Radical scavenging activity of phenolics and flavonoids in some medicinal plants of India

R.K. Choudhary, Ajaya Eshha Saroha and P.L. Swarnkar
Department of Botany, University of Rajasthan, Jaipur 302 004 India

ABSTRACT

Stem bark of Acacia nilotica and leaves of Mentha arvensis had maximum amount of phenolic content as well as radical scavenging activity among all the plants studied.

Key words: Total phenolic content, Flavonoids, Antioxidant activity, DPPH, Superoxide radical.

INTRODUCTION

The evolution of aerobic metabolic processes such as respiration and photosynthesis led to the production of reactive oxygen species (ROS) in mitochondria, chloroplasts and peroxisomes. In general, ROS are potentially toxic and their uncontrolled production can result in oxidative damage of cellular components (Halliwell and Gutteridge, 2007).

Some ROS such as the superoxide radical and hydrogen peroxide (H₂O₂), at low concentrations, fulfill important roles in stress perception, photosynthesis regulation, pathogen recognition, programmed cell death, and plant development (Mittler et al., 2004). A common feature among the different ROS types is their capacity to cause oxidative damage to proteins, DNA and lipids. These cytotoxic properties of ROS explain the evolution of complex arrays of detoxification mechanisms in plants. These ROS are scavenged by various enzymatic and non-enzymatic antioxidants to avoid the oxidative damage. Antioxidants and ROS are an important interacting system with different functions in higher plants, which ensures themselves a highly flexible organism (Borland et al., 2006; Rio et al., 2006 and Shao et al., 2005). Antioxidants interfere with the production of free radicals and inactivate them (Dusinka et al., 1999). Antioxidants such as carotenoids, vitamins, phenols, flavonoids, and endogenous metabolites are naturally found in various parts of plants (Cao et al., 1997, Veligoglu et al., 1998).

Phenols comprising of phenolic acids, flavonoids, biflavonoids, anthocyanins and isoflavonoids possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, as well as ability to modify the gene expression (Nakamura et al., 2003; Tapiers et al., 2002). Phenolic compounds, characterized by hydroxylated aromatic rings are ubiquitous in plants. Simple phenolics and flavonoids are important constituents of plants. These compounds show a wide range of antioxidant activities in vitro (Rice-Evans et al., 1995) and are thought to exert protective effects against major diseases such as cancer and cardiac disease. The antioxidant capacity of phenolic compounds is determined by their structure, in particular the ease with which a hydrogen atom from an aromatic hydroxyl group can be donated to the free radicals. In the present investigation, extracts of stem-bark and leaves of selected medicinal plants were analyzed for their total phenolic content and free radical scavenging activity.

MATERIALS AND METHODS

Plant material

The stem-bark and leaves of selected plants were collected from Jaipur, Rajasthan, India during the month of July 2008. The samples were dried at room temperature, crushed in grinder and the powder was stored in polythene bags until subjected to analysis. For analysis of phenolics, flavonoids and total antioxidant activity, the dried plant materials were extracted with 80% aqueous methanol for 48 h, filtered through Whatman no. 1 filter paper and appropriately diluted with 80% methanol.

Determination of total phenolic content

Total phenolics were analyzed spectrophotometrically using a modified Folin-Ciocalteau colorimetric method (Dewanto et al., 2002). 125 µl of the standard Gallic acid solution or sample extract was mixed with 0.5 ml of distilled water in a test tube followed by 125 µl of Folin-Ciocalteau reagent. The samples were mixed well and allowed to stand for 6 min before 1.25 ml of a 7% of sodium carbonate was added. Water was added to adjust the final volume to 3 ml. After incubation at room temperature for 90 min, the absorbance was recorded at 760 nm. Reference curve was prepared using 10-400 µg/ml of Gallic acid (linear regression r² = 0.9975) and the results are presented as amount of phenolic content (Gallic acid equivalent, GAE) per g dry weight.

Determination of total flavonoid content

Flavonoid quantification was done using aluminium chloride colorimetric method (Chang et al., 2002). Plant extracts (0.5 ml) were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared using 12.5 to 100 µg/ml of Quercetin in methanol (linear regression r² = 0.9991) and the results are expressed as amount of flavonoid content (Quercetin equivalent, QE) per g dry weight.

Superoxide radical scavenging capacity

The superoxide radical scavenging capacity of plant extract was analyzed using a modified method of Beauchamp and Fridovich (1971), as described by Zhishen et al. (1999). The 2 ml of reaction mixture containing 3x10⁻⁴ mol/riboflavin, 1x10⁻² mol/methionine and 1x10⁻⁴ mol/nitroblue tetrazolium (NBT) in 0.05 M phosphate buffer (pH 7.8) was illuminated with two 20 W fluorescent lamps at 25°C for 25 min in an aluminium foil-lined box. The photochemically reduced riboflavin generated O₂⁻ which reduced NBT to blue formazan. The unilluminated reaction mixture was used as a blank. The absorbance (A₀) was measured at 560 nm. The plant extracts (0.2 ml) were added to the reaction mixture, which scavenged O₂⁻ generation, thereby inhibiting the NBT reduction. Absorbance (Aₗ) was measured and the decrease in O₂⁻ was calculated by A₀-Aₗ. The degree of the scavenging was calculated by the following equation:

Scavenging (%)= \frac{(Absorbance_{control}-Absorbance_{sample})}{Absorbance_{control}} \times 100

RESULTS AND DISCUSSION

Table 1 shows total phenolics and flavonoid content as well as DPPH radical scavenging activity and Superoxide radical scavenging activity in stem bark of Acacia nilotica, Delbergia sissoo, Delonix regia, Polysalthia longifolia, Prospis cineraria and Zizyphus mauritiana. The total phenolics and flavonoid content and DPPH and Superoxide radical scavenging activity in leaf extract of Catharanthus roseus, Mentha arvensis, Murraya koenigii, Ocimum basilicum, Tabernaemontana divaricata, Tinospora cordifolia and Tribulus terrestris are presented in table 2.

Total phenolic and flavonoid contents

Phenolic content varied from 4.45 ± 0.82 to 63.75 ± 4.24 mg/g GAE in the stem bark extract. Acacia nilotica with total phenolic contents of 63.75 ± 4.24 mg/g GAE had the highest (p < 0.0001) amount among all the plants studied. Other plants did not show significant differences in phenolic contents among themselves (values between 4.45 to 7.65 mg/g GAE DW). Leaf extracts of Mentha arvensis had the highest amount of total phenolic contents (31.25 ± 2.09 mg/g GAE). The flavonoid contents of the extracts in terms of quercetin equivalent (the standard curve equation: y = 0.0067x + 0.0132, r² = 0.9991) varied between 1.34 ± 0.36 mg/g and 5.02 ± 0.48 mg/g in stem bark and 2.10 ± 0.52 and 9.95 ± 1.08 mg/g QE in leaf extract. The highest flavonoid contents were observed in the leaf extracts of Mentha arvensis (9.95 ± 1.08 mg/g QE) followed by stem bark extracts of Acacia nilotica (5.02 ± 0.48 mg/g QE).
The result of the present study showed that the extract of BHT in scavenging of superoxide radical. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract.

In conclusion, the results of the present study suggest that tested plant materials have moderate to high free radical scavenging activity. The high antioxidant activity could be well attributed to the phenolic and flavonoid content of the plants. More studies are needed to characterize the active principle responsible for antioxidant activity in these plants. It also important to note that antioxidant activity measured by in vitro methods may not reflect in vivo effects of antioxidants (Wu et al., 2004). However, the findings of this study establish that these medicinal plants are promising sources of antioxidants and may be effective as preventive agents in some of the diseases.

ACKNOWLEDGEMENT

One of us (R.K. Choudhary) is grateful to University Grant Commission (UGC), New Delhi, India for the financial support.

REFERENCES