



## REVIEW

### *Mukia maderaspatana* (L.) M. Roemer-A Review of Its Global Distribution, Phytochemical Profile and Antioxidant Capacity

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*Mukia maderaspatana* (Linn.) M. Roemer, (family : Cucurbitaceae) is a wild functional food plant, traditionally used for human consumption by various cultures. Indigenous communities around the world utilize various parts of the plant to alleviate a number of human and livestock ailments. For the first time, this review attempts to critically assess the global distribution and vernacular names as well as the phytochemical profile and the antioxidant capacity of this potentially medicinal taxon.

**Key Words:** *Mukia maderaspatana*, Taxonomy, Nomenclature, Chemical constitution, Reactive species scavenging capacity.

## INTRODUCTION

Natural products, notably those from plant origin, have consistently been an important source of therapeutic agents since ancient times. Currently, *ca.* 25-30 % of all drugs available as therapeutics are derived from natural products or are derivatives of natural products. Recent evidence from the pharmaceutical companies indicate that for certain ailments natural products still represent an extremely valuable source for the production of new chemical entities. The main reason is that they represent privileged structures selected by evolutionary mechanisms over a period of millions of years<sup>1</sup>. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs<sup>2</sup>. Although modern medicine may be available in these countries, to the extent of *ca.* 80 % of world's population depends on traditional medicine for their primary health care needs<sup>3</sup>. During the past two decades, traditional systems of medicine have evolved as a topic of global importance. Concurrently, many people in developed countries also have begun to turn to alternative or complementary therapies, including medicinal herbs. In almost all the traditional systems of medicine, medicinal plants play a major role and constitute their backbone. Though a reliable figure for the total number of medicinal plants on earth is difficult to assess, around 35,000-70,000 plant species are being speculated to be used worldwide in health care systems<sup>4</sup>. A significant percentage of the population in developed countries

like Australia (48 %), Belgium (38 %), Canada (70 %), France (75 %) and USA (42 %) has used traditional and alternative remedies at least once for health care<sup>5</sup>. The global market of trade related to medicinal plants is estimated around US \$ 60 billion per year and is reported to grow at the rate of 7 % annually. Europe (33 %), Asia (26 %), North America (20 %), Japan (11 %) and the others (10 %) share the market<sup>6-8</sup>. Of the estimated 45,000 plant species in the Indian subcontinent, representing about 7 % of the global flora<sup>9</sup>, around 20,000 medicinal plants have been recorded to be used in traditional medical treatments<sup>10</sup>. Several of these medicinal plants are listed in various indigenous medicinal systems such as the Siddha (600 species), Ayurveda (700 species), Amchi (600 species) and Unani (700 species)<sup>11</sup>. The traditional communities use *ca.* 7,000-7,500 plants for curing different diseases<sup>12-14</sup>.

The focus of nutrition research, today, is moving towards preventive medicine and the study of health-related edible plants are gaining momentum<sup>15</sup>. Functional edible plants are an emerging field in food science due to their increasing popularity among health-conscious consumers. They represent any healthy food claimed to possess added physiologic benefits, which may reduce chronic disease risk or otherwise optimize health<sup>16</sup>. Nutraceuticals are bioactive compounds that confer protection from chronic disease *via* mechanisms that are well beyond simply providing nutrition. A food becomes functional when the levels of one or more nutraceuticals are present at concentrations such that their regular consumption elicits a positive biological effect. Nonetheless, nutraceuticals can also

be isolated from functional foods and added to other food matrices or concentrated for distribution in capsules or tablets. Similar to pharmaceutical agents, research clearly demonstrates that functional foods and nutraceuticals possess physiological and molecular targets that modulate clinical end-points associated with chronic disease. Consequently, functional foods have also become the topic of considerable interest in the food and nutrition industry, thereby presenting new economic opportunities. The present review relates to one such functional edible leafy vegetable, *Mukia maderaspatana* (Linn.) M. Roemer, (family: Cucurbitaceae). It is important for its numerous medicinal values in the Ayurveda, Siddha, naturopathy and folkloric traditional medicines of India as well as the indigenous medical systems of the Sub-Saharan African, Asian and Australian communities. An attempt has been made to provide a concise account of its global distribution, vernacular names and the phytochemical and antioxidant profile.

**Cucurbits:** Cucurbits popularly refer to the members of the family, Cucurbitaceae, commonly known as the gourd family. The taxon comprises of about 120-130 genera and ca. 800-900 species, widely distributed in tropical and subtropical/warm temperate regions of Africa including Madagascar, South, Southeast and East Asia, Australia and Central and South America<sup>17,18</sup>. Although most have Old World origins, many species originated in the New World and at least seven genera have origins in both hemispheres. There is a tremendous genetic diversity within the family and the range of adaptation for cucurbit species includes tropical and subtropical regions, arid deserts and temperate locations<sup>19</sup>. A few species are adaptable to production at elevations as high as 2000 m. Cucurbitaceae is a medium sized and botanically highly specialized family of mainly herbaceous, mostly climbing or trailing plants, usually with tendrils. Cucurbits are a well-recognized source of secondary metabolites. Therefore, they are among the largest and the most diverse plant families and are cultivated worldwide in a variety of environmental conditions. They are also among the most important plant families supplying humans with edible products and useful fibers. There are about 90 genera and 700 species used as food<sup>20</sup>. A number of cucurbit vegetables are also exported from India<sup>20</sup>. Cucurbits are associated with the origin of agriculture and human civilization. They are among the first plant species to be domesticated in both Old and New World. Wild and cultivated plants of the Cucurbitaceae have played an important role in Indo-Aryan food, medicine and culture (2000-200 B.C); over 300 words describing cucurbits are found in the Sanskrit texts<sup>21</sup>. One of the oldest Sanskrit texts to mention a recognized cucurbit is the second-millennium B.C. *Atharva-Veda*. About 37 genera and 90 species are reported from India. The cucurbits are very important for both tribals and non-tribals, as these are mainly vegetable plants and a source of food, medicine and are also related with their culture and customs.

**Taxonomy:** Taxonomists over the years have differed on the delimitation of *Cucumis*, which was first described by Linnaeus in 1753. Linnaeus recognized seven species in the genus, all of which were cultivated or economically useful. Three of these have been transferred to other genera, one is synonymized and only three are still maintained. The type of the genus is *C. sativus* L., the cucumber. Numerous taxonomic

treatments of *Cucumis* have been proposed since the work of Linnaeus<sup>22-27</sup>. The most comprehensive treatment of *Cucumis* was that of Kirkbride<sup>27</sup>. Consequently, the classification of the Cucurbitaceae has gradually been transformed from a system, based solely on gross morphological characters, to one emphasizing non-traditional characters derived from pollen, seed coat anatomy, phytochemistry and chromosome numbers.

Based on seed and stamen morphology, Jeffrey divided *Melothria* into four genera: *Melothria* L., *Mukia* Arn., *Solena* Lour. and *Zehneria* Endl<sup>23</sup>. All these genera have three stamens. In *Mukia* and *Solena*, two stamens are two-thecal and the remaining stamen is one-thecal whereas in *Zehneria*, the stamens are two-thecal. *Mukia* has straight anther-thecae and verrucose seeds, whereas *Solena* has oblique, curved anther-thecae and smooth seeds. Other genera associated with *Cucumis* include *Cucumella* Chiovenda, *Dicaelospermum* C.B. Clarke, *Mukia* Arnott, *Myrmecosicyos* C. Jeffrey and *Oreosyce* Hooker f.<sup>23,25,26,28,29</sup>. Jeffrey has listed these genera according to his opinion of their relations to *Cucumis* as follows: *Cucumella* most closely related, followed by *Oreosyce*, *Myrmecosicyos*, *Mukia* and *Dicaelospermum*<sup>25,26</sup>. In a new treatment, *Dicaelospermum* has been sunk into *Mukia*<sup>30</sup>. Using maximum parsimony, maximum likelihood and Bayesian analyses of sequence data from both the nuclear and chloroplast genomes, Ghebretinsae, Thulin and Barber have provided a comprehensive phylogeny of *Cucumis* and the traditionally related genera. According to them, *Cucumella*, *Dicaelospermum*, *Mukia*, *Myrmecosicyos* and *Oreosyce* are nested within *Cucumis*. Based on molecular phylogenetic research, Schaefer is also of the similar opinion<sup>31,32</sup>. Thus, recent studies have shown that the genus, *Cucumis* L., in its current sense is paraphyletic, with the five further genera, including *Mukia*, nested within it. A proposal to expand *Cucumis* to include these nested genera has therefore been made<sup>31-33</sup>. The nomenclatural changes that are needed to accommodate the currently recognized and more broadly defined taxa of the nested genera in *Cucumis* have also been published<sup>34</sup>.

*Mukia* consists of about nine species, distributed in the tropics of the Old World, viz., Sub-Saharan Africa, Yemen, Asia (from Pakistan to China in the east and south-east through Indo-China and Malaysia to New Guinea), Australia and New Zealand. *M. gracilis* (Kurz), *M. javanica* (Miq.) C. Jeffrey, *M. leioperma* (Wight & Arn.), *M. maderaspatana* (L.) M. Roem., *M. ritchei* (C.B. Clarke), *M. rumphiana* (Scheff.) are the taxa occurring in Asia<sup>30</sup>. *M. maderaspatana* extends up to Africa<sup>35</sup> and *M. maderaspatana*, *M. javanica* (Miq.) C. Jeffrey, *M. gracilis*, *Mukia* sp. A and *Mukia* sp. B (belonging in the genus *Cucumis*<sup>36</sup>) are reported from Australia<sup>37</sup>.

*M. maderaspatana* is a prostrate or climbing, much-branched, annual herb with spreading bristly hairs and simple tendrils (Fig. 1). The leaves are alternate and broadly triangular in outline. These leaves are 3-5 lobed, 3 to 11 cm in length and breadth. Their apices are acute, base deeply cordate, irregularly dentate, dark green coloured and scabrid above but pale green and hispid beneath. The petioles are hairy and 0.6 to 2.5 cm long. Flowers are small, pale yellow in colour. Male flowers are fascicled on very short peduncles while the female flowers are usually solitary and sessile. Calyx is hairy, bear a

tube of 2 mm long and narrowly campanulate. Corolla pubescent, segments ovate-oblong, rounded at the apex, 2 mm long. Fruits are popularly known as berry and are globose-ellipsoid, up to 1.5 cm in diameter. These are pale green in colour with longitudinal cream stripes and turn reddish when ripe. The seeds are 4 mm long and 2 mm broad and are present in numerous numbers. These are closely arranged, compressed and ellipsoid<sup>38-43</sup>.



Fig. 1. *M. maderaspatana* in its natural habitat

**Distribution:** A number of reports in literature points to the widespread occurrence of *M. maderaspatana* throughout the tropics and subtropics of the Old World: extending from the Sub-Saharan Africa and Madagascar through Southwest Asia (including Yemen, Pakistan, India, Bangladesh, Sri Lanka Andaman and Nicobar Islands and southern China), Southeast Asia (including the Mainland Southeast Asia and Maritime Southeast Asia, Ryukyu and Yaeyama islands) to New Guinea and Australia.

In the Far East (i) the Japanese islands, viz., Ryukyu and Yaeyama<sup>30,44-46</sup>, (ii) the Bac Huong Hoa<sup>47</sup>, Na Hang<sup>48</sup> and Muong Nhe nature reserves<sup>49,50</sup> of Vietnam, (iii) the islands in the Mekong river between Kratié and Stung Treng provinces of Northeast Cambodia<sup>51,52</sup>, (iv) Champasak and Xiangkhoang provinces and Luang Prabang of Laos in the Indochinese peninsula<sup>53</sup> and (v) the Republic of the Union of Myanmar (Burma)<sup>36</sup> are recorded to contain *M. maderaspatana*. Low land rocky places and woods in Thailand, particularly, Mae Hong Son; Chiang Mai (Doi Chiang Dao, Doi Inthanon); Nan; Lampang (Jae Sawn) in the north, Khon Kaen (Doi Phanok Khao) in the northeast, Phra Nakhon Si Ayutthaya; Bangkok; Saraburi (Phu Khae) in the central, Chon Buri (Ang Phak Nam) in the southeast, Kanchanaburi (Tham Tarn Lot) in the southwest and the Phuket island are rich in the plant species<sup>30,54</sup>. *M. maderaspatana* have also been reported to occur along the rocky mountain slopes/thickets (400-1700 m) of South China, commonly in Guangdong province, Guangxi autonomous region, Guizhou province, Yunnan province and in Taiwan<sup>55</sup>. The taxon is also accepted as *Coccinia cordifolia* (L.) Cogn. in that country<sup>56</sup>.

The food plant is recorded to occur all over India, right from the foot-hills of the southern peninsular tip of the Western Ghats to the sandy plains and sand dunes of the arid zone (Thar desert) in the north-west; to the wetlands of Samaspur

Bird Sanctuary in Uttar Pradesh and extending in the north to the Shivalik range<sup>57-71</sup> up to Nepal<sup>72,73</sup>. Its occurrence is also reported from the Dibang Valley, Lohit and Siang districts of Arunachal Pradesh, in the north east of India<sup>74</sup> as well as from the Andaman and Nicobar islands<sup>75</sup>. (i) Chotiari wetlands complex, (ii) Ubauro Taluk of Sukkur District, (iii) Kingri, Gambat, Khairpur, Sobhodero, Kotdiji, Thari Mirwah, Faiz Ganj and Nara of Khairpur district (iv) the Nara desert regions of the Sindh province and (v) the Keti Bundar, Keenjhar Lake, Chotiari Reservoir and Pai forest in the Indus ecoregion, are the geographical locations belonging to Pakistan, where the medicinal plant grows<sup>76-82</sup>. Several parts of Sri Lanka<sup>83-85</sup>, Gunung Halimun Salak National Park (West Java) in Indonesia<sup>86</sup>, Batan island, Lepanto and Bontoc subprovinces, Cavite and Laguna Provinces in Luzon, Masbate Palawan and Mindanao regions of Philippine<sup>87</sup> and Singapore<sup>88</sup> are also reported to be rich in the taxon.

The Flora of West Tropical Africa<sup>89</sup> and the useful plants of West Tropical Africa<sup>90</sup> provide a wealth of information on the native cucurbits of the West Tropical Africa, including *M. maderaspatana*, its folk medicinal properties and the vernacular names in common use among the natives of Gambia, Sierra Leone, Senegal and Nigeria. According to Burkill<sup>90</sup>, the annual scandant or trailing herb occurs throughout the West African region in open and not forested localities. Pama, the capital of the province of Kompienga, in the South-East of Burkina Faso<sup>91</sup>, Gash Delta in Eastern Sudan<sup>92</sup>, Ulumba Mountain in Southern Malawi<sup>35</sup>, the Okavango Delta, Kalahari of north-western Botswana<sup>93</sup>, Kivu province of Congo<sup>94</sup>, Nigeria<sup>95</sup>, Kenya, Tanganyika, Uganda and Zanzibar<sup>96</sup> are reported in literature to possess the plant species in their floral wealth.

Widespread occurrence of *M. maderaspatana* in Australia could be inferred from literature. It stretches from the islands of the Dampier Archipelago and Pilbara region (in the west), through the Ord River Floodplain Ramsar Site and Purnululu National Park of Kimberley Region and the Gulf of Carpenteria, to the Inkerman and Molongle blocks of the Townsville Plains province (in the east)<sup>30,31,97-105</sup>.

**Nomenclature:** *Mukia maderaspatana* (Linnaeus) M. Roemer, (family: Cucurbitaceae) syn.: *Cucumis maderaspatanus* L.; *Melothria maderaspatana* (L.) Cogniaux; *Bryonia cordifolia* L.; *Coccinia cordifolia* (L.) Cogn.; *Bryonia scabrella* L. f.; *Mukia scabrella* (L.f.) Arnott. *Mukia maderaspatana* (L.) M. Roem. var. *scabrella* (L.) Kurz; *M. maderaspatana* var. *gracilis* Kurz *Bryonia rottleri* Spreng.; *Mukia rottleri* (Spreng.) M. Roem.; *Bryonia althaeoides* Seringe; *Mukia althaeoides* (Seringe) M. Roemer; *Melothria althaeoides* (Ser.) Nakai; *M. celebica* Cogn. var. *villosior* Cogn.; *M. leiosperma* auct. non (Wight & Arn.); *Cucumis maderaspatana* (L.); *C. pubescens* Willd.<sup>30,38,39,55,106-108</sup>. The common and local names of the plant among different cultures in various regions are summarized in Table-1.

**Chemical constitution:** Indigenous medical system, both past and present, often involves the prescription of specific foods, almost always plants or their potent derivatives, to treat a wide spectrum of illness. A preliminary phytochemical screening of the plant, collected from Tirunelveli hills of South India, has led to the paper chromatographic (PC) identification of the following aminoacids: L-glutamic acid, D-,L-alanine,



TABLE-1  
COMMON/LOCAL NAMES OF *M. maderaspatana*

Language	Common/Local Names
English	Madras pea pumpkin; Bristly bryony; Rough bryony; Wild cucurbit (Punjab-Pakistan)
Burmese	Sathakhiva; Thabwotkha
Chinese	Hong gua; Mao er gua; Mao hua ma jiao er (Taiwan)
Filipino	Melon-gubat
Hausa	Gautan zomo; Malami; Malami na mata
Japanese	Sango ju suzume uri
Mundari	Hringkaubutuki; Japaputus; Jhajinari
Nepalese	Kauasangga; Kaubutuki; Merommed
	Matyangre kankri; Sunkeshre laharo; Ladbhadi (Bantar); Nagilangiai (Tamang)
Sindhi	Bellari; Chirati
Sinhala	Gon-kekiri; Heen-kekiri <i>syn.</i> Hinkekiri
	Kekiri; Lene-kekiri <i>syn.</i> Lenkekiri
Tagalog	Melon-gubat
Thai	Taeng nok (Kanchanaburi); Taeng nu (Northern, Northeastern); Taeng phi pluk (Chai Nat); Taeng nu khon (Prachuap Khiri Khan); Taneng nuu
Urdu	Musmusa; Chibbher (Punjab-Pakistan); Chibhari Wal (Pakistan); Chirati (Pakistan)
Vietnamese	Câu qua ăn; Cau qua nhám
Bengali	Agmuki; Bilari; Patilalau (Bangladesh)
Gujarati	Chanak-chibhdi; Tindori
Hindi	Aganaki; Agumaki; Ankh-Phod; Ankh phutani bel; Aunkharo; Bilari; Gulya kakri; Laghumukhi; Musmusa; Paripushkara; Pindila; Setu
Kannada	Chitrati; Kaadu paavate balli; Mani toned <i>syn.</i> Mani tonde; Mani thonde <i>syn.</i> Manidonde; Sanna hindele kaayi
Konkani	Chirati
Kumaoni	Agumarki; Bilari; Gwalakakri
Malayalam	Aattanga; Chitrati; Mukkapeeram <i>syn.</i> Mukkapiram; Mukkalpeeram; Mukkaalpiram; Mukkapeeram; Mucca-pin; Mukkappiri; Mushumushka
Manipuri	Lam-thabi
Marathi	Bilavi; Chiraati; Ghugri; Kharwad; Meka
	Ringana vaela
Punjabi	Gwala kakri
Rajasthani	Ankh-Phutani ki bel
Sanskrit	Ahilaykhan; Ghantaali; Kritarandra; Krtarandhras; Musimusikkayi; Paripushkara; Pindila; Setu; Trikoshaki
Tamil	Musumusukkai; Mochumochukkai (Sri Lanka); Muchumuchukkai (Mosumosukkai); Aayilaiyam; Bommusutai; Cempucattumuli; Cunaikkoti; Elavalukam; Kattumucukkai; Kattuvellari; Maamooli; Nagilangiai; Paripuskarai
Telugu	Budama dosa; Chedupulla; Kutaru budama; Kuturu budam; Lingadonda; Musumusukaya; Nugudosa <i>syn.</i> Noogudosa; Potti budamu; Putribudinga
Tulu	Baana koralu; Mukkattere
Trade or popular name	Gwala Kakri
Bariba (Benin, West Africa)	Kobion
Pulaar and Fulfulde (Senegal, West Africa)	Pomey
Banda (Oubangui)	Akaya
Manja (Oubangui, Central African Republic)	Nya chindo
Swahili (Bushi area, Kivu province, Democratic republic of Congo)	Murhalagala

L-leucine, D-,L-serine, D-,L-aspartic acid, L-proline, L-tyrosine, D-,L-threonine, phenylalanine, D-,L-3,4-dihydroxyphenylalanine, L-hydroxyproline, D-,L-norleucine, D-,L-methionine,

L-arginine monohydrochloride, L-glycine and D-,L-valine<sup>109</sup>. The paper also reports the paper chromatographic detection of the following sugars, namely, arabinose, fructose, glucose, mannose, sucrose, xylose, galactose and ribose, together with uncharacterized steroids, triterpenes, alkaloids, phenols, flavones, catechins and saponins. The presence of spinasterol, 22,23-dihydrospinasterol, its 3-O- $\beta$ -D-glucoside,  $\beta$ -sitosterol, decosaenoic acid and triterpenes have also been reported from the leaf extract<sup>110,111</sup>. From the aerial parts of the taxon, collected from the herbal garden of Sri Ramachandra Medical College and Research Institute, Chennai, Iman *et al.*<sup>112</sup> have detected the presence of steroids, triterpenes, flavonoids, reducing sugars and glycosides. A systematic analysis of the flavonoid constitution of the aqueous alcoholic leaf-extract has resulted in the isolation and characterization of six C-glycoflavones, *viz.*, 6-C- $\beta$ -D-glucopyranosylapigenin (isovitexin), 6-C- $\beta$ -D-glucopyranosylluteolin (homoorientin), 8-C- $\beta$ -D-glucopyranosylapigenin (vitexin) and 8-C- $\beta$ -D-glucopyranosylluteolin (orientin), 7-O- $\beta$ -D-glucopyranosyl-6-C- $\beta$ -D-glucopyranosylapigenin (saponarin) and 7-O- $\beta$ -D-glucopyranosyl-6-C- $\beta$ -D-glucopyranosylluteolin (lutanarin)<sup>113,114</sup>. Ethyl alcohol extracts of the leaf and root of *M. maderaspatana* have been analyzed using silica gel 60F<sub>254</sub> HPTLC plates to contain three ( $R_f = 0.02, 0.20$  and  $0.87$ ) and five ( $R_f = 0.02, 0.20, 0.56, 0.75$  and  $0.87$ ) fluorescent spots, respectively<sup>115</sup>. Columbin has been isolated from its roots<sup>116</sup> and the seed oil (18.8 g per 100 g) has been reported to be rich in linoleic (50 %), oleic and palmitic acids<sup>117</sup>.

#### Antioxidant capacity

**Oxidative stress and its implications:** Oxidative stress resulting from an imbalance between antioxidant-oxidant status of a living system, may arise as a result of mitochondrial dysfunction, activation of enzymes, release of iron from chelating proteins and oxidation of various molecules such as glucose and catecholamines<sup>118</sup>. External factors including alcohol and drug consumption, over-exposition to sunlight, intense physical exercise and tobacco smoke may also contribute to oxidative stress. The human system normally tends to maintain a dynamic equilibrium between the reactive species generation and their quenching. The physiological defence systems to counteract these reactive species encompass (i) endogenous enzyme systems, such as catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase, as well as urate and coenzyme Q and (ii) exogenous factors, notably, the carotenoids, vitamins C and E, dietary phenolics and phytochemicals<sup>119</sup>. Natural antioxidants of plant origin have been demonstrated to be more effective in quenching reactive species levels compared to synthetic individual dietary antioxidants, due to the synergistic actions of a wide range of these biomolecules in food plants. The antioxidant capacities of these compounds arise as a result of their ability to transform the reactive species into stable and harmless metabolites or by scavenging reactive oxygen and nitrogen species *via* redox mechanisms. It is fairly well accepted today that oxidative stress is implicated in the aetiology of the development of aging and age-related disorders, including neurodegenerative diseases and ischemia-reperfusion disorders, as well as cancer, cataract, amyotrophic lateral sclerosis, acute respiratory distress

syndrome and lung oedema. Thus, reactive oxygen and nitrogen species have come to occupy an amazingly central role in addressing the pathogenesis of diverse human ailments.

**Antioxidant phytoconstitution:** Plant phenolics, particularly, the ubiquitous flavonoids, constitute the abundant class of antioxidants. The estimated total dietary intake may approach as high as 1 g/day, which is 10 times greater than the intake of vitamin C and 100 times that of vitamin E<sup>120</sup>. The antioxidant capacity of phenolic compounds has long been recognized for their strong chain-breaking actions and ability to scavenge radicals, thereby protecting cells against the detrimental effects of reactive species. The total amount of phenolics/100 g of the fresh leaves (FL) has been determined by the method described by Singleton, *et al.*<sup>121</sup> to be 292.4 mg gallic acid equivalents (GAE)<sup>113</sup>. Another study, adopting the method of Siddhuraju and Becker<sup>122</sup>, has evaluated the phenolic content of the methanol (MeOH) extract of the air-dried leaf (DL) to be 9.6 g/100g extract and the acetone (Me<sub>2</sub>CO) extract to be 12.2 g/100 g tannic acid equivalents (TAE). The phenolic contents of the stem, fruit and root have also been reported in the work. The contents are reported to vary from 4.6 to 19.7 g/100 g tannic acid equivalents of dried leaves for various extracts<sup>123</sup>. Balaraman *et al.*<sup>124</sup> have recently reported the phenolic content of the ethyl acetate (EAc) fraction of the shade-dried aerial parts of the plant as 10.71 mg/g gallic acid equivalents. Flavonoids are reported to be the predominant phenolics of this indigenous medicinal leaf, amounting to 247.1 mg of quercetin equivalents/100 g of fresh leaves<sup>113</sup> and 7.6 mg/g of dried leaves<sup>124</sup>. In plants, flavonoids are involved in a wide array of processes, including plant-pathogen interactions, pollination, light screening, seed development and allelopathy<sup>125</sup>. Many flavonoid-biosynthetic genes are induced under stress conditions. Consequently, the flavonoid levels tend to increase during exposure to biotic and abiotic stresses, such as wounding, drought, metal toxicity and nutrient deprivation. A common denominator in these environmental stress conditions is the production and accumulation of reactive oxygen species. Their accumulation leads to oxidative stress that can damage cellular components, such as DNA, lipids, proteins and sugars. To contain the oxidative stress-related traumas, reactive oxygen species homeostasis in plants is tightly regulated by a complex machinery of enzymatic and non-enzymatic antioxidants<sup>126</sup>. This accounts for the variations, commonly observed, in the contents of the phytometabolites. The tannin contents of the various fractions of the extract have been reported to vary from 0.1 to 8.3 g/100 g extract<sup>123</sup>. 100 g of the fresh *M. maderaspatana* leaf material is also determined to contain 17.1 mg of L-ascorbic acid, 0.194 mg of  $\alpha$ -tocopherol and 0.812 mg of  $\beta$ -carotene equivalents of vitamins C and E and total carotenoids respectively<sup>113</sup>.

**Reactive species scavenging capacity:** The *in vitro* antioxidant capacities of the plant extracts largely depend both on the complex nature of biological systems and on the conditions of the test system. They are also influenced by scores of other factors that cannot be fully described by one single method. Consequently, despite the wide popularity of antioxidant research during the past three decades, lack of standardized assays/universal method to compare research results of different research groups continue to be a major challenge.

Aqueous extract of the shade-dried leaves have been reported to potentially scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (IC<sub>50</sub> = 15.95  $\mu$ g/mL)<sup>127</sup>. The ethyl acetate extract of the air-dried aerial parts is reported to possess the maximum scavenging capacity (IC<sub>50</sub> = 2.95  $\mu$ g/mL) compared to the methanolic and chloroform extracts, which exhibited IC<sub>50</sub> = 58.65 and 90.32  $\mu$ g/mL respectively<sup>124</sup>. Methanolic extract of the stem is reported to have displayed the highest DPPH scavenging capacity of 123.8  $\mu$ g/mL, followed by the methanolic and dimethyl ether extracts of the root (130.1 and 136.9  $\mu$ g/mL respectively)<sup>123</sup>. The fruit extracts have been considerably poor in their activity (1506 and 1766.8  $\mu$ g/mL respectively for the Me<sub>2</sub>CO and MeOH extracts). According to another study, the air-dried whole plant extract could scavenge DPPH with an EC<sub>50</sub> of 1347.20 mg/mL<sup>128</sup>.

Another operationally simple and commonly employed assay involves the generation of the sensitive 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cationic oxidant (ABTS<sup>•+</sup>) followed by determining the capacity of the extract to scavenge the same. The aqueous leaf extract has been reported to scavenge ABTS<sup>•+</sup> concentration dependently (IC<sub>50</sub> = 23.65  $\mu$ g/mL). A more commercially adoptable mode of expressing the radical scavenging capacity is the Vitamin C equivalent antioxidant capacity (VCEAC)<sup>113</sup>. The total antioxidant capacity (TAC) of 100 g of aqueous alcoholic extract of the fresh leaves, determined by the said method, has been 301.9 mg<sup>113</sup>. Sowndhararajan *et al.*<sup>123</sup> have reported the methanolic and the Me<sub>2</sub>CO extracts of the root to exhibit a higher total antioxidant capacity (14161.4 and 13648.4  $\mu$ mol/g respectively). The stem extracts respectively followed these with 8572.5 and 5757.7  $\mu$ mol/g.

Hydroxyl radical (OH<sup>•</sup>) scavenging ability of *M. maderaspatana* aqueous leaf extract has been reported<sup>127</sup>, in terms of IC<sub>50</sub>, to be 29.14  $\mu$ g/mL. The ethyl acetate extract has been determined by Balaraman *et al.*<sup>124</sup> to be more potent (IC<sub>50</sub> = 57.52  $\mu$ g/mL) in scavenging OH<sup>•</sup>, compared to the chloroform (IC<sub>50</sub> = 66.99  $\mu$ g/mL) and methanol (IC<sub>50</sub> = 67.00  $\mu$ g/mL) extracts. The methanolic extract of the stem has been found to exhibit the highest OH<sup>•</sup> scavenging potential (42.6 % at 200  $\mu$ g/mL) while the methanolic extract of the fruit had displayed the least (18 %) at that concentration<sup>123</sup>.

The aqueous leaf extract has also been reported<sup>127</sup> to effectively scavenge superoxide anion radicals (O<sub>2</sub><sup>•-</sup>) with an IC<sub>50</sub> of 19.31  $\mu$ g/mL. The ethyl acetate extract was the more potent portion of the aerial parts of the plant (IC<sub>50</sub> = 41.04  $\mu$ g/mL)<sup>124</sup>. *M. maderaspatana* is also reported to be capable of scavenging H<sub>2</sub>O<sub>2</sub> in a dose-dependent manner and the scavenging capacity (IC<sub>50</sub> = 46.32  $\mu$ g/mL) has been better than  $\alpha$ -tocopherol at all concentrations, according to that study<sup>127</sup>.

Autoxidation of an aqueous emulsion system of  $\beta$ -carotene and linoleic acid is yet another test model to assay the antioxidant activities of plant extracts. Scavenging the radicals that are formed by linoleic acid oxidation in the emulsion by the antioxidant principles of the extract (inhibition of lipid peroxidation) forms the basis of this method. The aqueous alcoholic extract of the fresh leaves of *M. maderaspatana* has been evaluated to possess respectively 69.4 % and 32.6 % of the inhibitory capacity of standard  $\alpha$ -tocopherol at the end of 1 h and 2 h study<sup>113</sup>. The Me<sub>2</sub>CO extract of the root has been

recorded to display the highest inhibitory effect (29.4 %) while that of the fruit demonstrated only 12.3 % at the concentration of 200 µg/mL<sup>123</sup>. Both the stem and root extracts of the plant have been reported to exhibit strong and comparable activities.

Lipid peroxidation, initiated by reactive species, results in a number of secondary oxidation marker products, including malonaldehyde<sup>129</sup>. Among lipid peroxidation products used for antioxidant assays, malonaldehyde has been most widely used to evaluate the antioxidant activity of substances in lipid peroxidation systems. Malonaldehyde-thiobarbituric acid assay has become one of the popular assays for studies related to lipid peroxidation and is used widely to evaluate antioxidant activities of various natural products. However, thiobarbituric acid reacts with many different carbonyl compounds formed from lipid peroxidation. As a result, a more relevant parameter, the total carbonyl compounds reacting with thiobarbituric acid, called thiobarbituric acid-reacting substances (TBARS) has evolved. Among the tested fractions, ethyl acetate extract of the air-dried aerial parts of the plant has been reported to possess the highest antioxidant potential ( $IC_{50} = 7.70 \mu\text{g/mL}$ )<sup>124</sup>.

Ferric-reducing antioxidant power (FRAP) is another simple, rapid, versatile and inexpensive assay, frequently used to express the antioxidant capacity<sup>129</sup>. In this method, the antioxidant capacity is determined based on the ability of the antioxidants to reduce the yellow  $[\text{Fe}(\text{TPTZ})_2]^{3+}$  to blue  $[\text{Fe}(\text{TPTZ})_2]^{2+}$  (TPTZ = 2,4,6-tris(2-pyridyl)-s-triazine). The aqueous alcoholic extract of the fresh leaves of *M. maderaspatana* has been assessed to possess 187.5 mg VCEAC/100 g fresh leaves<sup>113</sup>. The MeOH and Me<sub>2</sub>CO root extracts of the plant have been reported to possess a ferric-reducing antioxidant power of 1470.0 and 1182.8 mmol Fe(II)/mg respectively<sup>123</sup>. These have been followed by the corresponding stem extracts (970.0 and 901.1 mmol Fe(II)/mg). The trend observed by the authors, according to the report, is as follows: root (MeOH > Me<sub>2</sub>CO) > stem (MeOH) > leaf (MeOH) > stem (Me<sub>2</sub>CO) > fruit (Me<sub>2</sub>CO) > fruit (MeOH) > leaf (Me<sub>2</sub>CO). The reducing power of the aqueous dried leaf extract was also determined by the method of Oyaizu<sup>130</sup>. The reducing power has been reported to be greater than the standard  $\alpha$ -tocopherol and increased in a concentration dependent manner<sup>127</sup>. The ethyl acetate extract, according to another study, has exhibited the highest reducing power compared to the chloroform and methanolic extracts and standard butylated hydroxytoluene (BHT) analyzed<sup>124</sup>.

An assay, very much related in its chemistry to the total phenol determination of Singleton, *et al.*<sup>121</sup>, is sometimes used to address the antioxidant activity of substances<sup>131</sup>. Various authors refer the protocol using different names, such as the total phenol assay or the phosphomolybdenum assay. The authors, in their article, have sequenced the relative reduction potentials of various extracts of the plant as: root (Me<sub>2</sub>CO) > leaf (Me<sub>2</sub>CO) > root (MeOH) > stem (Me<sub>2</sub>CO) > stem (MeOH) > leaf (MeOH) > fruit (MeOH) > fruit (Me<sub>2</sub>CO). Quantitatively they varied in the order: 41.0 > 33.0 > 28.8 > 28.0 > 26.0 > 22.8 > 20.4 > 18.5 g ascorbic acid equivalents/100 g of the extracts<sup>123</sup>.

Erythrocyte membranes are 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 membranes get damaged, leading to hemolysis. The inhibition of hemolysis, caused by the extracts of various parts of *M. maderaspatana*, have been reported to vary in the order: root (Me<sub>2</sub>CO) > stem (Me<sub>2</sub>CO) > leaf (Me<sub>2</sub>CO) > leaf (MeOH) > stem (MeOH) > root (MeOH) > fruit (Me<sub>2</sub>CO) > fruit (MeOH)<sup>123</sup>.

Though scavenging of reactive species by antioxidant metabolites is the generally accepted mechanism of their antioxidant activity, mechanisms involving metal binding have also been proposed and have gained due consideration. Considerable evidence has emerged from clinical studies to show that increases in cellular free iron concentrations have been associated with oxidative stress and that genetic and non-genetic iron misregulations in the brain contribute to neuronal death in certain neurodegenerative disorders, such as Alzheimer's, Parkinson's and Huntington's diseases and Hallervorden-Spatz syndrome. Even mildly elevated iron levels have been linked to increased cardiovascular disease and cancer incidences in humans and hence should be maintained within the optimum level. Since epidemiological evidences, presently available, indicates that regular intake of bioactive-rich plant foods promise a wide range of benefits, including the regulation of transition metals such as iron, determination of its chelating abilities has also gained significance. The chelating capacities of the constituents of the methanolic solution of the leaf extract is reported to be respectively 33.4 % and 52.9 % greater than those of  $\alpha$ -tocopherol and 61.17 % and 27.62 % higher than those of BHT at 25 and 50 µg/mL concentrations<sup>113</sup>. Extracts of various organs of *M. maderaspatana* have been in the order: fruit (MeOH) > stem (MeOH) > fruit (Me<sub>2</sub>CO) = root (Me<sub>2</sub>CO) > leaf (Me<sub>2</sub>CO) = stem (Me<sub>2</sub>CO) > root (MeOH) > leaf (MeOH)<sup>123</sup>.

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