00	47.60 ± 0.34	72.00	95.32	74.67	96.67	75.15	96.87	77.85	97.57
<i>Cassia fistula</i>	0.27	3.81	7.5	6.5	64.12	48.60 ± 0.17	64.12	77.37	69.32	82.75	77.57	92.00	79.10	94.40
<i>C. javanica</i>	0.50	3.92	7.5	6.5	20.27	45.83 ± 0.40	20.27	95.12	23.55	97.17	32.00	97.57	44.90	97.67
<i>C. occidentalis</i>	0.37	7.45	6.5	6.5	74.25	50.80 ± 0.88	74.25	74.95	79.37	75.70	87.52	78.82	91.67	84.42
<i>Catharanthus roseus</i>	0.42	7.26	6.5	7	76.80	42.66 ± 0.44	76.80	72.60	80.35	74.17	85.07	77.22	87.85	81.20
<i>Cleome gynandra</i>	0.76	0.25	7	7.5	74.22	47.66 ± 0.60	74.22	70.77	75.35	79.37	77.15	84.67	77.92	93.25
<i>Nerium indicum</i>	0.63	1.72	16	5.5	47.10	44.50 ± 0.82	47.10	98.10	51.75	98.55	52.90	98.72	55.40	99.02
<i>Parthenium hysterophorus</i>	3.13	7.46	7	6	89.12	47.25 ± 0.20	89.12	97.25	88.27	94.82	87.22	94.30	86.42	87.85
<i>Petalium murex</i>	0.52	2.76	NT	6	–	45.00 ± 0.00	–	82.82	–	88.00	–	96.15	–	96.65
<i>Peltophorum pterocarpum</i>	1.05	2.10	7.5	7.5	66.47	44.83 ± 0.33	66.47	65.95	68.97	66.87	71.90	80.35	78.70	83.25
<i>Tephrosia hamiltonii</i>	7.45	7.09	5.5	8	87.72	52.00 ± 0.41	87.72	61.65	88.20	62.57	91.75	68.92	92.30	70.30
<i>Thevetia peruviana</i>	0.45	5.42	6	7.5	84.30	41.00 ± 0.50	84.30	65.43	85.45	66.55	87.10	68.45	87.17	71.37
<i>Vitex negundo</i>	0.78	5.15	10	5	48.80	50.16 ± 0.17	48.80	95.51	51.12	95.52	52.55	97.00	56.90	97.37

Values are expressed as means ± S.E. of three parallel measurements; GAE, gallic acid equivalents. NT : not tested.

% Inhibition = $1 - (\text{absorbance of sample/absorbance of control}) \times 100$.

highest potential activity was recorded in *C. procera* ($RC_{50} = 4.5 \mu\text{g/ml}$) followed by *Vitex negundo* ($RC_{50} = 5 \mu\text{g/ml}$). Among the plants studied, members of Asclepiadaceae and Fabaceae families were the most effective in antioxidant potential while the members of Mimosaceae were rich in total phenolic contents.

Experimental

Flowers of 15 medicinally important arid zone plants were collected from the fields of University of Rajasthan, Jaipur. Fresh flowers (100 g) were ground and successively percolated in dichloromethane and methanol at 35 °C for 12 h. Later, the resultant extracts were dried using rotary evaporator. 2,2-Diphenyl-1-picrylhydrazyl (DPPH; molecular formula $C_{18}H_{12}N_5O_6$), gallic acid and ascorbic acid were purchased from Himedia, India. All other reagents used were of the highest available purity. Freshly prepared solutions were used for each experiment. The amount of total phenolic content were determined with the Folin-Ciocalteu reagent using the method of Bray and Thorpe¹¹ with some modifications and the results were expressed as mg of gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g FW). For antioxidant assay, method of Fogliano *et al.*¹² was adopted with suitable modifications to our particular circumstances. All the experiments were repeated (3 times) and the mean errors correspond to standard error ($\pm$ S.E.). Percent inhibition of DPPH was calculated by following equation :

$$\% \text{ Inhibition} = 1 - (A_1/A_2) \times 100$$

where, A_1 is the absorbance of the test sample and A_2 as the absorbance of control reaction.

Acknowledgement

Authors are highly thankful to Indian Council of Medical Research (ICMR), New Delhi, India, for providing financial support and facilities for this research work.

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