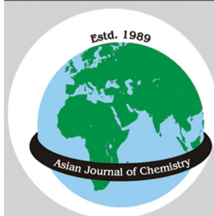

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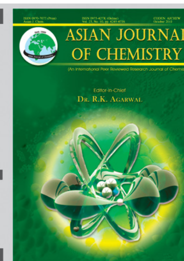

Antioxidant Flavonoid Metabolites of *Mukia maderaspatana* (L.) M. Roemer leaves

A.J.A. PETRUS[1,*] and N. BHUVANESHWARI[2]

[1]Department of Chemistry, Kanchi Mamunivar Centre for Post-Graduate Studies (Autonomous-College with Potential for Excellence), Puducherry-605 008, India
[2]Department of Chemistry, Dr. Pauls Engineering College, Vanur Tk., Villupuram-605 109, India

*Corresponding author: Tel: +91 413 2221419; E-mail: ajapetrus@hotmail.com

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Mukia maderaspatana (Linn.) M. Roemer, (Family: Cucurbitaceae) is an indigenous leafy vegetable, reported to offer protection against a number of oxidative stress-mediated ailments. Earlier studies have brought to light the total phenolic contents, reactive species (ROS/RNS) scavenging and metal chelating capacities of the leaf extract. 2,2'-Azino-*bis*(3-ethylbenzothiazoline-6-sulphonate ($ABTS^{\bullet+}$), super oxide ($O_2^{\bullet-}$) and NO scavenging capacities, ferric-reducing antioxidant power (FRAP) and ferrous chelating capacities of six C-glucoflavones, isolated and characterized from the edible leaves have been evaluated. 6-C-β-D-glucopyranosylluteolin (**4**) and 8-C-β-D-glucopyranosylluteolin (**6**) exhibited maximum ROS/RNS scavenging capacities, while the predominant flavone of the leaf, 7-O-β-D-glucopyranosyl-6-C-β-D-glucopyranosylapigenin (**2**) fared weakly. 6-C-β-D-glucopyranosylapigenin (**3**) was found to be a better scavenger of $ABTS^{\cdot+}$ whereas 8-C-β-D-glucopyranosylapigenin (**5**) scavenged $O_2^{\bullet-}$ better and 7-O-β-D-glucopyranosyl-6-C-β-D-glucopyranosylluteolin (**1**) scavenged NO better, in addition to possessing a good ferric-reducing antioxidant power. The ferrous chelating capacities were in the order **1 > 6 > 4 > 2 > 5 > 3**, depending upon the metal-binding motifs of their molecular structures. **1**, **4** and **6** were found to be only 10 % less potent to chelate Fe(II) compared to deferoxamine, the hydroxamic siderophore, widely employed in the treatment of patients constantly submitted to blood transfusion. The potential ROS/RNS scavenging and metal chelating antioxidant capacities of the C-glucoflavones may contribute considerably to the total antioxidant capacity as well as to the reported protective properties of this functional leafy vegetable.

Key Words: *Mukia maderaspatana*, C-glucoflavone, Antioxidant, Scavenging capacities, Metal chelating capacities.

INTRODUCTION

Antioxidants derived from fruits, vegetables, spices and cereals, in the form of flavonoids, phenolic acids and alcohols, stilbenes, tocopherols and tocotrienols, ascorbic acid and carotenoids have been the subject of extensive research for their potentials to ameliorate oxidative stress and its consequences[1]. Presently, these dietary phytometabolites are emerging as pharmacologically active prophylactic and therapeutic solutions[2]. The regulation is often *via* one or more mechanisms that include reduction, radical-scavenging, pro-oxidant metal chelation and quenching of singlet oxygen. *Mukia maderaspatana* (Linn.) M. Roemer, (Family: Cucurbitaceae) syn. *Melothria maderaspatana* (L.) Cogn.; *Cucumis maderaspatana* (L.); *Mukia scabrella* (L.f.) Arn.; *Bryonia scabrella* (L.f.), (Siddha/Tamil: Musumusukkai; Ayurveda: Ahilekhana, Trikoshaki), is a prominent medicinal herb[3]. Locally, the leaves are cooked and consumed in different forms[4]. Folkloric traditional medicine claims that the leaves and tender shoots are useful as aperient, diuretic, stomachic, antipyretic, antiflatulent, antiasthmatic, antitussive, antihistaminic, antibronchitic and as an expectorant, in addition to its prescription against vertigo and biliousness[3-5]. The leaf-tea is administered for the alleviation of jaundice[6] 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 flu[7]. The leaf extract has also been shown to possess immunomodulatory[8], hepatoprotective[9-12], antiinflammatory[13], antimicrobial[14], hypotensive and vasodialatory[15-18] and antihyperglycemic activities[19-21] and the ethyl acetate fraction has been reported to possess a dose-dependent antiplatelet activity[22]. The leaf extract has also been evaluated to possess potent antioxidant capacity *in vitro*[4,23,24] and *in vivo*[25]. To the best of our knowledge, no report of the flavonoid antioxidant profile of this Indian medicinal leaf is available in literature, except for the isolation and determination of the predominant phenolic metabolite of the leafy vegetable, *viz.*, 7-O-β-D-glucopyranosyl-6-C-β-D-glucopyranosylapigenin[4].

Characterization of the dietary antioxidants and their capacities offer better insight into their functionality, as these are necessary to cope up with/inhibit the initiation or propa-

TABLE-1
^{1}H-^{13}C COSY SPECTRAL DATA (DMSO-d_6) OF COMPOUNDS **1** AND **2** ISOLATED FROM *M. maderaspatana* LEAVES

Position of C		Compound **1** (δ, ppm)		Compound **2** (δ, ppm)	
		^{1}H, Multiplicity (*J*, Hz)	^{13}C	^{1}H, Multiplicity (*J*, Hz)	^{13}C
Flavone	2		163.92		164.10
	3	6.82, s	103.08	6.88, s	103.08
	4		182.29		182.00
	5		160.25		159.25
	6		110.86		110.44
	7		161.30		162.36
	8	6.77, s	93.31	6.91, s	93.64
	9		156.10		156.36
	10		105.26		104.77
	1’		120.82		120.82
	2’	7.47, d (2.2)	114.81	7.98, d (9.0)	128.51
	3’		149.20	6.95, d (9.0)	115.93
	4’		156.61		161.30
	5’	6.92, d (8.5)	115.90	6.95, d (9.0)	115.93
	6’	7.47, dd (2.2, 8.5)	118.60	7.98, d (9.0)	128.51
Glucoside at C-6	1”	4.63, d (9.0)	73.21	4.66, d (9.5)	72.55
	2”	3.95, t (9.0)	72.81	4.07, t (9.5)	70.76
	3”	3.27, t (9.0)	78.80	3.35, t (9.5)	73.68
	4”	3.19, m	70.22	3.25, m	69.41
	5”	3.14, m	81.36	3.17, m	80.82
	6”	3.28, m	60.57	3.58, m	60.57
		3.58, m		3.74, m	
Glucoside at O-7	1”’	5.00, d (7.0)	100.76	4.98, d (7.5)	101.09
	2”’	3.31, m	73.68	3.35, m	75.66
	3”’	3.42, t (9.0)	75.66	3.52, t (9.4)	77.11
	4”’	3.33, m	70.76	3.93, t (9.4)	70.08
	5”’	3.21, m	75.90	3.24, m	78.80
	6”’	3.52, m	61.72	3.62, m	60.23
		3.78, m		3.77, m	

gation of reactive oxidants. Such informations are also essential to validate the safety and traditional uses and to standardize preparations of these plants. Furthermore, this information may be used to establish flavonoid databases. The flavonoid contents of some western foods have been reported and archived in the USDA flavonoid database[26]. But little is known about the polyphenolic/flavonoid constitution and non-nutritive bioactivity of foods from developing nations that may contribute to their medicinal functionality. With this motive, leaves of *M. maderaspatana* have been analyzed systematically for their flavonoid antioxidant composition and reported in this article.

EXPERIMENTAL

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 Science of the Centre. The separated leaves were then dried sufficiently in shade and kept packed in air-tight polythene packs till sufficient amount of the sample was collected for the analysis.

2,2'-Azino*bis*(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, ferrous chloride, rutin and deferoxamine methanesulfonate were obtained from Sigma-Aldrich Inc. and Sephadex LH-20 from Pharmacia. Silica gel (60-120 mesh for column chromatography), microcrystalline cellulose for thin layer chromatography and methanol for spectroscopy were procured from Merck Specialities Private Limited. All other chemicals/reagents were of analytical/laboratory grades from Himedia/Merck/Loba Chemie. Melting points have been determined in open capillaries and are uncorrected. Shimadzu UV-160 spectrophotometer was used for electronic spectral measurements and the NMR spectral recordings were performed on Bruker DRX-300 spectrometer, using DMSO-d_6 solutions. ESI-MS was recorded in Micromass Quattro II triple quadrupole mass spectrometer and the data acquired in the negative ionization mode.

Isolation and characterization of the phenolic metabolites

Air dried and powdered leaves of *M. maderaspatana* (3.7 kg) were extracted exhaustively with boiling 90 % aq. EtOH (3 × 40 L, 6 h), filtered through Whatman No. 3 filter paper and concentrated under reduced pressure at 50 ºC. The aqueous ethanolic concentrate was subsequently fractionated into benzene, diethyl ether (Et_2O), ethyl acetate (EtOAc) and 2-butanone (EtCOMe) solubles by solvent extraction. The 2-butanone residue was further fractionated, using a column of Sephadex LH 20 and eluting with 90 % aq. methanol (MeOH), into 16 fractions. The eluates were analyzed by paper chromatography (Whatman No.1, distilled water, ascending, 28 ºC) for their phenolic composition. Based on the paper chromatography features, fractions 5-9 and 10-16 were combined and concentrated as before to get respectively fractions F-1 and F-2. The former was again passed through the same column and eluted with 90 % aq. MeOH to separate a further eight fractions. Fractions 3-6 were combined together, concentrated and its repeated passage through the column resulted in the isolation of compounds **1** and **2**, which were recrystallized

from MeOH. Fractions 7, 8, F-2 and EtOAc fraction, whose paper chromatography characteristics appeared similar, and the Et_2O fraction were combined together and fractionated into 10 fractions using the column of Sephadex LH 20 as before. After rejecting fractions 1-3, the others were pooled together, concentrated and subjected to separation by normal phase SiO_2 column chromatography. The separation was effected by gradient elution using binary mixtures of $CHCl_3$: MeOH of increasing polarities. The fractions eluted with 1:3 was found to be a mixture containing four compounds, **3-6**, as analyzed by paper chromatography (Whatman No. 1, 15 % aq. HOAc (acetic acid), ascending, 28 ºC). The compounds were then separated using preparatory TLC (cellulose, 20 × 20 cm, 0.5 mm, 15 % aq. HOAc) into **3** (R_f 0.50), **4** (R_f 0.36), **5** (R_f 0.31) and **6** (R_f 0.23), recrystallized from MeOH.

Compound **1**, $C_{27}H_{30}O_{16}$ (m.p. 239-241ºC), gave yellow colour with NH_3, Na_2CO_3 and NaOH, pink with Mg + 5 M HCl, olive green with neutral Fe(III) and a positive Molisch's test. UV (λ_{max}, nm) MeOH: 256, 267 sh, 347.5; 259, 265 sh, 358 sh, 398 (+NaOAc); 259.5, 371.5 (+NaOAc + H_3BO_3); 263, 302 sh, 395 (+NaOMe); 273.5, 298 sh, 329 sh, 431 (+$AlCl_3$); 273.5, 295 sh, 354, 388 (+$AlCl_3$ + HCl). ESI-MS (*m/z*, %) 609 $[M-H]^-$ (38), 447 $[(M-H)-162]^-$ (100), 357 $[luteolin+71]^-$ (16), 327 $[luteolin + 41]^-$ (48). ^1H and ^{13}C NMR (Table-1).

Compound **2**, $C_{27}H_{30}O_{15}$ (m.p. 232 ºC), colour reactions characteristically similar to compound **1**. UV (λ_{max}, nm) MeOH: 270.5, 327.5; 270, 330, 398 sh (+NaOAc); 269.5, 331.5 (+NaOAc + H_3BO_3); 273.5, 304, 385.5 (+NaOMe); 277.5, 300.5, 343, 390 sh (+$AlCl_3$); 278, 298.5, 342, 388 sh (+$AlCl_3$ + HCl). ESI-MS (*m/z*, %) 593 $[M-H]^-$ (47), 473 $[(M-H)-120]^-$ (25), 431 $[(M-H)-162]^-$ (34), 341 $[apigenin+71]^-$ (12), 311 $[apigenin+41]^-$ (98). ^1H and ^{13}C NMR (Table-1).

Determination of *in vitro* antioxidant capacity

Vitamin C equivalent antioxidant capacity: ABTS radical cation ($ABTS^{\bullet+}$) scavenging capacity and ferric-reducing antioxidant power (FRAP) of 10 mg/L of each isolate were determined by the procedures described previously[4] and expressed as vitamin C equivalent antioxidant capacity (VCEAC). Vitamin C standard curves were constructed by plotting the absorbances of 1.25 - 20 mg/L of L-ascorbic acid (L-AA) against the corresponding concentrations. The vitamin C equivalent antioxidant capacity of 10 mg/L of the isolates/standard rutin were determined from the standard graph and expressed as percentage relative to 10 mg/L of L-ascorbic acid. All data were recorded as mean ± SD, computed from three replications.

Superoxide anion radical scavenging capacity: Superoxide radicals ($O_2^{\bullet-}$) were generated based on the method of Beauchamp and Fridovich and the scavenging capacities were determined, as described by Zhishen et al[27]. The photochemically reduced riboflavins generated $O_2^{\bullet-}$ which reduced NBT to form blue formazan. The unilluminated reaction mixture was used as a blank. The absorbance (A) was measured at 560 nm. Samples/standards (L-ascorbic acid and rutin) were added to the reaction mixture, in which $O_2^{\bullet-}$ was scavenged, thereby inhibiting the NBT reduction. Absorbance (A_1) was measured and the decrease in $O_2^{\bullet-}$ was represented by (A - A_1). The scavenging capacity was determined, in triplicate, using the relation: $O_2^{\bullet-}$ scavenging (%) = $[(A - A_1)/A] \times 100$.

Nitric oxide scavenging capacity: Nitric oxide generated from sodium nitroprusside (SNP) was measured using the Griess reagent[28]. Briefly, 100 µL of aq. ethanolic solutions of the isolates/standard (0.1 mg/mL) were added to 0.2 mL of 10 mM sodium nitroprusside and 1.8 mL of phosphate buffer (KPB), pH 7.4. The reaction mixtures were incubated at 37 ºC for 3 h. 1.0 mL of the incubated mixture was then removed and diluted with 1.0 mL of Griess reagent (1 % sulphanilamide and 0.1 % naphthylethylenedi-amine dihydrochloride in 5 % H_3PO_4). The absorbance of the chromophore formed as a result of the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylenediamine was observed at 540 nm with reference to the absorbance of a standard solution of sodium nitrite treated in the same way with Griess reagent, as percentage. L-Ascorbic acid and rutin were the positive standards taken and all analyses were carried out in triplicate.

Transition metal ion chelating capacity: Metal ion chelating capacities of the isolates/standards were evaluated using Fe(II) and the percentage inhibition of the ferrozine-Fe(II) complex formation was calculated adopting the protocol described earlier[4], with certain modifications, in triplicate. The ubiquitous flavonoid, rutin and the hydroxamic siderophore, deferoxamine, employed for the treatment of iron over-load, were used as positive standards. Briefly, 50 µL of samples/standards (6 mM) were mixed with a mixture of 50 µL of $FeCl_2$ (2 mM) and 50 µL of ferrozine (6 mM) and the total volume was adjusted to 4.0 mL with KPB (pH 7.4). After allowing the reaction mixture to remain at room temperature for sufficient time [10 min for samples/rutin and 0.5 h for deferoxamine to permit completion of autoxidation of Fe(II)[29]], the 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 sample/standard. The blank contained only $FeCl_2$, ferrozine and KPB.

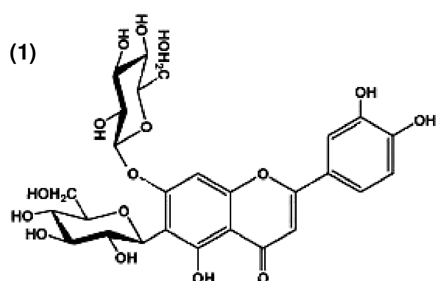
RESULTS AND DISCUSSION

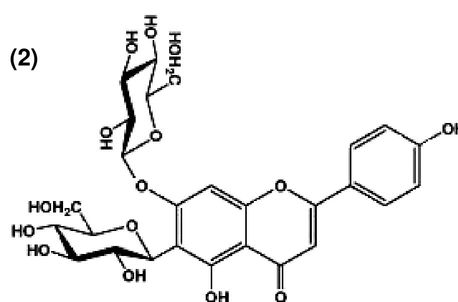
Currently there has been an increased interest globally to identify dietary antioxidants that are pharmacologically potent for use in preventive and therapeutic medicine and also in the food industry. Since plants are known to synthesis a variety of antioxidants in response to oxidative stress, induced by environment (including photons), they represent a potential source of these pharmacologically active metabolites. Studies have also emphasized 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 essential to have a clear idea of the major phenolic families of the plants consumed and their levels contained therein[30]. This has stimulated the systematic analysis of the flavonoid constitution of *M. maderaspatana*, an indigenous leafy vegetable that has been investigated to possess potent antioxidant/radical scavenging and metal chelating capacities[4, 23-25].

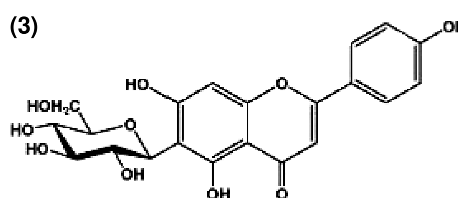
Solvent extraction and subsequent chromatographic separations have lead to the isolation of six C-glycoflavones, **1-6**. The identities of compounds **3-6** were established respec-

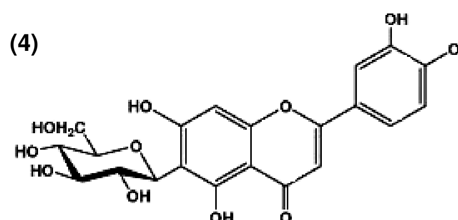
tively as 6-C-β-D-glucopyranosylapigenin (isovitexin), 6-C-β-D-glucopyranosylluteolin (homoorientin), 8-C-β-D-glucopyranosylapigenin (vitexin) and 8-C-β-D-glucopyranosylluteolin (orientin) (Fig. 1) by direct comparison of the chemical, spectral and chromatographic features with those of the authentic samples isolated from *Ocimum sanctum*[31] and *Parkinsonia aculeate*[32]. Compounds **1** and **2** exhibited chemical and UV fluorescence behaviour characteristic of flavone glycosides. Acid hydrolysis[33] (2 M HCl, 100 ºC, 2 h) of flavone **1** resulted in the Wessely-Moser rearranged C-glucoflavone pairs, agreeing in all respects to 8-C-β-D-glucopyranosylluteolin and 6-C-β-D-glucopyranosylluteolin[32] and D-glucose in equal proportions. Presence of two anomeric proton doublets at δ 5.00 (J = 7.0 Hz) and 4.63 (J = 9.0 Hz) in the ^{1}H NMR spectrum of flavone **1** confirmed the O-glucopyranosyl- and C-glucopyranosyl-moieties. ESI-MS exhibited the quasi-molecular ion $[M-H]^-$ peak at *m/z* 609 and $[(M-H)-162]^-$ at 447 corresponding to the loss of an hexose, $[\text{luteolin} + 71]^-$ at 357 and $[\text{luteolin} + 41]^-$ at 327, characteristic of O-glucosylated homoorientin. This O-glucosylation was at 7-OH could be confirmed from the characteristic absorption maxima of the bands in the UV spectra of its methanolic solution and NaOAc and NaOMe added spectra[33]. Comparison of the ^{1}H NMR and ^{13}C NMR spectral features of compound **1** with those reported previously from *Hordeum vulgare* leaves led to the conclusive characterization of compound **1** as 7-O-β-D-glucopyranosyl-6-C-β-D-glucopyranosylluteolin[34] (lutonarin) [Fig. 1]. Acid hydrolysis of **2**, as before, resulted in the C-glucoflavone pairs, agreeing in all respects to 8-C-β-D-glucopyranosylapigenin and 6-C-β-D-glucopyranosylapigenin and D-glucose in equal proportions. Presence of two anomeric proton doublets at δ 4.98, (J = 7.5 Hz) and 4.66 (J = 9.5 Hz) in the ^{1}H NMR spectrum of compound **2** confirmed the O-glucopyranosyl- and C-glucopyranosyl-moieties. ESI-MS exhibited the quasimolecular ion $[M-H]^-$ peak at *m/z* 593 and $[(M-H)-162]^-$ at 431 corresponding to the loss of an hexose, $[\text{apigenin} + 71]^-$ at 341 and $[\text{apigenin} + 41]^-$ at 311, characteristic of O-glucosylated isovitexin. The 7-O-glucosylation was again confirmed from the characteristic absorption maxima of the bands in the UV spectra of its methanolic solution and NaOAc and NaOMe added spectra[33]. Based on the ^{1}H–^{13}C COSY NMR spectral features of **2** and comparing ^{13}C NMR data reported by Markham and Mitchell[35] compound **2** was characterized as 7-O-β-D-glucopyranosyl-6-C-β-D-glucopyranosylapigenin (saponarin) (Fig. 1).

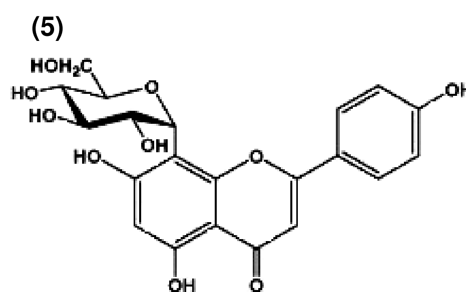
In living organisms, ROS/RNS are continuously produced *in vivo* and these reactive transients are both toxins as well as beneficial constituents. A delicate balance between these two antagonistic characters is clearly an important aspect of life. At low or moderate levels, they are essential for detoxification,











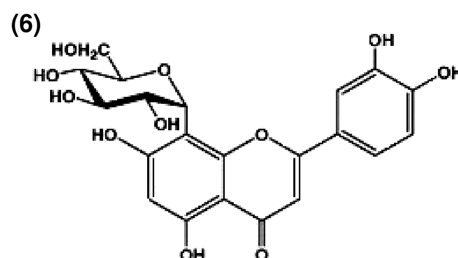


Fig. 1. Structures of the C-glucoflavones isolated from *M. maderaspatana* leaves

cellular responses and immune function. However, over production of these reactive species, induced by exposure to external stimuli, including chemical carcinogens, ultraviolet radiation, bacterial or viral infections, elicit oxidative stress and the consequent deleterious effects on human health. Development of chronic and degenerative ailments including aging, arthritis, autoimmune disorders, cancer, as well as cardiovascular and neurodegenerative diseases, are the familiar ones among these deleterious effects[36]. The human body has evolved with considerable mechanisms to counteract oxidative stress with the help of antioxidants, which are either naturally

TABLE-2
FLAVONOID COMPOSITION OF THE LEAVES OF *M. maderaspatana* AND THEIR CAPACITIES TO SCAVENGE REACTIVE SPECIES AND TO CHELATE Fe(II) IONS

Compound	Yield (%)	Contribution to total leaf flavonoid (%)	Relative percentage[a]				
			VCEAC		Superoxide scavenging	NO scavenging	Fe(II) chelation
			ABTS	FRAP			
1	0.00177	0.707	68.5 ± 5.3	73.8 ± 5.1	40.2 ± 1.8	47.1 ± 0.9	81.3 ± 3.7
2	0.22100	88.298	62.8 ± 4.2	55.9 ± 4.1	31.1 ± 3.0	41.0 ± 2.3	29.2 ± 1.9
3	0.01830	7.311	71.4 ± 3.7	65.5 ± 3.4	36.4 ± 1.7	42.8 ± 2.8	25.9 ± 2.2
4	0.00243	0.971	84.2 ± 5.5	85.1 ± 4.9	50.2 ± 2.4	52.0 ± 1.1	79.4 ± 2.8
5	0.00397	1.586	69.1 ± 5.7	60.4 ± 8.8	44.3 ± 0.8	41.3 ± 1.2	28.9 ± 1.4
6	0.00075	0.300	85.6 ± 4.6	79.7 ± 2.2	48.9 ± 1.8	58.2 ± 0.8	80.6 ± 2.9
L-ascorbic acid			100	100	38.3 ± 2.7	51.3 ± 1.8	nd
Rutin			83.3 ± 4.4	81.8 ± 4.1	51.8 ± 2.0	50.5 ± 1.5	74.4 ± 0.8
Deferoxamine			nd	nd	nd	nd	90.4 ± 3.2

[a]Mean ± standard deviation (n = 3); nd: not determined; VCEAC = Vitamin-C equivalent antioxidant capacity.

produced *in situ* or exogenously acquired through foods and/or supplements. The food choices, dietary patterns and life style transformations have resulted in the manifestation of decreased immune responses and an outburst of oxidative stress. Consequently, the significance of dietary antioxidants has regained due relevance in the present context.

The association between diet and disease has persisted in the history of medicine even during the days of Hippocrates. Records of the prescription of various foods, including vegetables, for the treatment of ailments, including cancer, by his followers exist[37]. Despite the vast interest in diet as a mode to prevent chronic and degenerative human disorders, it was not until the late 20th century that the mechanism of action of diet-derived chemoprevention began to unravel. The beneficial effects of fruits and vegetables have often been attributed to, among other things, the high content of bioactive phytoconstituents[38]. Current evidences have established that these bioactive compounds contribute significantly to the prevention of chronic diseases[39]. Polyphenols are among the ubiquitous class of secondary metabolites in nature and the antioxidant potentials of several of these are reported to be superior to those of the other well-known antioxidants, such as vitamins C and E and β-carotene. Interest in the redox properties of phytophenols has motivated studies focusing on the mechanism of their protection against ROS and to evolve the strategies to manage the pathogenesis of a number of degenerative diseases[40-42]. Consequently, dietary natural products have been and are being screened for their antioxidant capacity, especially for their ROS-/RNS-scavenging properties. This broad interest has led to the development of various assays to determine the antioxidant capacity[43, 44]. Yet, there exists no convenient or standard assay to evaluate the antioxidant capacity of an extract/an isolated chemical principle[43,44]. Further, the antioxidant capacity of a plant extract/isolate is also influenced by a plethora of factors[44] that cause considerable difficulties in comparing the antioxidant capacities of various dietary constituents. These factors have necessitated the measurement of more than one type of antioxidant capacity determination to take into account the various mechanisms of antioxidant action.

Flavonoids are among the major antioxidant constituents of our diet[45]. Their daily intake almost equals the daily doses of all other antioxidants, including the carotenoids, vitamins C and E, put together. They have been proven to possess an effect on antioxidant activity and thereby prevent cardiovascular and other free radical-mediated diseases, including hemorrhoidals, immune disorders and neurodegenerative diseases[45]. Three factors generally dictate the capacity of flavonoids to act as effective antioxidants: (i) the metal-chelating potential that is strongly dependent on the relative arrangement of the phenolic functions and the carbonyl group of the pyrone ring, (ii) the presence of H^+/e^- donating substituents that quench free radicals and (iii) the ability of the flavonoid ring systems to delocalise the unpaired electron leading to the formation of a stable phenoxyl radical.

The ROS-/RNS-scavenging capacities of the isolated C-glucoflavones were evaluated using $ABTS^{\bullet+}$ by the procedure described earlier[4] with some alterations. Compounds **4** and **6** scavenged the radical cation as effectively as the standard rutin (Table-2), while the predominant flavonoid of the leaf (**2**), exhibited *ca.* 63 % of the capacity of L-ascorbic acid. All the tested isolates, in general, possessed fairly good antiradical capacity (Table-2). Despite the weak radical scavenging capacity of **2**, the compound contributed maximum to the measured total vitamin-C equivalent antioxidant capacity of 301.926 ± 0.809 mg/100 g fresh leaf[4] since the other potentially scavenging glucoflavones constituted only 11.3 % to the total leaf flavonoid composition (Table-2). The measured antioxidant/reducing capacities, adopting the FRAP assay, varied from 56 % (for **2**) to 85% (for **4**). Flavone **2** expressed the minimum capacity in this protocol too (Table-2).

Though the leaf flavonoids were not an effective scavenger of $O_2^{\bullet-}$ when compared to their capacities to scavenge $ABTS^{\bullet+}$, barring **2** and **3** the other flavones were found to scavenge better than the standard L-ascorbic acid. They were only marginally weaker than the common flavonol, rutin (Table-2). Almost similar trend could be realized in their capacities to scavenge NO and here again, the predominant polyphenol, **2**, demonstrated weakly (Table-2). Thus, flavone **2** and **3**, which accounted for 95.6 % of the total leaf flavonoid expressed lower ROS-/RNS-scavenging capacity and the other potential constituents contributed only to the extent of 4.4 % to the flavonoid content of this functional[46] leafy vegetable.

Determination of metal-chelating antioxidant capacity

During normal iron homeostasis, circulating iron is bound to transferrin. When a state of iron overload occurs, for instance, as a result of (i) long-term transfusions in patients with anemia of genetic disorders such as β-thalassemia, sickle cell disease and Diamond Blackfan syndrome and (ii) bone-marrow failures such as aplastic anemia and myelodysplastic syndromes, iron content exceeds the capacity for transferrin to bind and non-transferrin-bound iron results. As there is no active mechanism to excrete iron from the body, a progressive accumulation of body iron easily occurs[47]. Such redox-active iron can be taken up by liver, cardiac and endocrine cells through uptake mechanisms that are independent of the transferrin receptor[48] and the excess iron in parenchymal tissues can cause serious clinical sequelae, such as cardiac failure, liver disease, diabetes and eventual death. An increase in intracellular non protein-bound iron concentration has also been implicated in a number of oxidative stress-related pathways and conditions in humans. The same has been demonstrated to be the primary generator of H_2O_2 and $^{\bullet}OH$ that damage lipids, DNA and other biomolecules[49]. Since iron-mediated damages have been implicated in disease development, exploration of iron-chelating mechanism of polyphenol antioxidant activity has become the necessity in addition to radical-scavenging behaviour. Iron-mediated oxidative damage is just not limited only to living organisms. Due to the presence of iron in the environment, iron generated $^{\bullet}OH$ is also responsible for food spoilage and wood rotting. Hence, polyphenolics of natural origin have been widely studied for use as preservatives for food, cosmetics and pharmaceuticals.

Per cent Fe(II) chelating capacities of none of the isolated glucoflavones were found to be superior to the standard deferoxamine. Deferoxamine is a trihydroxamic siderophore used for the treatment of iron overload and its high affinity for Fe(III) renders it a suitable substance for the comparison of iron-chelating activity. It also chelates Fe(II) with high affinity under aerobic conditions, probably due to the formation of the more stable Fe(III)-deferoxamine[29]. Flavones **1, 4** and **6** have been found to be inferior only by about 10 % but these were marginally better chelators when compared to the dietary flavonol, rutin. Unlike the radical scavenging capacities of flavones **2, 3** and **5** expressed weak Fe(II) chelating capacities, probably due to the structural variations in the iron-binding motifs[50] of the two sets of glucoflavones (Fig. 1).

Conclusion

The wisdom of the traditional healers about the potential resources of the wild food plants of ethnic communities has enabled them to meticulously utilize this natural resource for the health care of mankind. The focus today is on the experiments aimed at preventing, inhibiting or intercepting the harmful products of oxidative stress. Dietary flavonoids, as potent reactive species-scavengers and metal-chelators, have attracted a tremendous interest as chemopreventives and possible therapeutics against the prevalent radical-mediated human ailments. The present investigation has attempted to analyze the antioxidant flavonoid constitution of *M. maderaspatana* leaves, which biosynthesise C-glucoflavones. Radical scavenging capacities of a set of structurally related metabolites are on par with the dietary flavonol, rutin and their metal-binding capacities are only *ca.* 10 % less than that of deferoxamine and thus the hydroxamic siderophore, widely employed in the treatment of patients constantly submitted to blood transfusion.

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