

Antioxidative constitution of *Mukia maderaspatana* (Linn.) M. Roem. leaves

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Received 10 February 2010; Accepted 22 November 2010

Indigenous leafy vegetables of the traditional rural communities are potentially nutritious and contain a vast array of beneficial phytochemicals that offer protection against several chronic and age-related human ailments. The present study was planned to assess the antioxidative constitution of the fresh leaves of *Mukia maderaspatana* (Linn.) M. Roem. (Family: Cucurbitaceae). The total phenolic, flavonoid, carotenoid and vitamin E contents were determined using specific spectrophotometric methods and the amount of ascorbic acid by titrimetry. The medicinal leafy vegetable was found to be a good dietary source of the low molecular weight antioxidants analysed. The leaf extract exhibited potent *in vitro* antioxidant/radical scavenging (ABTS^{•+} and FRAP assays) and metal (Ferrous ion) chelating activities and also inhibited β -carotene bleaching. Saponarin has been isolated and determined as the major phenolic antioxidant by HPLC. The amount of phenolics (292.392 ± 1.087 mg GAE), flavonoid (247.079 ± 0.705 QE), carotenoids (0.812 ± 0.075 mg), vitamins C (17.046 ± 0.839 mg) and E (0.194 ± 0.068 mg) and saponarin (220.800 ± 0.944 mg) present and the antioxidant capacities (301.926 \pm 0.869 mg vitamin C equivalent antioxidant capacity) are reported for 100 g of fresh leaves. The protective/therapeutic significance of these antioxidant components is also highlighted.

Keywords: *Mukia maderaspatana*, Cucurbitaceae, Antioxidant, Phytochemicals, Dietary source, Leafy vegetable, Saponarin.

IPC code; Int. cl. (2011.01)—A61K 36/00, A61P 39/06.

Introduction

Indigenous leafy vegetables play an important role in providing food security, health care and longevity for the rural population, especially in countries, where modern health care is still a far reaching goal due to economic constraints. Insufficient vegetable and fruit consumption annually causes 2.7 million deaths worldwide and is one of the top ten risk factors contributing to human mortality. Food and Agricultural Organisation has reported that at least one billion people are thought to use wild foods in their diet¹. In India, Malaysia and Thailand, about 150 wild plant species have been identified as sources of emergency food and similarly, about 1400 edible plant species in South Africa¹. Therefore, exploiting the locally grown edible wild and semi-cultivated plant resources, which are a treasure trove of the most affordable health-promoting phytochemicals and micronutrients and considering their incorporation in the diet of nutritionally marginal populations or to certain vulnerable groups within populations,

especially in developing countries, where poverty and climatic changes are causing havoc to the rural populace, may prove worthy. Unfortunately, the knowledge of the potent medicinal properties of several indigenous leafy vegetables happens to remain confined to the traditional rural communities and has not been exposed to the modern societies. *Mukia maderaspatana* (Linn.) M. Roem. syn. *Melothria maderaspatana* (Linn.) Cogn.; *Cucumis maderaspatana* (Linn.); *Mukia scabrella* (Linn. f.) Arn.; *Bryonia scabrella* Linn. f., (Locally known as: Musumusukkai) (Family: Cucurbitaceae), is an annual monoecious, climbing vine or prostrate herb, densely covered with white hairs (Plate 1) and found commonly in waste places and vacant fields, extending from the plains of the coast, ascending up to 1800 m in the hills, almost throughout India and in China, Taiwan, Malaysia, Australia, New Zealand and in Africa^{2,3}. Recipes commonly prepared by the local communities using the leaves of *M. maderaspatana* include (i) grinding three handful of the leaves mixed with 0.25 Kg of rice, soaked in water to get a batter suitable to cook as *dosa* (crispy savoury pancake consumed as a staple food) and (ii) roasting required amount of the leaves in ghee or gingili oil and then

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grinding the mass with a roasted mixture of fresh coriander leaves, curry leaves, dhal, pepper, red chillies, garlic and salt, to get a delicious paste that can be used as *chutney*^{4,5} (a side dish to add taste) for the local recipes like cooked rice, *idli* (a savoury cake, also consumed as a staple food), *dosa*, etc.

The leaves of *M. maderaspatana* find a prominent place in the Siddha and Ayurvedic systems of medicine for over three centuries. Folkloric traditional medicine claims that the leaves and tender shoots are useful as aperient, diuretic, stomachic, antipyretic, antifatulent, antiasthmatic, antitussive, antihistaminic, antibronchitic and as an expectorant, in addition to its prescription against vertigo and biliousness²⁻⁶. The leaf-tea is administered for the alleviation of jaundice⁷ and *mupatena* tea, the extract of the leaves and bark is reported to be a good decongestant and a very good remedy for cough, cold and flu⁸. The leaf extract has also been shown to possess immunomodulatory⁹, hepatoprotective¹⁰⁻¹³, antiinflammatory¹⁴, antimicrobial¹⁵, hypotensive and vasodialatory activities^{16,17} and the ethyl acetate fraction has recently been reported to possess a dose-dependent antiplatelet activity¹⁸. The leaf-tea is also claimed to possess hypotensive properties in human subjects with concomitant beneficial effects on serum antioxidant potential, plasma lipid profile, fibrinogen, serum bilirubin and body mass index¹⁷.

Dietary phytochemicals, especially polyphenols such as the ubiquitous flavonoids, polyunsaturated fatty acids, tocopherols, vitamin C and carotenoids, have been the subject of extensive research for their potential benefits in disease prevention and also as therapeutic agents. In this article, we report the results of our attempt to determine the capacity of the leaf extract to scavenge/inhibit potentially reactive oxygen species that have come to occupy an amazingly central role in the pathogenesis of an array of human ailments and the composition of the predominant low molecular weight antioxidant phytochemicals elaborated by the leaves of the taxon.

Materials and Methods

Plant material and preparation of the leaf extract

Fresh leaves of *M. maderaspatana* were collected from their natural habitat in the village, Rauthankuppam, Vanur Thaluk of Tamil Nadu, India, during March and the identity was established by the Department of Plant Sciences of the Centre. Fresh leaves (FL) were utilised for the determination of vitamins C and E, total carotenoids, and for the



Plate 1—*Mukia maderaspatana* plant

determination and isolation of the predominant phenolic metabolite. For the determination of the total phenolic and flavonoid compositions, and *in vitro* antioxidant capacity measurements, methanolic solution (leaf extract/LE), prepared in the following manner was used. A precisely weighed amount of fresh leaves (10 g) were extracted with 80% aqueous methanol (3 × 100 ml, 40°C, 24 h), cooled to room temperature and centrifuged at 4500 rpm for 15 min. The supernatants were quantitatively recovered and made up to the mark in 100 ml volumetric flask.

Chemicals and Instruments

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) diammonium salt, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 3-(2-pyridyl)-5,6-di(4-phenylsulphonic acid)-1,2,4-triazine (ferrozine) sodium salt, ethanol anhydrous (EtOH), ferrous chloride, (±)- α -tocopherol and β -carotene were obtained from Sigma-Aldrich Inc. Acetone and methanol for chromatography/spectroscopy were procured from Merck Specialities Private Limited. All other chemicals/reagents were of analytical/laboratory grades from Himedia/Merck/Loba Chemie. HPLC analysis was carried out using Zorbax Eclipse XCB-C8 column (250 × 4.6 mm, 5 μ m) and Shimadzu UV-160 Spectrophotometer was used for electronic spectral measurements. NMR spectral recordings were performed on Bruker DRX-300 spectrometer, using DMSO-d₆ solutions.

Determination of the antioxidant phytoconstituents

Total phenolics and flavonoids

The amount of phenolics was determined by the spectrophotometric method¹⁹ with minor modificat-

ions. Briefly, an aliquot (1 mL) of appropriately diluted LE or standard solutions of gallic acid monohydrate (10-100 mg/l) were added to 25 ml volumetric flasks, each containing 9 ml of distilled water. A reagent blank was prepared using distilled water. Folin–Ciocalteu phenol reagent/FCR (1 mL) was added to the mixture and shaken. After 5 min, 7% Na₂CO₃ solution (10 mL) was added with mixing. The solution was immediately diluted to volume (25 mL) with distilled water, mixed thoroughly and then allowed to stand for 90 min. The absorbance of the solutions *versus* prepared blank was measured at 765 nm. Total phenolic content of the LE was determined from the standard curve and was expressed as mg gallic acid equivalent (GAE)/100 g FL. The determination was repeated three times and the mean \pm SD of the three measurements was taken.

The total flavonoid concentration was measured using the colorimetric assay²⁰. Appropriately diluted LE (1 mL) or standard solutions of quercetin (10-100 mg/L) were added to 10 mL volumetric flasks, each containing 4 ml distilled water. A reagent blank was also prepared using distilled water. At time zero min, 5% NaNO₂ (0.3 mL) was added to each flask. After 5 min, 10% AlCl₃ (0.3 mL) was added and at time 6 min, 1M NaOH (2 mL) was added to the mixture. The solutions were diluted to volume (10 mL) immediately with distilled water and then thoroughly mixed. The absorbances of the pink solutions were measured at 510 nm against the prepared blank and the samples were analysed in triplicate. Total flavonoid content of the LE was expressed on a fresh weight basis as mg quercetin equivalent (QE)/100 g using a standard quercetin calibration curve.

Polyphenolic constitution

Studies have emphasised the significance and putative modes of action of specific flavonoids as bioactive components of the diet in both *in vivo* and *in vitro* models and hence, it is important to have a clear idea of the major phenolic families of the plants consumed and their levels contained therein²¹. With this motive, fresh leaves of *M. maderaspatana* (500 g) were extracted exhaustively with boiling 90% aq. EtOH (3 \times 6 l, 6 h), filtered through Whatman No. 3 filter paper and concentrated under reduced pressure at 50°C to get 100 mL of aqueous concentrate. An aliquot (1 mL) of the concentrate was diluted and subjected to the analysis of the polyphenolic composition by HPLC. Gradient elution involving binary mobile phase was used, and the eluates monitored at 270 nm. The mobile phase consisted of a mixture of Solvent A = Water: MeOH:

HOAc = 95.0:5.0:0.5 and Solvent B = MeOH: Water: HOAc = 95.0:5.0:0.5, varied in a gradient manner with an elution rate of 1 ml/min as follows: time (min) - A:B (ml) = 0-75:25; 10-50:50; 15-25:75; 20-10:90; 25-90:10 (Fig. 1). Quantitative determination of the isolate was effected after a solvent blank run, using a standard solution of saponarin.

Vitamins C and E and carotenoids content

The ascorbic acid content was determined by titrimetry²² using a standardised solution of 2, 6-dichlorophenolindophenol (DCPIP). Briefly, an aqueous solution of DCPIP sodium salt (50 mg/100 mL) was standardised using a standard solution of ascorbic acid (L-AA), prepared by dissolving L-ascorbic acid (50 mg) in 20% metaphosphoric acid (60 mL) and diluting with distilled water to make up to 250 ml in a volumetric flask. The acidified aqueous solution of DCPIP was reddish and when reduced by ascorbic acid, it turned colourless and the reaction was specific for ascorbic acid at pH 1.0 to 3.5. Thus, when the reddish solution was added to a standard solution of L-AA or LE containing vitamin C, the resulting solution remained colourless till the equivalence point was reached. Further, addition of the dye rendered a faint reddish colour to the solution, which marked the end point of the titration. Fresh leaves (25 g) were soaked in benzene (3 \times 250 mL, ambient, 6 h) and filtered through Whatman No. 3 filter paper. The leaf material was then homogenised with 20% metaphosphoric acid (50 mL) followed by extraction at room temperature for 15 min and centrifuged at 4000 rpm for 10 min. The residue was re-extracted with 25 mL of extracting solution, again centrifuged and the combined extract was filtered under suction and then made up to 100 mL. 20 mL of this solution was pipetted out into a conical flask, acetone (2.5 mL) was added and titrated against the standardised solution of DCPIP to get the faint reddish end point that persisted for 10 s. The amount of Vitamin C (mg/100 g) available in the fresh leaves was then determined from the mean of three such determinations.

Vitamin E content was determined according to the procedure of Tsen²³. Briefly, extract (0.5 mL) prepared²⁴ or standard ethanolic solutions of α -tocopherol of varying concentrations (0.5 mL) were taken in a stoppered 10 ml amber bottle, 6.0 mM ethanolic solution of 4,7-diphenyl-1,10-phenanthroline (0.5 mL) were added and diluted to 4.0 ml with EtOH. The contents were mixed well and 1.0 mM

fresh ethanolic FeCl₃ (0.5 mL) followed by 40 mM ethanolic H₃PO₄ (0.5 mL) were introduced exactly after 15 s. The absorbance was measured at 534 nm against a prepared blank and the vitamin E content was expressed in mg/100 g FL.

Fresh leaves (25 g) were homogenised with acetone (100 ml), vacuum-filtered, and concentrated. The residue was dissolved in 20% aq. acetone and made up to 25 ml. The total carotenoid content of the leaf extract was determined by the protocol described by Lichtenthaler and Buschmann²⁵. Accordingly, in the extract that contained carotenoids (carotenes and xanthophylls = $c + x$) and chlorophylls, the absorbance at $\lambda_{\text{max}} = 470$ nm (A_{470}) [extract diluted to give $A_{470} \sim 0.7$] corresponded to the sum of the specific absorbances for Chlorophyll *a* (α_{Chla}), Chlorophyll *b* (α_{Chlb}), and total carotenoids ($\alpha_{(c+x)}$). i.e. $A_{470} = A_{(c+x)470} + A_{\text{Chla}470} + A_{\text{Chlb}470}$. According to Beer-Lambert law, $A_{(c+x)470} = \alpha_{(c+x)470} \times C_{(c+x)} \times d$, where, $C_{(c+x)}$ corresponded to the molar concentration of the carotenoids and d was the path length of the cuvette. Similarly, $A_{\text{Chla}470} = \alpha_{\text{Chla}470} \times C_{\text{Chla}} \times d$ and $A_{\text{Chlb}470} = \alpha_{\text{Chlb}470} \times C_{\text{Chlb}} \times d$. For a cuvette of path length 1 cm, applying the 20 % aq. acetone -specific extinction coefficients, the total carotenoid concentration was determined using the relation, $C_{(c+x)} (\mu\text{g ml}^{-1}) = [1000 A_{470} - 1.82 C_{\text{Chla}} - 85.02 C_{\text{Chlb}}] / 198$. From this the total carotenoid content of the leaf in mg/100 g weight was arrived.

Determination of *in vitro* antioxidant capacity

ABTS radical cation scavenging activity

The determination of ABTS^{•+} scavenging activity was done according to the improved ABTS^{•+} decolourising assay of Re *et al*²⁶. Briefly, ABTS^{•+} was produced by reacting 7.0 mM ABTS stock solution with 2.45 mM potassium per sulphate (final concentration) and maintained in the dark at room temperature for 16 h. Prior to use in the assay, the concentration of the solution was adjusted by dilution with EtOH, so that the ABTS^{•+} blue-green chromogen with a characteristic absorption at 734 nm had an absorbance of 0.650 ± 0.020 . The solution was then equilibrated at 30°C. The sample solution and standard L-AA solutions were diluted appropriately to provide 20–80% inhibition of the blank [consisted of 50% aqueous EtOH (20 μL) and diluted ABTS^{•+} (980 μL)] absorbance. The reaction was then initiated by the addition of the diluted ABTS^{•+} (980 μL) to each sample/standard solution (20 μL) and the mixture was allowed to stand at room temperature for 10 min, at the end of which the absorbance was measured

immediately at 734 nm. Determinations were repeated three times for each sample solution.

Ferric-reducing/antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie and Strain²⁷. Briefly, the FRAP reagent was prepared from 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM hydrochloric acid and 20 mM ferric chloride solution in proportions of 10:1:1 (v/v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37°C in a water bath prior to use. An aliquot of the LE (50 μL) was added to the FRAP reagent (1.5 mL) and the absorbance of the reaction mixture was recorded at 593 nm after exactly 6 min against a blank, which consisted of 120 μL water and 900 μL reagent. The assay was carried out in triplicate.

Determination of VCEAC

The *in vitro* antioxidant capacity of the extract, determined by the above two assays, was expressed in the familiar units based on the dietary constituent, Vitamin C, as Vitamin C equivalent antioxidant capacity²⁸ (VCEAC). Vitamin C standard curves that relate the concentration of L-AA (1 to 20 mg/L) and the amount of absorbance reduction (for ABTS^{•+} scavenging) or absorbance increase (for FRAP) caused by L-AA were plotted. The absorbance reduction/increase produced by the extract was then correlated to that of L-AA standards using the standard curve and the results were expressed in mg vitamin C equivalents (VCE)/100 g of FL. All data were recorded as mean \pm SD, computed from three replications.

Inhibition of β -carotene bleaching

The antioxidant activity, based on coupled oxidation of β -carotene and linoleic acid, was measured by the method of Pratt²⁹. Linoleic acid (20 mg) and Tween 20 (200 mg) were taken in a flask and then a solution of β -carotene (2 mg in 10 ml of chloroform) was added. After removal of chloroform, distilled water saturated with oxygen (50 ml) was added. Aliquot (200 μL) of the extract, dissolved in EtOH to give a 15 $\mu\text{g/ml}$ solution, was added to the flask with shaking. A blank without the addition of the extract and three control samples, viz., 2, -di-*tert*-butyl-4-methylphenol (BHT/butylated hydroxytoluene), α -tocopherol and quercetin, added as standards, were also prepared in separate flasks. Samples were subjected to oxidation by placing them in an oven at 50°C for 2 h. The absorbances were read at 470 nm at regular intervals. The antioxidant activity

(AA) was expressed as inhibitory ratio, calculated using the relation, $AA = [1 - (A_0 - A_t) / (A_{00} - A_{0t})] \times 100$, where, A_0 and A_t , respectively corresponded to the absorbances at the beginning of the incubation and at time t , with the extract/standards, while A_{00} and A_{0t} similarly corresponded to the absorbance at the beginning of the incubation and at time t , without the extract/standards.

Transition metal ion chelating capacity

Chelation of transition metal ions by the polyphenolic constituents of the extract and standards were evaluated by determining the chelating activity of Fe (II) using the method of Dinis *et al.*³⁰. Briefly, extract containing 25 and 50 $\mu\text{g/ml}$ (0.4 ml each) or standards were added to a solution of 2 mM ferrous chloride (0.05 ml). The reaction was initiated by the addition of 5 mM of ferrozine (0.2 ml) and the total volume was adjusted to 4.0 ml with methanol. The mixture was then shaken vigorously and allowed to stand at room temperature for 10 min. Absorbance of the solutions were measured spectrophotometrically at 562 nm. All tests and analyses were run in triplicate. The percentage inhibition of the ferrozine-Fe (II) complex formation was calculated using the relation, Fe(II) chelating capacity (%) = $[(A_0 - A_1) / A_0] \times 100$, where, A_0 was the absorbance of the blank and A_1 was the absorbance in the presence of extract/standard. The blank contained only ferrous chloride, ferrozine and methanol. The water soluble versatile hexadentate chelating ligand, disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA), α -tocopherol and the commercial antioxidant, butylated hydroxytoluene (BHT) were used as positive standards.

Isolation of the predominant polyphenolic metabolite

The aqueous concentrate of the leaf extract obtained as described earlier was fractionated into n-hexane, benzene, diethyl ether and 2-butanone (EtCOMe) solubles. The more polar EtCOMe fraction, when kept in a refrigerator for a week, yielded an off-white solid, which was purified using a column of Sephadex LH 20, 90% aq. MeOH and subsequently recrystallised from MeOH.

Results and Discussion

Determination of the antioxidant phytoconstituents

Phenolics and flavonoids

Plants have been used traditionally for the treatment and prophylaxis of several human ailments. The protection has often been attributed to antioxidant phytoconstituents, especially the polyphenols,

vitamins C and E and carotenoids. These dietary antioxidants contribute considerably to the defence against oxidative stress and the consequent risk of chronic diseases. Epidemiologic data have suggested that high intake of fruits, vegetables, cereals, dry legumes, cocoa products, and beverages that are rich in polyphenols, is inversely related to mortality from coronary artery disease, cancers, neurodegenerative diseases, diabetes, and osteoporosis³¹⁻³³. Flavonoids are polyphenolics that are more frequently encountered as integral components of flowering plants, in general and food plants, specifically. Their remarkable antioxidant properties arise from the redox and chelating capabilities of the phenolic groups conjugated to the stable delocalised π -electron clouds of the aromatic ring system. Several polyphenolic compounds are reported to regulate the genes that are critical for the control of proliferation, cell cycle and apoptosis pathway in cancer cells. The total amount of phenolics, present in 100 g of the leaves, is found to be 292.392 mg GAE (Table 1), which is less than the amounts reported from *Cleome gynandra* Linn.³⁴ (321.000 mg) and *Mallotus stenanthus* Muell.-Arg.³⁵ (394.968 mg) but more than that from *Leucas aspera* Spreng³⁶ (231.000 mg). Flavonoids constituted the predominant phenolics of this indigenous medicinal leaf, amounting to 247.079 mg QE (Table 1), which is 84.5% of the total phenolics determined in the present study and may contribute significantly to the folkloric claims. This amount is found to be greater compared to that recorded for other local dietary/potentially medicinal leaves such as *C. gynandra*³⁴ (207.000 mg), *M. stenanthus*³⁵ (186.879 mg) and *L. aspera*³⁶ (188.000 mg) and hence the required antioxidant phytoconstituents and the associated health benefits may conveniently be derived by the consumption of the leafy vegetable.

Polyphenolic composition

As a result of the innumerable studies that have been concluded, which has established the abilities of the antioxidants to counteract oxidative stress in biological systems and thereby ameliorate several chronic human illness, there is a considerable interest of quantification of the antioxidants and determination of antioxidant capacities of specific food constituents. Hence, the polyphenolic composition of the edible leaf was analysed by HPLC (Figure 1) and the predominant polyphenol has been characterised as 7-O- β -D-glucopyranosyl-6-C- β -D-glucopyranosylapigenin (saponarin). To our

knowledge, this is the first reported of the isolation of the compound from *M. maderaspatana* leaves. Using a standard solution of saponarin, the amount of the predominant leaf flavonoid was found to be 220.800 mg/100 g FL (Table 1), amounting to 75.52% of the total polyphenolics and 89.36% of the total flavonoids.

Determination of vitamins C and E and carotenoids content

Evidences have suggested that the plasma levels of L-AA in large sections of the population are sub-optimal for the health protective effects of vitamin C (L-AA/ascorbate)³⁷. This vitamin cannot be synthesised by human system and are, thus, entirely dependent upon dietary sources to meet the needs. 100 g of the fresh *M. maderaspatana* leaf material is

found to contain 17.046 mg of L-AA. Though the L-AA content is much less when compared to 70.670 mg and 64.950 mg reported to be present respectively in *C. gynandra*³⁴ and *Amaranthus* sp.³⁸, it is substantially more than the contents present in *Centella asiatica* (Linn.) Urban. (15.180 mg) and *Beta vulgaris* Linn. (4.900 mg)^{38, 39}. The other potentially beneficial antioxidant vitamin, viz. vitamin E, has been determined to the extent of 0.194 mg/100 g of α -tocopherol in the FL, which is an amount much higher than the reported 0.040 mg from *B. vulgaris*³⁹ but lower than the one from *Talinum fruticosum* (Linn.) Juss.⁴⁰ (33.420 mg/100 g of tocopherols and tocotrienols). Major researches on the biological activities of tocopherols during the past decades have focused predominantly on α -tocopherol, occurring in significant quantities in green leafy vegetables, nuts and seeds. In addition to its antioxidant activities, vitamin E might be involved in anti-inflammatory processes, inhibition of platelet aggregation, enhanced immune function and help prevent or delay coronary heart disease, cancer, age-related macular degeneration and cataracts, cognitive decline and neurodegenerative diseases, such as Alzheimer's disease. Carotenoids not only play a crucial role as precursors of vitamin A but are themselves powerful antioxidants and are considered as preventive factors against cardiovascular diseases, carcinogenesis and

Table 1—Antioxidant constituents of *M. maderaspatana* leaves^a

Constituents	Contents ^b
Total phenolics (GAE)	292.392 ± 1.087
Total flavonoids (QE)	247.079 ± 0.705
Saponarin	220.800 ± 0.944
Ascorbic acid	17.046 ± 0.839
α -tocopherol	0.194 ± 0.068
Total carotenoids ^c	0.812 ± 0.075

^aMean ± standard deviation of three determinations

^bmg/100 g of fresh leaves)

^c β -carotene equivalents

GAE-gallic acid equivalent

QE-queracetin equivalent

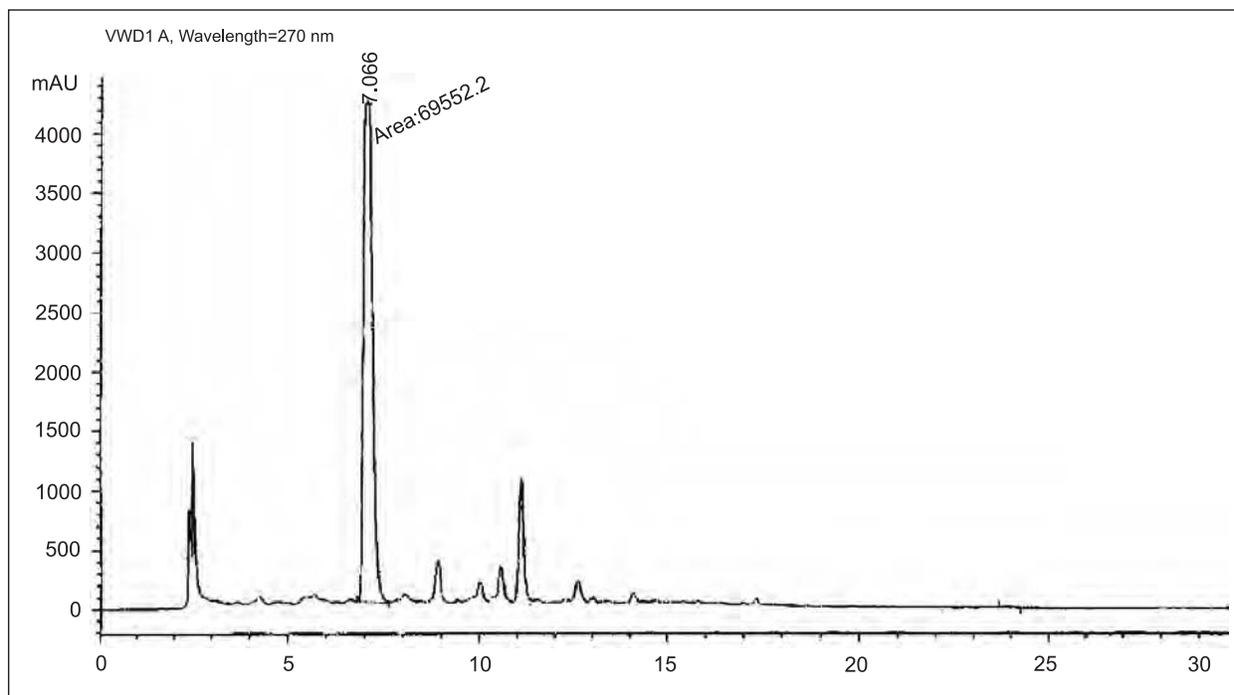


Fig. 1—HPLChromatogram of *Mukia maderaspatana* leaf extract

vision related abnormalities, rheumatism, Parkinson disease and infertility. Investigation of the potentially beneficial carotenoid-containing foods is therefore of great relevance, especially, in the context of the manifestation of the countless number of diseases today and the fresh leaves of *M. maderaspatana* has been investigated to contain 0.812 mg of β -carotene equivalents of total carotenoids/100g FL. This amount is considerably less than the one reported from *Amaranthus* sp.³⁸ (5.310 mg) and *C. asiatica*³⁸ (5.330 mg) presumably due to the difference in the methodologies adopted for the determination.

Studies have shown that additive and synergistic combinations of scores of phytochemicals⁴¹, which are either directly or indirectly involved in various redox processes, are responsible for the observed health benefits of fruits and vegetables. A diet rich in plant foods can provide over 25,000 phytochemicals that cannot be supplied by a typical dietary pattern based on refined grains, added oils, sugar and salt. Only traditional plant-based diets are the best sources of several of these phytoconstituents.

Determination of *in vitro* antioxidant capacity

The significance of reactive species (RS) in the aetiology and pathophysiology of several human diseases has attracted increasing attention over the past two decades. Various mechanisms have been proposed to contribute to the generation of these RS in living organisms. Oxidative damage induced to biomolecules by RS vary considerably and have been implicated in the pathogenesis of more than a hundred odd diseases, including cardiovascular diseases, immune deficiency syndromes, inflammatory conditions, diabetes mellitus, cancer, gastric ulcer, aging and neurodegenerative disorders. Antioxidants may act as (i) physical barriers that prevent generation of RS or prevent RS from accessing important biological sites (ii) chemical sinks that quench RS by trapping energy and electrons, (iii) catalytic systems that neutralise or divert RS, (iv) metal ion chelators, and (v) chain-breaking antioxidants. These appear to work in synergy with each other and protect against various RS.

Though several methods have been developed and tested over the decades to assess the antioxidant capacities of plant extracts, no consensus has yet been reached to conclude at the most convenient or a standard method for claiming total antioxidant capacity. The antioxidant capacity of a plant extract is also influenced by a number of factors⁴² that cause

considerable difficulties in comparing the antioxidant capacities of various dietary constituents and hence it has become a necessity to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action. In the present study, therefore, the antioxidant capacity of the leaf extract has been evaluated by measuring its capacity to scavenge RS (ABTS^{•+}), to reduce and/or chelate Fe (II) ions, and to inhibit autoxidation.

One of the most commonly employed and operationally simple assay of antioxidant capacity measurements involves the generation of the coloured radical cationic oxidant ABTS^{•+} and determining the ability of an extract/a metabolite to scavenge the same²⁶. Though the radical used in the assay is not found in mammalian biology, the decolourisation assay is widely recommended for plant extracts since (i) the λ_{\max} used for monitoring the stable blue-green chromogen (734 nm) eliminates colour interference in the extracts, (ii) the absorbance reduction tends to become a constant in <10 min, and (iii) ABTS^{•+} is soluble in both aqueous and organic phases and is not affected by ionic strength, thus capable of reacting with both lipophilic tocopherols, carotenes and flavonoids and hydrophilic polyphenolic glycosides and phenolic acids. VCEAC of 100 g of the fresh leaves of *M. maderaspatana*, determined by the said method, is found to be 301.926 ± 0.869 mg, which is less than the capacity of *C. gynandra*³⁴ (523.670 mg) and *M. stenanthus*³⁵ (886.493 mg) but significantly more than that reported for *Asparagus officinalis* Linn.⁴³ (82.780 mg) and *Brassica oleracea* Linn.⁴³ (30.530 mg).

The FRAP assay underestimated the antioxidant/reducing ability of the extract by a factor of ca. 1.6 (only 187.492 ± 0.583 mg VCE/100 g FL) probably because (i) FRAP cannot detect compounds that act by radical quenching and (ii) the reducing power appears to be more related to the degree of hydroxylation and the extent of conjugation in polyphenols. A similar trend but of much lesser magnitude (553.994 mg VCE, amounting to a factor of only 0.6) was observed for *M. stenanthus*³⁵. It may be inferred from Figure 2 that the leaf extract is found to possess only 33.2% of the activity of the commercial antioxidant, BHT at end of the 2 h study and 32.6% of that of α -tocopherol and 47.6% of that of quercetin. Though the chelating abilities of the constituents of the extract was only 55.56% of the

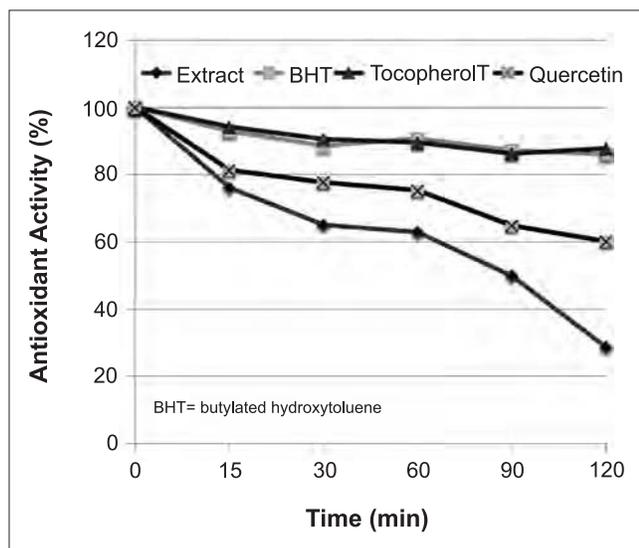


Fig. 2—Antioxidant activities of the extract and standards determined by β -carotene bleaching assay

capability of the strongly chelating Na_2EDTA at the concentration of 25 $\mu\text{g/ml}$ and 67.83% at 50 $\mu\text{g/ml}$, they were found to chelate Fe(II) more efficiently than both α -tocopherol and BHT at the two concentrations taken for the study (Figure 3). Their chelating efficiencies were respectively, 33.36% and 52.85% greater than those of α -tocopherol and 61.17% and 27.62% higher than those of BHT at the two concentrations.

Conclusion

Human food choices and dietary patterns, today, have been driven largely by necessity and economics and have been much influenced by the promotion, based more on their merits of taste, cost or convenience, with little significance to their nutritional merits or health values. In an era of western cultures over ruling the tradition, food habits and life style transformations are manifested in decreased immune responses leading to an outburst of chronic ailments. In such a context, the benefits of the potentially nutritive and antioxidative leafy vegetable can be exploited as a convenient source of chemopreventive dietary constituent, especially for the less privileged section of the population, who could also be encouraged to cultivate such nutritive native leafy vegetables for their livelihood. Saponarin, the major phenolic antioxidant of the leaves has been reported to possess hepatoprotective, hypoglycemic and antimicrobial properties^{44,45} and hence, its presence in significant amounts in the leaf, may

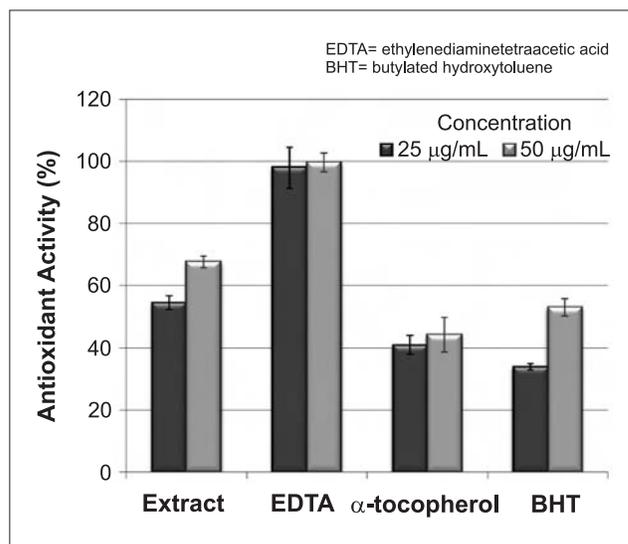


Fig. 3—Ferrous ion chelating capacities of the extract and standards

probably explain the claims of the indigenous drug in the traditional medical practices.

Acknowledgements

The authors are grateful to Dr. P. Sambasiva Rao, Head and Dr. R. Venkatesan, Reader, Pondicherry University, Puducherry and also to True Analytica Lab Private Limited, Chennai for the analytical facilities.

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