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In Vitro Determination of Antioxidant Activity of *Murraya koenigii* (L.) Spreng

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ABSTRACT

Murraya koenigii (Linn.) Spreng, locally known as "curry patta" or "mitha neem" (curry leaf tree) belonging to the Rutaceae family, is commonly used as raw material for traditional medicinal formulation in India. Its aromatic leaves contain essential oil and it is used as a condiment. The fresh leaves of *M. koenigii* have been reported to possess anti-microbial, mosquitocidal, topo-isomerase inhibition and antioxidant properties. The present paper aims to undertake a comparative study of the antioxidant potential of extracts of plant parts and fruits/seeds in different stages of biological development and ripeness. Eight different plant materials were tested to determine antioxidant activity, including roots, leaf, un-ripened (green colored) fruit and its seed, half ripened fruit and its seed, ripened fruit, and its seed. Stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) was used for *in vitro* determination of free radical scavenging activity of the extracts. The ripened fruit exhibited highest phenol (16.4 $mg.g^{-1}$) and flavonoid (2.8 $mg.g^{-1}$) contents (fresh weight basis) and lowest activity in half-ripened fruit. The antioxidant activity of fruit extracts changed with their biological development and ripeness. This investigation is the first report on the comparative *in vitro* analysis of antioxidant potential of extracts of leaves, roots, fruits and seeds of *M. koenigii*.

Keywords: aromatic plants, curry leaf tree, flavonoids, medicinal, phenols

INTRODUCTION

Reactive free radicals entering the human system through environmental pollution or generated within the body due to physical stress are known to be associated with cellular and metabolic injuries. These have been reported to cause oxidation of lipids, proteins and nucleic acids leading to the depletion of the human immune system (Ames 1983). In addition, many other harmful effects of free radicals in the human system have been documented (Ames *et al*. 1993; Ciencewicki *et al.* 2008; Kell 2010). Epidemiological studies have shown that consumption of fresh fruits and vegetables is strongly associated with a decreased risk of cardiovascular diseases and certain cancers (Kris-Etherton *et al*. 2002; Serafini *et al*. 2002). To combat the harmful effects of free radicals in the human body, supplementation of antioxidants in a diet could be a long-term strategy to prevent life style-induced non-communicable diseases such as hypertension, cardiovascular diseases and cancers.

Murraya koenigii (Linn.) Spreng, locally known as "curry patta" or" mitha neem" (curry leaf tree), belonging to the Rutaceae family, is commonly used as the raw material for traditional medicinal formulations in India. Its aromatic leaves contain essential oil and it is used as a condiment. The fresh leaves of *M. koenigii* have been reported to possess anti-microbial, mosquitocidal, topo-isomerase inhibition and antioxidant properties (Salah *et al.* 1995; Kumpulainen and Salonen 1999; Powers *et al.* 2004; Gomes *et. al*. 2006). This paper aims to undertake a comparative biochemical investigation of *M. koenigii* to determine total phenols, flavonoids and the antioxidant potential of extracts of fresh roots, leaves and fruits and seeds of different stages of biological development and ripeness.

MATERIALS AND METHODS

M. koenigii plant material was collected from field-grown trees around the Ajmer area (26° 27' 0" North, 74° 38' 0" East). The extracts prepared from fresh materials were used for analyzing total phenols, flavonoids and antioxidant activity *in vitro*. Eight plant materials tested for determination of antioxidant activity were roots (R), leaves (L), un-ripened fruits (green colored) (URF) and its unripened seeds (URS), half-ripened fruit (HRF) and its half-ripened seeds (HRS), ripened fruit (RF), and its ripened seed (RS). 1 g of plant material was extracted in 10 ml of 80% methanol by maceration (10-15 min). The solvent was then centrifuged at 14,000 rpm for 30 min at room temperature. The extract obtained was used for analysis.

All solvents used were of analytical grade. 1,1-diphenyl-2-picryl hydrazyle (DPPH) and quercetine were procured from Sigma-Aldrich Inc., (St. Louis, USA); gallic acid and ascorbic acid were procured from Merck Co. (Darmstadt, Germany); Folin Ciocalteau reagent, aluminum chloride, methanol, sodium carbonate and potassium acetate were purchased from Qualigens Fine Chemical Co. (Mumbai, India).

Absorbance was measured on a Spectroscan-50, UV-VIS spectrophotometer (Biotech. Engineering Management Co. UK).

Data were statistically analyzed by calculating the standard deviation of their mean values. Taking 0% inhibition the regression analysis was used to produce regression equation by plotting a graph between the concentrations of the extracts and percentage inhibitions of free radicals. The IC_{50} values (concentration of extracts required to scavenge 50% DPPH free radicals) were calculated by using regression equations.

Determination of total phenols

Total phenols were determined by the Folin Ciocalteau reagent method (McDonald *et al*. 2001). An aliquot of each plant extract (0.5 ml 1:10 mg l^{-1}) or gallic acid (standard phenolic compound) was added with Folin Ciocalteau reagent (5 ml 1:10 diluted with distilled water) and 4 ml of a 1M solution of Na_2CO_3. The mixture was allowed to stand for 30 min at room temperature and absorbance was measured at 710 nm on a Spectroscan-50, UV-VIS

Received: 21 January, 2010. Accepted: 29 June, 2011.

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spectrophotometer (Biotech. Engineering Management Co., UK).

Total phenolic contents of extracts were expressed as mg gallic acid equivalent (GAE)/g fresh weight (FW). All samples were analyzed in triplicate.

Determination of total flavonoids

Total flavonoid content was analyzed by the aluminum chloride method (Chang *et al.* 2002). Each plant extract (0.5 ml of 1:10 g l^{-1}) was mixed with 1.5 ml methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M potassium acetate and 2.8 ml distilled water. The mixture was allowed to stand for 30 min at room temperature and absorbance was measured at 415 nm. Total flavonoid content was expressed as mg quercitin equivalents (QE) g^{-1} FW. Samples were analyzed in triplicate.

Determination of DPPH-free radical scavenging activity

Stable DPPH was used for *in vitro* determination of free radical scavenging activity of the extracts (Koleva *et al.* 2002). Different concentrations of each extract were mixed with a methanolic solution of DPPH (0.004%). The mixture was allowed to stand for 15 min. The scavenging of free radicals by each extract was evaluated spectrophotometrically at 517 nm against the absorbance of DPPH radicals. The percentage discoloration was calculated by following formula:

DPPH radical scavenging activity (%) = $[AC_{517} - AE_{517}/AC_{517}] \times 100$

where AC_{517} is the absorbance of the DPPH solution without extract, AE_{517} is the absorbance of the tested plant extract with DPPH. The degree of discoloration indicates the free radical scavenging efficiency of the substances. Ascorbic acid was used as a free radical scavenger reference compound.

RESULTS

Total phenol and flavonoid contents of methanolic extracts from different parts of *M. koenigii* as well as their free radical scavenging effect of the corresponding extracts were determined spectrophotometrically.

RF extract exhibited the highest content of both GAE and QE (**Fig. 1A**), and also the highest antioxidant capacity, i.e., AAE (**Fig. 1B**). The highest percentage of discoloration (81.26%) of DPPH was observed in the RF extract (**Fig. 1C**).

Regression equations to derive the IC_{50} values (concentration of extracts required to scavenge 50% DPPH-free radicals) showed an inverse relationship between IC_{50} value and percentage scavenging potential of a sample. The strongest DPPH radical scavenging activity was exhibited by the RF extract with an IC_{50} = 0.307 mg ml^{-1} (**Fig. 1D**).

DISCUSSION

The present investigation comprehensively profiles the antioxidant activity of extracts of different plant parts and fruit/seeds of an important aromatic plant, *M. koenigii*, focusing on phenols and flavonoids. Natural antioxidants of plant origin are important in health, food and preventive medicine (Halliwell *et al.* 2005). The antioxidant potential of different plant parts, more importantly in the fruits and seeds of different stages of development and ripeness, differed. RF of *M. koenigii* could be used as an important antioxidant supplement. Although many studies support that total phenols and flavonoids contribute significantly to the total antioxidant potential of many fruits and vegetables (Gerber *et al.* 2002; Katalinic *et al.* 2006), our observations that scavenging potential as well as total quantity of phenols and flavonoids are maximum in RF adds precision to the available knowledge in this area of work. In addition, the observations are likely to sensitize further basic physiological research on the possible association of process of ripening of fruits and enhance their antioxidant activity. Reports are also available on analysis and isolation of antioxidant vitamins and active carbazole alkaloids from fresh leaves of *M. koenigii* (Ramsewak *et al.* 1999; Tachiibana *et al.* 2001; Palaniswany *et al.* 2003) but the present investigation reports the first comparative analysis of the antioxidant potential of extracts from leaves, roots, fruits and seeds of different stages of biological development and ripeness of *M. koenigii.*

ACKNOWLEDGEMENT

Authors gratefully acknowledge the Desert Medicine Research Center, Jodhpur, India for providing research facilities.

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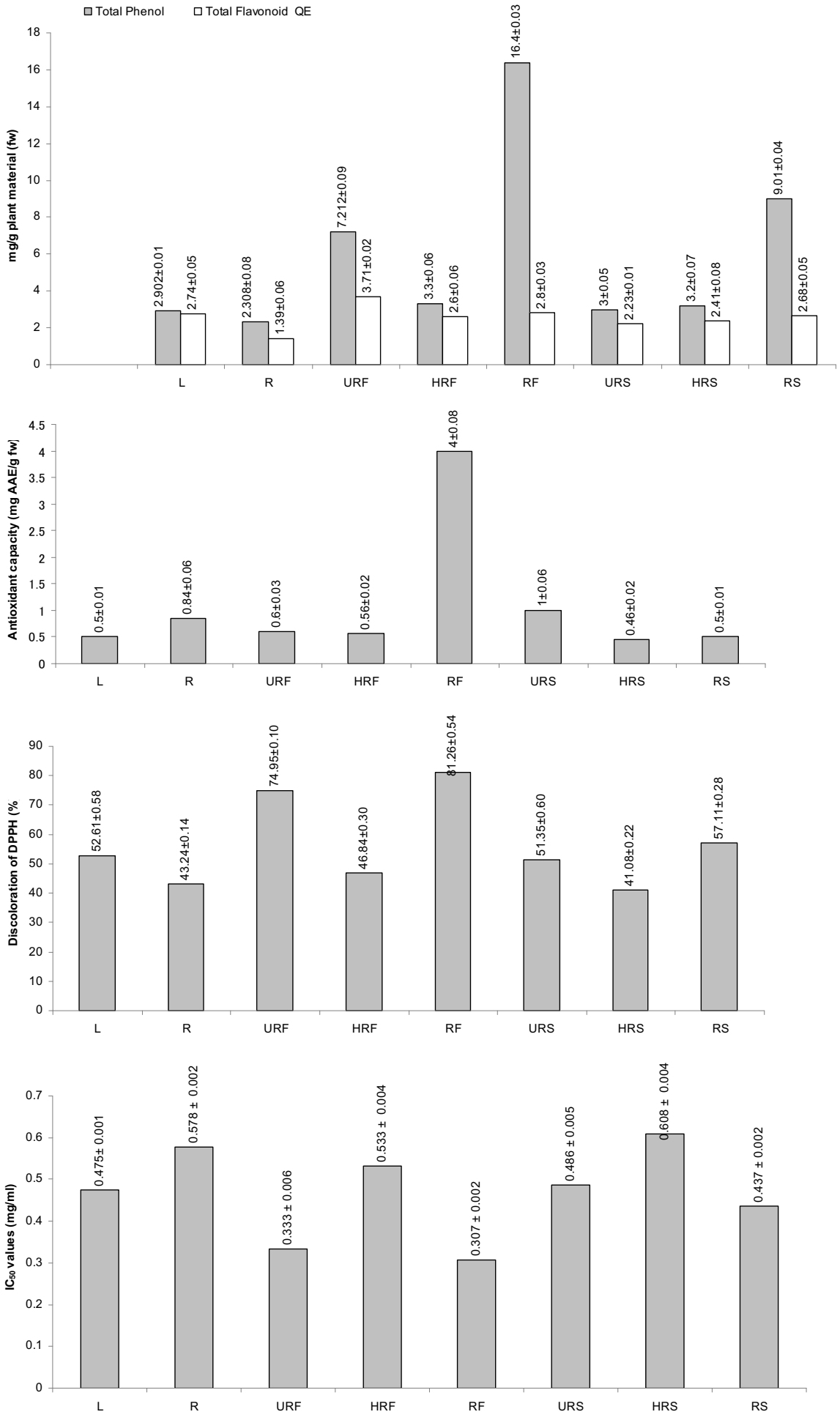

Fig. 1 Analysis of (**A**) total phenol and flavonoids, (**B**) antioxidant capacity, (**C**) discoloration of DPPH (%), (**D**) IC_{50} values. Values represent mean ± standard deviation.