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Research Article

IN VITRO ANTIOXIDANT CHARACTERISTICS OF DIFFERENT PARTS OF *MELOTHRIA MADERASPATANA* (L.) COGN.

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ABSTRACT

The antioxidant capability and total phenolic contents of acetone and methanol extracts from the leaves, stem, fruits and roots of *Melothria maderaspatana* (L.) Cogn. were evaluated. Generally, methanol gave higher extract yields than acetone. Among the various extracts studied, root extracts gave higher values of total phenolics (19.7 and 18.3g/100g acetone and methanol extracts respectively), tannins (8.0 and 8.3g/100g acetone and methanol extracts respectively) and antioxidant activity in terms of FRAP (1182.8 and 1470.0 mmol Fe (II)/ mg acetone and methanol extracts respectively) and $ABTS^{\bullet+}$ (13648.4 and 14161.4μmol/g acetone and methanol extracts respectively) followed by stem extracts. The DPPH free radical scavenging activity was well established in methanol extract of stem (IC_{50} at 123.8μg/ml). However, inhibition of hemolysis (76.31% at 500μg) and phosphomolybdenum reduction (41.0mg ascorbic acid equivalent/ 100g extract) were found to be higher in acetone extract of roots. Further, the methanol extract of stem exhibited sustainable hydroxyl ($HO^{\bullet}$) radical scavenging potential and a noticeable iron chelating potential. Owing to these properties, this plant have the potential as natural source of antioxidants, capable of protecting against free radical mediated damage and may have applications in preventing and curing various diseases.

Keywords: *Melothria maderaspatana*; antioxidant; polyphenols; FRAP; $DPPH^{\bullet}$; $ABTS^{\bullet+}$

INTRODUCTION

Oxidation processes are intrinsic in the energy management of all living organisms and are therefore kept under strict control by several cellular mechanisms[1]. However, the excessive production of free radicals and the unbalanced mechanisms of antioxidant protection result in the onset of numerous diseases and accelerate ageing. The putative protective effects of antioxidants against these deleterious oxidation-induced injuries have received increasing attention in recent times, especially within biological, medical, nutritional, and agrochemical areas. Among dietary antioxidants, phenolic compounds, the secondary metabolites from plants, are the most abundant natural antioxidants [2], which act as reducing agents, hydrogen donators, free radicals scavengers, and singlet oxygen quenchers and therefore, as cell saviors[3].

Melothria maderaspatana (L.) Cogn. (synonyms: *Mukia maderaspatana* (L.) Roem; *M. maderaspatana* Arn.; *Bryonia scabrella; Cucumis maderaspatana* L.) (Family: Cucurbitaceae) is traditionally used as a leafy vegetable and to cure several ailments in South India. The plant has expectorant properties and is prescribed against chronic diseases with cough as a predominant symptom. Decoction of root is masticated for relief from toothache. Tender shoots and leaves are used as a gentle aperient and recommended in vertiligo and biliousness. Decoction of seeds is sudorific, crushed and applied on aching bodies, especially on strained backs. The plant is useful in vitiated conditions of pitta, burning sensation, diabetics, flatulence, colic, constipation, ulcers, asthma, neuralgia, nostalgia and odontalgia[4]. This plant extract has also been shown to have hepatoprotective[5], immunomodulatory[6] activities in rat models. *M. maderaspatana* leaf extract has antihypertensive, antioxidant and antihyperlipidemic properties in hypertensive patients[7,8]. Further, Raja and Pugalendi[9] reported the *in vitro* antioxidant property of aqueous extract of leaves from *M. maderaspatana*. Based on the traditional knowledge of medicinal system, the present study was carried out to evaluate the antioxidant activity of acetone and methanol extracts of leaves, stem, fruits and roots of this traditional edible species which occurs in wild as well as cultivated in kitchen gardens.

MATERIALS AND METHODS

Chemicals

Butylated hydroxyanisole (BHA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium ferricyanide, 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^{\bullet}$), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), potassium persulfate, 2,2'-azinobis(3-ethylbenzothiozoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$), 2,4,6-tripyridyl-*s*-triazine (TPTZ), linoleic acid, ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylenediamine tetraacetic acid (EDTA) disodium salt, trichloroacetic acid (TCA), Tween 40 and ferric chloride were obtained from Himedia, Merck or Sigma. All other reagents used were of analytical grade.

Preparation of extracts

Fresh leaves, stem, fruits and roots of *M. maderaspatana* were collected from Coimbatore, Tamil Nadu state, India during the month of June 2008, authenticated and deposited in the Botany Herbarium, Bharathiar University with voucher number BUBH-2898. The freshly collected plant materials were washed thoroughly in tap water, shade dried at room temperature (25°C), powdered and used for solvent extraction.

The plant samples were successively extracted with petroleum ether (for disposing lipid and pigments), acetone and methanol using soxhlet apparatus. Each time before extracting with the next solvent, the material was dried in hot air oven at 40°C. The solvents were evaporated using a rotary vacuum-evaporator (Yamato RE300, Japan) at 50ºC and the remaining water was removed by lyophilization (VirTis Benchtop K, USA). The extract recovery in different solvents was expressed as percent of the plant sample dry matter. The freeze-dried extracts thus obtained were dissolved in the respective solvents at the concentration of 1mg/ml and used for assessment of antioxidant capacity through various chemical assays.

Determination of total phenolic and tannin contents

The total phenolic content was determined according to the method described by Siddhuraju and Becker[10]. Aliquots of each extracts were taken in test tubes and made up to the volume of 1ml with distilled water. Then 0.5ml of folin-ciocalteu phenol reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents (TAE). Using the same extract the

tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP). One hundred milligrams of PVPP was weighed in a 100 × 12mm test tube and to this 1.0ml of distilled water and then 1.0ml of tannin containing phenolic extract were added. The content was vortexed and kept in the test tube at 4ºC for 4h. Then the sample was centrifuged (3000g for 10min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured, as monitored above and expressed as the content of non-tannin phenolics on a dry matter basis. From the above results, the tannin content of the sample was calculated as follows: Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%).

Ferric-reducing/antioxidant power (FRAP) assay

The antioxidant capacity of phenolic extracts of samples was estimated according to the procedure described by Pulido *et al.*[11]. FRAP reagent (900µl), prepared freshly and incubated at 37°C, was mixed with 90µl of distilled water and 30µl of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37°C for 30 min in a water bath. The final dilution of the test sample in the reaction mixture was 1/34.

The FRAP reagent contained 2.5ml of 20mmol/l TPTZ solution in 40mmol/l HCl plus 2.5ml of 20 mmol/l $FeCl_3 . 6H_2O$ and 25ml of 0.3mol/l acetate buffer (pH 3.6) as described by Siddhuraju and Becker [10]. At the end of incubation, the absorbance readings were taken immediately at 593nm, using a spectrophotometer. Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2000µmol/l, ($FeSO_4.7H_2O$) were used for the preparation of the calibration curve. The values are expressed as mmol Fe (II) per milligram extract.

Antioxidant activity by the ABTS$^{\bullet+}$ assay

The total antioxidant activity of the samples was measured by ABTS radical cation decolorization assay according to the method of Re *et al.*[12]. ABTS$^{\bullet+}$ was produced by reacting 7mM ABTS aqueous solution with 2.4mM potassium persulfate in the dark for 12–16h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to give an absorbance at 734 nm of 0.700 ± 0.02.

The stock solution of the sample extracts were diluted such that after introduction of 10µl aliquots into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1ml of diluted ABTS solution to 10µl of sample or Trolox standards (final concentration 0–15µM) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated of the blank absorbance at 734 nm and then was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts on dry matter.

Metal chelating activity

The chelating of ferrous ions by acetone and methanol extracts of *M. maderaspatana* was estimated by the method of Dinis *et al.*[13]. Briefly the extract samples (250µl) were added to a solution of 2mmol/l $FeCl_2$ (0.05ml). The reaction was initiated by the addition of 5mmol/l ferrozine (0.2ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto *et al.*[14]. An aliquot of 100µl of sample solution was combined with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in a 4ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents/100g extract.

Free radical scavenging activity on DPPH$^{\bullet}$

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH**,** according to the method of Blios[15]. Sample extracts at various concentrations was taken and the volume was adjusted to 100µl with methanol. 5ml of a 0.1mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

% DPPH radical scavenging activity = (Control OD – Sample OD / Control OD) X 100.

Hydroxyl radical scavenging activity

The scavenging activity of acetone and methanol extracts of *M. maderaspatana* on hydroxyl radical was measured according to the method of Klein *et al.*[16]. Various concentrations (50, 100, 150 and 200µg) of extracts were added with 1.0ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA solution (0.018%), and 1.0ml of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5ml of ascorbic acid (0.22%) and incubated at 80–90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1.0ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0g of ammonium acetate, 3.0ml of glacial acetic acid, and 2ml of acetyl acetone were mixed and raised to 1L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity is calculated by the following formula:

% HRSA = from $[(A_0 - A_1)/A_0]$ X100, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

The β-carotene/linoleic acid antioxidant activity

One millilitre of a β-carotene solution in chloroform (1mg/10ml) was pipetted in to a flask containing 20mg of linoleic acid and 200mg of Tween 40. The chloroform was removed by rotary vacuum evaporator at 45°C for 4 min and, 50ml of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion.

A 5ml aliquot of the emulsion was added to a tube containing 200µl of the antioxidant (extracts, BHA or α-T) solution at 1mg/ml concentration and the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without β-carotene[17]. The tubes were placed in a water bath at 50°C and the absorbance was monitored at 15 min intervals until 180min. All determinations were carried out in triplicate. The antioxidant activity of the sample extracts and standard was evaluated in terms of bleaching of β-carotene using the following formula: AA = $[1- (A_0 - A_t)/(A^1_0 - A^1_t)]$ X 100, where A_0 and A^1_0 are the absorbance measured at zero time of incubation for the test sample and control, respectively, and A_t and A^1_t are the absorbances measured in the test sample and control, respectively, after incubation for 180 min.

Antihemolytic activity

Antihemolytic activity of the extracts was assessed as described by Naim *et al.*[18]. The erythrocytes from cow blood were separated by

centrifugation and washed with phosphate buffer (0.2M, pH 7.4). The erythrocytes were then diluted with phosphate buffered saline to give 4% suspension. 500μg of extract/ml of saline buffer were added to 2ml of the erythrocyte suspension and the volume was made up to 5ml with saline buffer.

The mixture was incubated for 5 min at room temperature and then 0.5ml of H_2O_2 solution in saline buffer was added to induce the oxidative degradation of the membrane lipids. The concentration of H_2O_2 in the reaction mixture was adjusted to bring about 90% hemolysis of blood cells after 240 min. After incubation the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of hemolysis was determined by measuring the absorbance at 540nm corresponding to hemoglobin liberation.

Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($p<0.05$) using statistica (Statsoft Inc., Tulsa, USA).

Values expressed are means of three replicate determinations ± standard deviation.

RESULTS AND DISCUSSION

Recovery percent, total phenolics and tannin contents

The yield percent, total phenolics and tannins content of the extracts obtained from *M. maderaspatana* are shown in Table 1. Methanol gave higher yield, with the stem and fruit recording equal and maximum recovery percentages (4.7g/100g extract). Among the extract of *M. maderaspatana*, roots contained higher levels of total phenolics (19.7g/100g acetone extract; 18.3g/100g methanol extract) followed by stem (14.7g/100g methanol extract; 12.6 g/100g acetone extract) in terms of tannic acid equivalents. The tannin contents of the extracts of *M. maderaspatana* also showed similar trend with the roots registering higher content (8.0 and 8.3g/100 g acetone and methanol extracts respectively) followed by stem (6.6 and 6.2 g/100g acetone and methanol extracts respectively). However the total phenolic (4.6g/100g) and tannin (0.1g/100g) contents of acetone extracts of fruits were much lower. Some phenolic compounds present in natural products have higher antioxidant activities than those of synthetic antioxidants[19]. Higher amount of phenolic compounds in acetone and methanol extracts of *M. maderaspatana* roots make it a strong free radical scavenger, indicating that this plant is a good source for natural antioxidants and can prevent free radical mediated oxidative damage.

Table 1: Extract yield percentage total phenolics and tannin contents of acetone and methanol extracts of *M maderaspatana*

Sample	Extract yield (%)	Total phenolics (g/100g extract)	Tannin (g/100g extract)
LA	0.9	12.2 ± 1.0	5.4 ± 0.5
SA	1.2	12.6 ± 0.3	6.6 ± 0.6
FA	2.9	4.6 ± 0.1	0.1 ± 0.1
RA	0.7	19.7 ± 1.1	8.0 ± 0.2
LM	4.3	9.6 ± 0.5	5.0 ± 0.1
SM	4.7	14.7 ± 0.6	6.2 ± 0.2
FM	4.7	10.4 ± 0.3	6.1 ± 0.1
RM	3.9	18.3 ± 1.0	8.3 ± 0.2

Values are mean of three replicate determinations (n=3) ± standard deviation.
LA, SA, FA and RA are acetone extracts of leaf, stem, and fruit and root respectively.
LM, SM, FM and RM are methanol extracts of leaf, stem, and fruit and root respectively.

Ferric reducing antioxidant power

FRAP assay directly measures antioxidants or reductants in a sample that react with ferric tripyridyltriazine (Fe^{3+} TPTZ) complex and produce a coloured ferrous tripyridyltriazine (Fe^{2+} TPTZ). The antioxidant capacities of sample extracts of *M. maderaspatana* varied significantly ($p<0.05$) (Table 2) and the root extracts showed the higher FRAP antioxidant activity (1470.0 and 1182.8mmol Fe (II)/mg methanol and acetone extracts, respectively) followed by stem extracts (970.0 and 901.1mmol Fe (II)/mg methanol and acetone extracts respectively). The order of FRAP activity of respective sample extract is as follows: RM > RA > SM > LM > SA > FA > FM > LA. Rice-Evans *et al.*[20] reported that phenolic compounds exhibited redox properties by acting as reducing agents, hydrogen donators and singlet oxygen quenchers. Oktay *et al.*[21] also observed a highly positive relationship between total phenolics and antioxidant activity in many plant species. Therefore, the sample extracts can act as electron donors and react with free radicals and convert them to stable products, thus terminating the radical chain reaction.

ABTS$^{\bullet+}$ radical scavenging activity

ABTS$^{\bullet+}$ assay is an excellent tool to determine the antioxidant activity of hydrogen donating and chain breaking antioxidants. In ABTS$^{\bullet+}$ cation radical scavenging method, the activity of tested sample extracts are expressed as the micromolar equivalent of Trolox solution, having an antioxidant capacity equivalent to 1g dry matter of the extract under the experimental investigation. The effect of acetone and methanol extracts of *M. maderaspatana* on ABTS radical cation scavenging activity is presented in Table 2. Though all the samples exhibited strong ABTS$^{\bullet+}$ radical scavenging activity, the root extracts exhibited higher TAA (14161.4 and 13648.4μmol/g methanol and acetone extracts respectively) followed by stem extracts (8572.5 and 5757.7μmol/g methanol and acetone extracts respectively). Pietta *et al.*[22] evaluated the total antioxidant potential of commonly used medicinal plants and concluded that the phenolic compounds play a vital role in scavenging of ABTS$^{\bullet+}$. The methanol and acetone extracts *M. maderaspatana* roots indicate that they could terminate the oxidation process by converting free radicals to the stable form.

Metal chelating activity

Presence of transition metal ions in a biological system could catalyse the Haber–Weiss and Fenton-type reactions, resulting in generation of hydroxyl radicals ($OH^{\bullet}$). However, these transition metal ions could form chelates with the antioxidants, which result in the suppression of $OH^{\bullet}$ generation, and inhibit ion of peroxidation processes of biological molecules. In this assay, the presence of chelating agents in the extracts of *M. maderaspatana* disrupt the ferrozine–Fe^{2+} complex formation, thus decreasing the red colour. The metal ion scavenging effects of various extracts were in the following order: FM > SM > FA=RA> LA=SA > RM > LM (Table 2). It is reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion[23]. The data presented in this study indicated that *M. maderaspatana* ability for iron binding and could reduce the generation of hydroxyl radicals.

Phosphomolybdenum assay

Various extracts of *M. maderaspatana* were also used to determine their antioxidant capacities by the formation of green phosphomolybdenum complex. The formation of the complex at 95°C was measured by the intensity of absorbance (695 nm) in

extracts at the concentration of 100μg/ml as shown in Table 2. The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of green phosphate/Mo(V) complex with the maximal absorption at 695 nm. Being simple and independent of other antioxidant measurements commonly employed, the assay was extended to plant polyphenols. In the present study, the phosphomolybdenum reduction potential of various sample extracts were in the order of RA > LA> RM > SA> SM > LM > FM > FA with the respective values of 41.0 > 33.0 > 28.8 > 28.0 > 26.0 > 22.8 > 20.4 > 18.5 g ascorbic acid equivalents/100g of the extract. The results indicate that the acetone extract of *M. maderaspatana* roots (41.0g AA/100g extract) has powerful antioxidant activity than other sample extracts. Though both the acetone and methanol extracts of *M. maderaspatana* roots showed superior and comparable activities in other assays (FRAP, ABTS and DPPH) in the present study, only the acetone acetone extract of roots showed powerful antioxidant activity in phosphomolybdenum assay than other extracts. This may be explained by the fact that the transfer of electrons/hydrogen from antioxidants occurs at different redox potential in various assay systems and the transfer also depends on the structure of the antioxidants[24].

Table 2: FRAP, ABTS•+, metal chelating and phosphomolybdenum activity of acetone and methanol extracts of *M. maderaspatana*

Sample	FRAP[A] (mmol Fe (II)/mg extract)	TAA[B] (μmol/g extract)	Metal chelating property (mg EDTA/g extract)	AEAC[C] (gAA/100g extract)
LA	317.8 ± 58.3^{e}	4083.7 ± 1490.0^{d}	1.2 ± 0.4^{b}	33.0 ± 1.4^{b}
SA	01.1 ± 324.3^{c}	5757.7 ± 543.9^{c}	1.2 ± 0.5^{b}	28.0 ± 0.4^{c}
FA	514.4 ± 13.9^{d}	3786.7 ± 403.5^{e}	2.0 ± 0.4^{a}	18.5 ± 0.7^{d}
RA	1182.8 ± 33.3^{b}	13648.4 ± 2237.4^{a}	2.0 ± 0.9^{a}	41.0 ± 4.7^{a}
LM	955.6 ± 20.0^{c}	3240.0 ± 458.6^{e}	0.7 ± 0.2^{c}	22.8 ± 0.3^{c}
SM	970.0 ± 7.3^{c}	8572.5 ± 390.7^{b}	2.4 ± 0.7^{a}	26.0 ± 0.2^{c}
FM	505.6 ± 6.9^{d}	2227.5 ± 112.7^{f}	2.9 ± 0.7^{a}	20.4 ± 0.6^{d}
RM	1470.0 ± 152.6^{a}	14161.4 ± 597.1^{a}	1.1 ± 0.3^{b}	28.8 ± 2.9^{c}

Values are mean of three replicate determinations (n=3) ± standard deviation. LA, SA, FA and RA are acetone extracts of leaf, stem, fruit and root respectively. LM, SM, FM and RM are methanol extracts of leaf, stem, and fruit and root respectively.

[A] - Concentration of substance having ferric-TPTZ reducing ability as equivalent to 1 mmol Fe(II).
[B] - Total antioxidant activity (μmol equivalent Trolox performed by using ABTS•+ radical cation).
[C] - Ascorbic acid equivalent antioxidant capacity (g equivalent of ascorbic acid per 100 gram extract) through the formation of phosphomolybdenum complex.

Mean values followed by different superscript in a column are significantly different (p<0.05).

DPPH• radical scavenging activity

DPPH• radical scavenging activity has been widely used to evaluate the antioxidant activity of plant extracts and foods [25]. The presence of antioxidant in the sample extract react with DPPH, which is a stable free radical, and convert it to 1,1-diphenyl-2-(2,4,6-trinitrophenyl) hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant compounds which can be detected spectrophotometrically at 517 nm. Fig 1 shows the DPPH radicals scavenging capacity of various extracts of *M. maderaspatana* with BHA and α-tocopherol as references. Concentration of the sample necessary to decrease initial concentration of DPPH• by 50% (IC_{50}) under the experimental condition was calculated. Therefore lower value of IC_{50} indicates a higher antioxidant activity. The experimental data indicate that methanol extract of *M. maderaspatana* stem displayed the highest DPPH• scavenging effect (123.8μg/ml) followed by the methanol and acetone and extracts of roots (130.1 and 136.9μg/ml respectively). In contrast, the fruits extracts were found to have lesser antioxidant activity (1506 and 1766.8μg/ml of acetone and methanol extracts respectively). The radical scavenging activity of the extracts could be related to the nature of phenolics and their hydrogen donating ability[26].

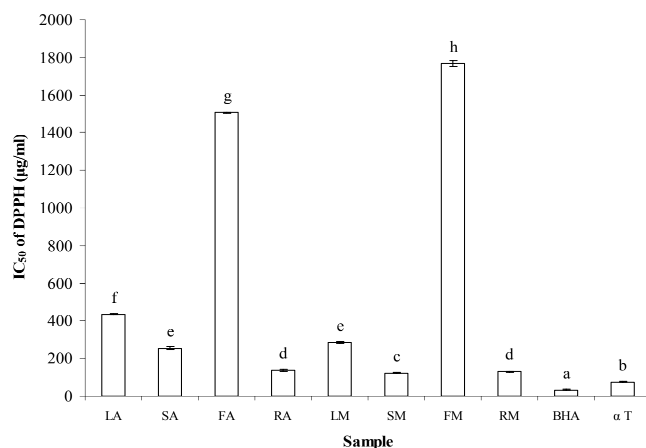

Fig. 1: DPPH• radical scavenging activity of acetone and methanol extracts of *M. maderaspatana*

LA, SA, FA and RA are acetone extracts of leaf, stem, fruit and root respectively.
LM, SM, FM and RM are methanol extracts of leaf, stem, fruit and root respectively BHA - Butylated hydroxyl anisole; α T- α-tocopherol.
Values are mean of three replicate determinations (n=3) ± standard deviation.
Bars having different letters are significantly different (p<0.05).

Hydroxyl radical scavenging activity

Scavenging of OH• is an important antioxidant activity because of its very high reactivity which can easily cross the cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing OH• is very important for the protection of living systems[27]. Fig 2, shows the OH• scavenging effect of *M. maderaspatana* extracts at different dose levels (50, 100, 150 and 200µg/ml. All the extracts generally registered good hydroxyl radical scavenging activity in a concentration dependent manner. Among them, the methanol extract of stem showed the highest OH• scavenging potential (42.6% at 200µg/ml concentration). The ability of the *M. maderaspatana* extracts to quench hydroxyl radicals seems to directly relate to the prevention of propagation of the process of lipid peroxidation, and seem to be good scavengers of active oxygen species thus reducing the rate of chain reaction.

β-Carotene bleaching (BCB) assay

Assessment of antioxidant activity using the BCB assay is based on *in vitro* bleaching of β -carotene, caused by radicals released upon the oxidation of linoleic acid in the emulsion[28]. The effectiveness of antioxidants in suppressing the action of radicals towards β-carotene was evaluated by monitoring the colour reduction spectrophotometrically.

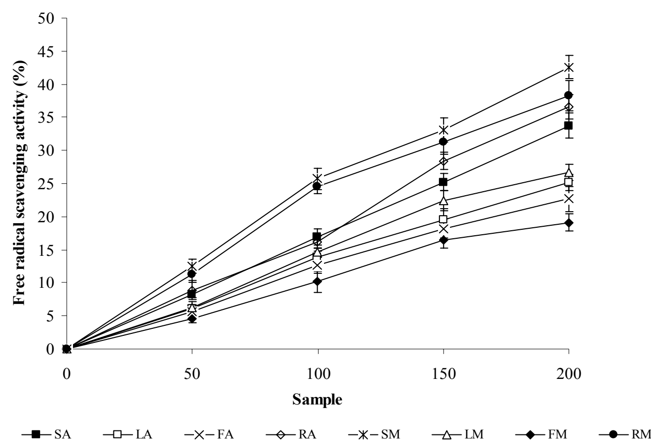

Fig. 2: Hydroxyl radical scavenging activity of acetone and methanol extracts of *M. maderaspatana*

LA, SA, FA and RA are acetone extracts of leaf, stem, fruit and root respectively.
LM, SM, FM and RM are methanol extracts of leaf, stem, fruit and root respectively
Values are mean of three replicate determinations (n=3) ± standard deviation.

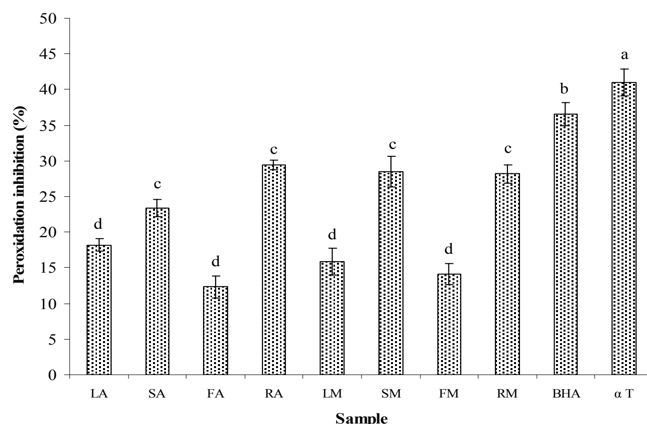

Fig. 3: β carotene/ linoleic acid peroxidation inhibition activity of acetone and methanol extracts of *M. maderaspatana*

LA, SA, FA and RA are acetone extracts of leaf, stem, fruit and root respectively. LM, SM, FM and RM are methanol extracts of leaf, stem, fruit and root respectively BHA - Butylated hydroxyl anisole; α T - α-tocopherol.
Values are mean of three replicate determinations (n=3) ± standard deviation.
Bars having different letters are significantly different ($p<0.05$).

As seen in Fig 3, both stem and roots of *M. maderaspatana* exhibited strong and comparable antioxidative activities in emulsion. The acetone extract of roots displayed the highest inhibition effect (29.4%), whereas the acetone extract of fruits demonstrated the lowest activity (12.3%) at 200 µg/ml plant material. Meanwhile BHA (36.6%) and α- tocopherol (41%) registered higher peroxidation inhibition than the sample extracts. Overall, all extracts displayed moderate potential of quenching linoleate free radicals (generated

from linoleic acid peroxidation) and shielding of the carotenoid from bleaching. The bleaching of β-carotene could be inhibited by antioxidants, which are capable of reducing the rate of chain reaction initiated during lipid peroxidation and transforming the reactive end product to a more stable form.

Antihemolytic activity

Erythrocyte membrane is rich in polyunsaturated fatty acids which are susceptible to free radical-mediated peroxidation. Since peroxidation of membrane lipids is a free-radical chain reaction, the erythrocyte membrane is quickly damaged, leading to hemolysis. In this study, we used a biological test based on free radical-induced erythrocyte lysis in cow blood. This assay is useful for screening studies on various molecules and their metabolites, especially on the one hand molecules have an oxidizing or antioxidizing activity and on the other hand molecule having a long-term action[29]. Lipid oxidation of cow blood erythrocyte membrane mediated by H_2O_2 induces membrane damage and subsequently hemolysis. The antihemolytic activity of the acetone and methanol extracts of *M. maderaspatana* leaves, stem, fruits and roots are presented in Fig 4. At the concentration of 500µg of extract in the final reaction mixture, acetone extract of *M. maderaspatana* root and stem samples registered comparable activity (76.31% and 73.14% respectively) which is higher than the standard BHA. Interestingly, LA, FA, LM, SM, and RM were found to have higher level of inhibition of hemolysis than the positive control α tocopherol (56.3%). The prevention of hemolysis by the extracts were in the following order: RA > SA >BHA> LA > LM > SM > RM > FA > αT > FM. Pronounced antihemolytic activities have also been reported from the extracts of *Oudneyna africanan*, *Artemisia arboresens, Globularia alpyu*[29], *Camellia sinensis, Ficus bengalensis* and *Ficus racemosa*[30]. Likewise, the antioxidant activities of chlorogenic acid and related catechols have already been studied in both cell free systems (such as DPPH microassay) and in cell models (as erythrocytes)[31]. Phenolic compounds of *M. maderaspatana* present in the incubation medium may have ability to quench hydroxyl radicals before the radicals attack the biomolecules of the erythrocyte membrane to cause oxidative hemolysis.

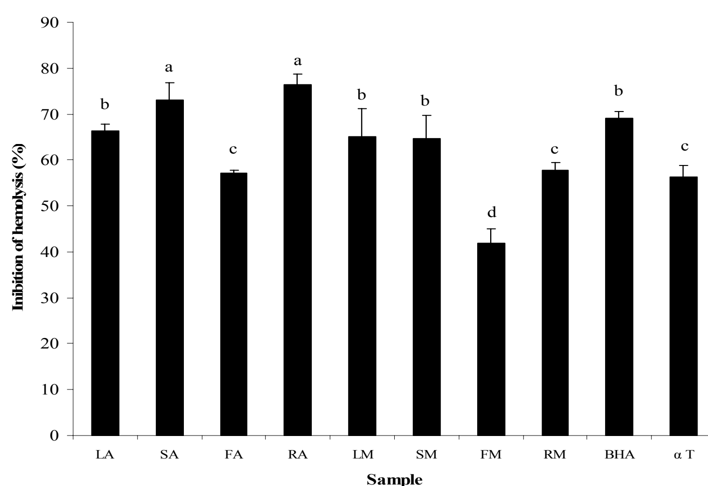

Fig. 4: Antihemolytic activity of acetone and methanol extracts of *M. maderaspatana*

LA, SA, FA and RA are acetone extracts of leaf, stem, fruit and root respectively.
LM, SM, FM and RM are methanol extracts of leaf, stem, fruit and root respectively BHA - Butylated hydroxyl anisole; α T- α-tocopherol.
Values are mean of three replicate determinations (n=3) ± standard deviation.
Bars having different letters are significantly different ($p<0.05$).

CONCLUSION

The results of the present study indicate that acetone and methanol extracts of *M. maderaspatana* stem and roots are high in phenolic contents and these extracts exhibit strong antioxidant activities. The scavenging activities observed against DPPH and hydroxyl radicals, peroxidation inhibition as well as the protective activities against erythrocyte damage, lead us to propose *M. maderaspatana* roots and stem as promising natural sources of antioxidants suitable for application in nutritional/pharmaceutical fields, in the prevention of free radical- mediated diseases. Further studies are needed to explore the potential phenolics compound(s) from *M. maderaspatana* and *in vivo* studies are needed for better understanding their mechanism of action.

REFERENCES

1. Halliwell B, Gutteridge, JMC. Free radicals in biology and medicine. Oxford: Oxford University Press; 2007.
2. Fiorentino A, D'Abrosca B, Pacifico S, Mastellone C, Piscopo V, Caputo R, Monaco P. Isolation and structure elucidation of antioxidant polyphenols from quince (*Cydonia vulgaris*) peels. J. Agric. Food Chem. 2008; 56: 2660-2667.
3. Fattouch S, Caboni P, Coroneo V, Tuberoso CIG, Angioni A, Dessi, S, Marzouki N, Cabras P. Antimicrobial activity of tunisian quince (*Cydonia oblonga*) pulp and peel polyphenolic extracts. J. Agric. Food Chem. 2007; 55: 963-969.
4. Nair RV. Indian medicinal plants. Vol 4, Chennai: Orient Logman Private Limited; 2002. p. 73.
5. Thabrew MI, Silva KTD, Labadie RP. Immunomodulatory activity of three Sri- Lankan medicinal plants used in hepatic disorders. J. Ethnopharmacol. 1991; 33: 63-66.
6. Thabrew MI, Christopher D, Robin D. Protection against galactosamine and tert-butyl hydroperoxide induced hepatocyte damage by *Melothria maderaspatana* extract Phytother. Res. 1995; 9: 513-517.
7. Raja B, Kaviarasan K, Arjunan MM, Pugalendi KV. *Melothria maderaspatana* leaf extract for treating hypertension: Chemistry and effects on biomarkers, J. Alter. Comp. Ther. 2005; 11: 264-268.
8. Raja B, Kaviarasan K, Arjunan MM, Pugalendi KV. Effect of *Melothria maderaspatana* (Linn.) leaf extract on blood pressure, lipid profile, anthropometry, fibrinogen, bilirubin and albumin levels in hypertensive patients. J. Alter. Comp. Med. 2007; 13: 349-354.

9. Raja B, Pugalendi KV. Evaluation of antioxidant activity of *Melothria maderaspatana in vitro*, Cent. Eur. J. Biol. 2010; 5(2): 224-230.
10. Siddhuraju R, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic orgins of Drumstick tree (*Moringa olifera* Lam.) leaves. J. Agric. Food Chem. 2003; 51: 2144-2155.
11. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric. Food Chem. 2000; 48: 3396-3402.
12. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free. Radic. Bio. Med. 1999; 26: 1231-1237.
13. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Pharm. Res. 1994; 315: 161-169.
14. Prieto P, Pineda M, Aguilar M. Spectophotometric quantitative of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem. 1999; 269: 337-341.
15. Blios MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 26: 1199-1200.
16. Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. Biochemistry 1991; 20: 6006-6012.
17. Taga MS, Miller EE, Pratt DE. Chia seeds as a source of natural lipid antioxidants. J. Am. Oil Chem. Soc. 1984; 61: 928-931.
18. Naim M, Gestener B, Bondi A, Birk Y. Antioxidant and antihemolytic activities of soyabean isoflavones. J. Agric. Food Chem 1976; 24: 1174-1177.
19. Lu Y, Foo Y. Antioxidant and radical scavenging activities of polyphenols from apple pomace. Food Chem. 2000; 68: 81-85.
20. Rice-Evans CA, Miller NT, Paganga G. Antioxidant properties of selected fruit seeds. Food Chem. 1997; 88: 411- 417.
21. Oktay M, Culcin I, Kufrevioglu OI. Determination of *in vitro* antioxidant activity of fennel (*Foenniculum vulgare)* seed extracts. Lebensm. Wiss. Technol. 2003; 36: 263-271.
22. Pietta P, Simonetti P, Mauri P. Antioxidant activity of selected medicinal plants. J. Agric. Food Chem. 1998; 46: 4487- 4490.
23. Gardner PT, White TC, McPhail DB, Duthie GG. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chem. 2000; 68: 471-474.
24. Loo AY, Jain K, Darah I. Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. Food Chem. 2008; 107: 1151-1160.
25. Soares JR, Dinis TCP, Cunha AP, Almedia LM. Antioxidant activities of some extracts of *Thumus zygis*. Free. Radic. Res. 1997; 26: 469- 478.
26. Shimada KK, Fujikawa KY, Nakamura T. Antioxidative properties of xanthone on autoxidation of soybean oil in cyclodextrin. J. Agric. Food Chem. 1992; 40: 945-948.
27. Yang J, Guo J, Yuan J. *In vitro* antioxidant properties of rutin. Lebensm. Wiss. Technol. 2008; 41: 1060-1066.
28. Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem. Anal. 2002; 13: 8-17.
29. Djeridane A, Yous M, Nadjemi B, Vidal N, Lesgards JF, Stocker P. Screening of some Algerian medicinal plants for the phenolic compounds and their antioxidant activity. Eur. Food Res. Technol. 2006; 224: 801-809.
30. Manian R, Anusuya N, Siddhuraju P, Manian S. The antioxidant and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. Food Chem. 2008; 107: 1000-1007.
31. Lekse JM, Xia L, Stark J, Morrow JD, May JM. Plant catechols prevent lipid peroxidation in human plasma and erythrocytes. Mol. Cell. Biochem. 2001; 226: 89–95.