Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 1, 2013

Research Article

TOTAL PHENOLIC, TOTAL FLAVONOID CONTENT AND THE DPPH FREE RADICAL SCAVENGING ACTIVITY OF *MELOTHRIA MADERASPATANA* (LINN.) COGN.

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Received: 06 Nov 2012, Revised and Accepted: 10 Dec 2012

ABSTRACT

Melothria maderaspatana (Linn.) Cogn. belonging to family Cucurbitaceae, is comprised mainly of slender scadentor prostrate annual herbs. It is used as a vegetable in India. In present investigation, shade dried plant parts (leaves, stem and fruits) were used for quantitative estimation of the total phenolic contents as Gallic acid equivalent (GAE) per gram dry weight and flavonoid as Quercetin equivalent (QE) per gram dry weight. The DPPH free radical scavenging activities were also estimated in different concentrations (25-100 µg per ml) of plant parts used. The total phenolic contents were ranging between 21.1±1.11 to 24.7±0.46 mg GAE/gm DW while flavonoid were found maximum 7.1±1.24 mg QE/gm DW in leaves. The DPPH radical scavenging capacity was found maximum in leaves and it was totally dose dependent and increased with increased concentrations.

Keywords: *Melothria maderaspatana*, DPPH radical, Total phenolic contents, Total flavonoid contents.

INTRODUCTION

In modern society, herbal medicine continues to flourish and play a pivotal and indispensable role in public healthcare. Exploring the bioactive constituents represents a promising approach toward discovery of new drugs. In India, different medicinal systems make use of a number of plants in the treatment of hypertension. The ability of some phenolic compounds to act as antioxidants has been demonstrated in the literature. Free radical that generate inside, the body is responsible for oxidative stress and compounds that can scavenge free radicals have great potential in ameliorating these disease processes [1]. Oxidative stress has been shown to play a very crucial role in some disease state like liver cirrhosis, atherosclerosis, cancer, diabetes, etc. Antioxidants have ability to protect the human body against damage by reactive oxygen species [2].

There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoid, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid [3]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity [4,5].

Melothria maderaspatana (Linn.) Cogn belonging to family Cucurbitaceae is reported as expectorant and has been used traditionally from a long time for a number of ailments. The tender shoots and bitter leaves are used as a gentle aperient and prescribed in vertigo and biliousness [6]. *M. maderaspatana* has been shown to exert hepatoprotective, immunomodulatory [7,8], antioxidant [9], anti-inflammatory [10], antimicrobial activity [11] and antiplatelet activity [12]. The consumption of *M. maderaspatana* leaf tea decreased the blood pressure and showed beneficial effects on lipid profile, fibrinogen, and bilirubin and body mass index in human volunteer [13]. However, no reports on quantification of total phenolic contents and flavonoid have been done so far.

MATERIALS AND METHODS

Plant material

The stem, leaves and fruits of selected plant was collected from Jaipur, Rajasthan, India during the month of December, 2011 and authenticated as RUBL as 21071 by Herbarium, Department of Botany, University of Rajasthan, Jaipur. The samples were dried at room temperature, crushed in grinder and the powder was extracted with methanol for 48h, filtered through Whatmann no. 1 filter paper and appropriately diluted with methanol.

Determination of total phenolic content

Total phenolic content were analyzed spectrophotometrically using a modified Folin-Ciocalteu colorimetric method [14]. 125µl 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-Ciocalteu 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 3ml. After incubation at room temperature for 90min, the absorbance was recorded at 760nm. All experiments were repeated three times for precision and values were expressed in mean ± standard deviation in terms of phenolic content (Gallic acid equivalent, GAE) per g dry weight. Reference curve was prepared using 10- 400 µg/ml of Gallic acid (linear regression $r^2 = 0.9975$).

Determination of total flavonoid content

Flavonoid quantification was done using aluminium chloride colorimetric method [15]. 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 415nm. All experiments were repeated three times for precision and values were expressed in mean ± standard deviation in terms of flavonoid content (Quercetin equivalent, QE) per g dry weight. The calibration curve was prepared using 12.5 to 100µg/ml of Quercetin in methanol (linear regression $r^2 = 0.9991$).

DPPH radical scavenging capacity

The antioxidative activity of the extracts was elucidated by 2, 2-diphenyl-1- picryhydrazyl (DPPH) free radical scavenging capacity of the extracts [16]. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) as free radical form (90% purity) was obtained from Sigma–Aldrich. DPPH solution (0.004% w/v) was prepared in methanol. Two ml of this solution was added to a sample solution (0.1ml, 1mg/ml in methanol). After 30 min, absorbance at 515nm was measured and the percentage of radical scavenging activity was calculated from the following equation:

% Radical scavenging = (1-Abs. sample/Abs. control) ×100

Abs. control is the absorbance of the DPPH solution without sample and Abs. sample is the absorbance of the tested sample.

RESULTS AND DISCUSSION

Natural plant extracts or pure compounds are safe ingredients, which do not have any toxic effects. Plant extracts can be characterized by polyvalent formulations and interpreted as additive, or, in some cases, potentiating. First, the therapeutic benefit of medicinal plants is usually attributed to their antioxidant properties and oxidative stress is a prominent feature of these diseases [17,18].

The screening of plant parts revealed that the amount of the total phenolic contents were higher in the fruit tissues (24.7±0.46 mg GAE/gm DW), while lower amount (21.1±1.11 mg GAE/gm DW) was observed in stem part of this plant. Flavonoids are regarded as one of the most the widespread groups of natural constituents found in plants. The amount of flavonoid content was decreased as leaf > stem > fruit tissues and ranging between 2.7±0.35 to 7.1±1.24 mg QE/gm DW. The results are shown in table 1.

According to Chanwitheesuk [19] antioxidants can act as either reducing agents, or by free radical scavengers or singlet oxygen quenchers. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. Medicinal plants can protect against harmful effects of ionizing radiation. The use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers.

The DPPH radical scavenging activity was decreased as leaf> fruit> stem. The screening of the leaf, stem and fruit of the plant indicates that the presence of high phenolic compounds may be due to the presence of tannins and flavonoid which are known to possess antioxidant activities [20,21,22]. The DPPH radical scavenging activities are shown in table 2.

In our earlier studies the similar results were obtained in *Spilanthus acemella* and *Tricosanthes cucumerena* Linn. [23,24].

In conclusion *Melothria maderaspatana* (Linn.) Cogn. have different concentrations of the total phenolic contents and flavonoid in various plant parts which possess the antioxidant activity of the plant. It has been shown that the scavenging effects on the DPPH radical scavenging activity increased with the increasing concentration of the samples to a certain extent and hence are said to be strongly dependent on the extract concentration. A strong correlation has been observed between the flavonoid and the DPPH radical scavenging. We further isolate the bioactive compounds which are responsible for the antioxidant activity.

Table 1: Total phenolic and total flavonoid content in different plant parts of *Melothria maderaspatana* (Linn.) Cogn.

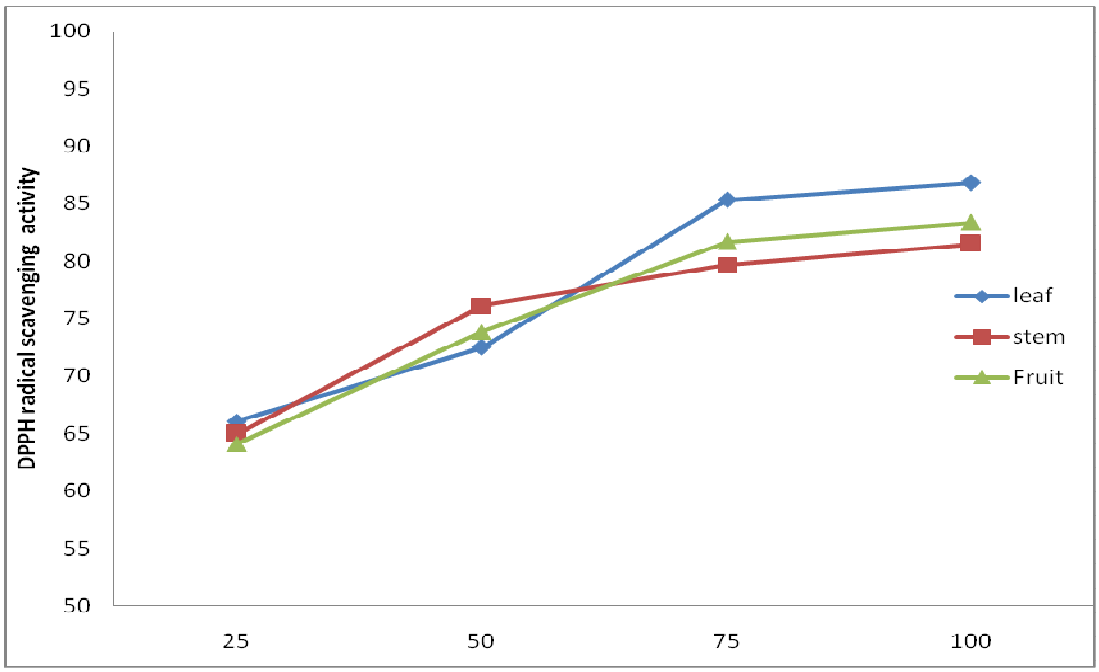
Plant part	Total Phenolic content (mg GAE/gm DW)	Total Flavonoid content (mg QE/gm DW)
Leaf	22.3±0.54	7.1±1.24
Stem	21.1±1.11	5.9±0.86
Fruit	24.7±0.46	2.7±0.35

Mean ±Standard Deviation

Table 2: The DPPH free radical scavenging activity (%) of *Melothria maderaspatana* (Linn.) Cogn.

Concentration (µg)	Leaf	Stem	Fruit
25	66.09±0.29	65.00±1.13	64.09±0.45
50	72.50±0.45	76.09±0.85	73.84±0.52
75	85.34±0.63	79.67±0.53	81.67±1.48
100	86.84±0.77	81.50±0.95	83.34±0.67

Mean ±Standard Deviation



Graph 1: The DPPH free radical scavenging activity (%) of *Melothria maderaspatana* (Linn.) Cogn.

ACKNOWLEDGEMENT

The one of the authors Babeet Singh Tanwer is grateful to the UGC for providing financial support as meritorious student fellowship file no. F. 4-1/2006 (BSR)/7-219/2009(BSR).

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