

Phyto-Nutrient Diversity in *Morinda Citrifolia* L. Genotypes of Andaman Islands, India

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Abstract

The Indian mulberry or Noni (*Morinda citrifolia* L.) is one of the emerging sources of natural antioxidants for herbal and pharmaceutical industry. The genus *Morinda* has more than 150 species in which *M. citrifolia* is identified as most important for health and economic point of view. Present study revealed significant ($p < 0.05$) diversity in 33 genotypes of *M. citrifolia* from Andaman and Nicobar Islands (India) for phyto-constituents. The promising genotypes viz. FRG-14, JGH-5, TRA-1, TRA-2 and HD-6 were identified for commercial uses. Correlation analysis in *M. citrifolia* germplasm showed strong correlation between carotenoids and ascorbic acid ($r^2 = 0.973$; $p < 0.05$), tannin ($r^2 = 0.598$; $p < 0.05$), flavonoids ($r^2 = 0.691$; $p < 0.05$) and phenol ($r^2 = 0.598$; $p < 0.05$). The genotypes showed wide range for antioxidant capacity which showed positive correlation with carotenoids ($r^2 = 0.335$; $p < 0.05$), flavonoids ($r^2 = 0.249$; $p < 0.05$) and Cu ($r^2 = 0.953$; $p < 0.05$), Mn ($r^2 = 0.953$; $p < 0.05$) and Mg ($r^2 = 0.582$; $p < 0.05$). The diversity analysis is useful for designing breeding strategies for phyto-nutrient rich genotypes for better recovery in health products.

Keywords: Phytochemicals; Micronutrients; DPPH activity; Pharmaceutical industry; *Morinda citrifolia*

Introduction

Now-a-days, natural antioxidants are much talked compounds for their health benefits. Noni (*Morinda citrifolia* L.; Rubiaceae) is an underexplored phyto-nutrient rich medicinal plant from tropical region [1]. It is a small evergreen tree or shrub, bears lumpy, green to yellowish-white fruits. *Morinda* fruits (Figure 1) have been used in different traditional health systems of Nicobarese, Chinese, Australians and Polynesians [2]. It is native of Andaman & Nicobar and adjoining regions in Indian Ocean and distributed to other regions of the Indian and Pacific Oceans [3]. Around 150 species were reported in genus *Morinda* which spread across the India, Pacific and tropical island regions and *Morinda citrifolia* L. is most commonly used species in herbal and pharmaceutical industry [1,4]. The *M. citrifolia* is also used for traditional medicine, roasted food and fried vegetable by primitive tribes of Andaman and Nicobar Islands [5].

Researchers reported around 200 phytochemicals including phenolics, carotenoids, polysaccharides, flavonoids, iridoids, fatty acids, scopoletin, catechin, beta-sitosterol, damnacanthal and alkaloids [3,6]. These compounds contribute in number of biological activities such as anti-angiogenesis, antioxidant, cyclooxygenases-1 and -2 inhibition and tyrosine kinase inhibition and curing simple to complex ailments such as arthritis, diabetes, high blood pressure, muscle aches, menstrual difficulties, headaches, heart disease, immunodeficiency, cancer, gastric ulcers, sprain, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems and drug addiction [3,4,6].

These compounds contribute in free radical scavenging capacity of plant extracts but their kind and concentration varies among plant species or their genotypes [2,7,8]. Further, morphological and genetic diversity in *Morinda germplasm* could affect physiological processes, resultantly phyto-nutrient diversity in *M. citrifolia* [9,10]. Thus, information on such diversity in *M. citrifolia* was essential for developing genotypes with higher recovery of antioxidants and micronutrients in *Morinda* based herbal and pharmaceutical products. Therefore, present study conducted to analyze diversity in *M. citrifolia* for phyto-nutrients and to find correlation between such compounds for use in further breeding programme.



Figure 1: Ripe fruits of *Morinda citrifolia* L. (HD-6)

Materials and Methods

Sample collection and preparation

Fresh healthy mature fruits of 33 genotypes of *Morinda citrifolia* L. were collected from Noni Germplasm Block, Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands, India. Sample preparation for phytochemicals and antioxidant activity estimation was done as per the procedure described by Singh, *et al.* (2012a) with minor modifications. In brief, 2 g sample from homogenized fruit pieces were ground in 10 ml methanol in mortar and pestle till sample become colorless [8]. The extract was centrifuged (Heraeus Biofuge, Taylor Scientific Pvt. Ltd., Missouri) at $8000 \times g$ for 10 min and filtered through Whatman No. 1 filter paper. It was concentrated by rotary evaporator (Cyber Lab Corp., Millburg, USA) and kept in -20°C for further analysis. Sample for anthocyanin estimation were prepared in Methanol: Formic acid: Water (70:2:28) solution. Carotenoid estimation was done with 80% acetone extract while 4% oxalic acid was used for Ascorbic acid estimation.

Phytochemical estimation

Total polyphenol content in fruit samples of *M. citrifolia* was determined by Folin-Ciocalteu reagent method (10%, v/v) with gallic acid as standard (mg/100 g fresh weight) and taking absorbance at 765 nm by UV-spectrophotometer (Elico SL-164, Pvt Ltd, Hyderabad, India). Flavonoid content was determined using the procedure described by Chang, *et al.* (2002) and expressed as mg rutin equivalent (mg/100 g fresh weight) [11,12]. The pH differential method was used for anthocyanin estimation and concentration was expressed as mg cyanidine-3-glucoside (C3GE) /100g fresh weight. Ascorbic acid, tannin and total carotenoids were estimated using standard volumetric methods described by [13,14].

DPPH antioxidant activity

The hydrogen atom or electron-donation ability of *M. citrifolia* fruit extracts was determined using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) as per procedure described by Singh, *et al.* (2012a) in underutilized fruits. In brief, 0.1 ml methanolic extract of fruit added to 3 ml of 0.001M DPPH methanol solution and took absorbance at 517 nm using UV-visible spectrophotometer (Elico Pvt., Hyderabad, India) after 30 min of incubation period [8]. The per cent inhibition of activity was calculated using the standard formula: $[(A_o - A_e)/A_o] \times 100$ (A_o , absorbance without extract; A_e , absorbance with extract).

Proximate composition

Carbohydrate and acidity in fruits from 33 genotypes were estimated by process described by Sadasivam and Manikam (1996) [15]. Crude protein was determined by formula, CP (%) = $N \times 6.25$; where N is total nitrogen in fruits estimated by digestion and distillation units. Fat content in fruits was estimated by soxlet apparatus crude fibre content was estimated by acid-base digestion method and total soluble solids in the fruits were determined by refractometer (PAL 1, Atago, Tokyo Tech., Japan) [15,16].

Anti-nutritional factors

Phytate, oxalate and nitrate content in fruits of *M. citrifolia* genotypes were determined by the procedure described by Hassan, *et al.* (2008) while saponin content was estimated as per AOAC (1995) method [16,17].

Micronutrient estimation

Magnesium, calcium, copper and manganese content in fruits of 33 genotypes of *M. citrifolia* were determined through Atomic

Absorption Spectrophotometer (AAS; Shimadzu AA 6200). For this, the sample were converted into ash by muffle furnace and dissolved in millipore water and the samples were then diluted to a suitable volume.

Statistical analysis

The observed data were analysed for mean, STDEV and range using EXCEL software 2007. Pearson correlation coefficient for phyto-nutrients was tested for significance ($P < 0.05$; $P < 0.01$) using WINKS SDA software.

Results and Discussions

Phytochemical contents

Phytochemicals are important bioactive compounds from plant kingdom and play key role in human health and food. Now-a-days, it has been accepted that the natural bio-actives such as phenolics, carotenoids, flavonols, ascorbic acid, tannin, anthraquinones and anthocyanin are key factors for longevity. They neutralize, scavenge or inhibit the free radicals generated as by-products of biochemical reactions in body. However, their concentration in plants is affected by genetic, environmental and estimation methods and researchers identified phytochemical rich plant sources [2,8,18].

The results for phytochemical contents in 33 genotypes of *Morinda citrifolia* showed great extent of diversity as summarized in Table 1. The *M. citrifolia* was reported as one of the richest source of natural antioxidants which is supported by the findings of present study [6,8].

Genotypes*	Poly-phenol (mg/100g)	Flavonoid (mg/100g)	Tannin (mg/100g)	Antho-cyanin (mg/100g)	Carot-enoid (mg/100g)	Ascor-bic acid (mg/100g)	Anti-oxidant Activity (DPPH) (%)	Nitrate (mg/100g)	Phytate (mg/100g)	Oxalate (mg/100g)	Saponin (mg/100g)
Average	261.8	344.1	236	248.3	455.2	85.1	62.89	18.2	239	9.2	88.3
STDEV	58.8	166.4	72.9	37.9	142.4	7.1	11.64	59	619.4	49.5	258.5
Range	58.8-370.3	47.8-656.2	72.9-395.2	37.9-340.4	114.7-696.3	7.1-98.2	11.6-88.6	18.2-98.8	185.1-968.0	9.2-67.1	88.3-440.0

Table 1: Statistics of phytochemical diversity in *Morinda citrifolia* L. genotypes

Total polyphenol content in fresh fruits of genotypes was ranged from 165.40 mg/100 g ('MBAY-6') to 370.26 mg/100 g (in 'TRA-1'). The 'CHTAP-13', 'HD-6' and 'HD-6A' also found to be rich in polyphenol. *Morinda* fruits were rich source of flavonoids and present study observed significant ($p < 0.05$) diversity among the tested genotypes of *M. citrifolia* [14]. It ranged from 47.57 to 656.18 mg/100 g, maximum in 'JGH-5' while minimum in 'CHTAP-13' while other genotypes rich in flavonoid content were 'TRA-1', 'PBAY-7', 'TRA-2' and 'JGH-1'. The significant variations in *M. citrifolia* genotypes for phyto-nutrients could be due to genetic variations as deoxyribonucleic acid (DNA) which was reported by Singh, *et al.* (2011a; 2013) [9,19]. Here, the DNA play crucial role in regulating the transcription and translation processes associated with the enzymatic activities for synthesis of secondary metabolites in cell system [20].

Tannin contribute in strong free radical activity of plant extracts and present study showed wide variation among the genotypes of *M. citrifolia* for its concentration in fresh fruits which ranged from 88.92 mg/ 100g in 'MEM-3' to 395.20 mg/100 g in 'HD-6'. Other tannin rich genotypes were 'TRA-1', 'CHTAP-13', 'HD-6A', 'JGH-1' and 'JGH-5' [18]. *Morinda* was reported as poor source of anthocyanin but difference could observe in present study which showed it range from 163.25 mg/100g ('MEM-3') to 340.39 mg/100 g ('MANJ-1') [8]. Total carotenoids are crucial factor for antioxidant activity of the plant extracts and existing diversity of *M. citrifolia* showed an opportunity for selection of carotenoid rich genotypes for use in breeding programme [18]. The study showed wide variation among *M. citrifolia* genotypes for carotenoid content which ranged from 114.68 to 696.26 mg/100 ml, with 'LH-12', 'AHD-1', 'TRA-2', 'MEM-2' and 'WAND-4' as carotenoid rich genotypes. However, *M. citrifolia* genotypes did not show significant ($p < 0.05$) variations for ascorbic acid content which ranged from 71.0 (MBAY-16) to 98.20mg/100g (GAH-1). Thus, identified genotypes could help in breeding of phyto-nutrient genotypes as well as in higher recovery of phytochemicals from same quantum of fruits.

DPPH Antioxidant activity

The phytochemical constituents exhibit their free radical scavenging capacity during *in vitro* studies [6,8,18]. Similarly, the fruit extract from 33 genotypes of *M. citrifolia* showed great variation in DPPH activity (Table 1). The genotypes showed high antioxidant activities were identified as 'GAH-1' (92.23%; $IC_{50} = 114.57 \mu\text{g/ml}$) and 'HD-6' (88.83 %; $IC_{50} = 115.60 \mu\text{g ml}^{-1}$) while lowest activity was recorded from methanol extract of 'MEM-3' fruits (66.77%; $IC_{50} = 245.84 \mu\text{g ml}^{-1}$). The higher antioxidant activity of *M. citrifolia* could be due to higher contents of phytochemicals which showed strong correlation and presence of other natural antioxidants like flavones, flavonols and proanthocyanidins [8,18,21]. The observations for strong correlation between antioxidant activity with phyto-nutrients are in conformity with the previous reports of Katalinic, *et al.* (2006) and Singh, *et al.*

(2012a, 2012b) [8,18,22]. The low antioxidant activity of 'MEM-3' will be fruits due to the reasons: First, it has been reported that reaction of DPPH with certain phenols such as eugenol and its derivatives is reversible, resulting in low readings for antioxidant activity (% disappearance). The second possible reason could be due to the slow rate of the reaction between DPPH and the substrate molecules [23,24].

Proximate composition

The proximate analysis of fruits of 33 genotypes of *M. citrifolia* also supported wide range diversity as presented in Table 2. Fruit juice recovery affected by various factors and present study revealed wide variation among the test genotypes. It was highest in 'HD-6A' (65.5 %) while lowest in 'MEM-1' (22.4 %). Breeding for higher recovery of crude fibre from *Morinda citrifolia* was big challenge and present study identified source genotypes as 'MEM-1' (9.97 %) and 'BRJ-19' (7.62 %). Fresh fruits of *M. citrifolia* are poor in fat content which ranged from 0.09% in 'JGH-5' to 0.24% 'MANJ-1' (Table 2) [5]. The study showed possibilities for selection of appropriate genotypes in *M. citrifolia* for protein as 'FRG-14' (6.0%) and 'MANJ-9' (2.9%); total soluble solids like 'ABH-1' (9.8°Brix); 'CHLD-17' (0.41%) for titrable acidity and 'LH-1' (930.4 mg/100 g) for carbohydrate recovery.

Genotypes*	Juice (%)	Crude fiber (%)	Fat (%)	Protein (%)	TSS (°Brix)	Acidity (%)	Carbohydrate (mg/100g)	Mn	Ca	Cu	Mg
Average	9.2	0.6	0.05	0.7	1.1	0.1	181.9	68.6	2534.8	2.9	378.7
STDEV	36.2	8.6	0.2	4.5	7.8	0.2	653.7	88.9	2279.2	8.2	507.8
Range	9.2-65.5	0.6-10	0.05-0.2	0.7-6.0	1.1-9.8	0.1-0.4	181.9-930.4	4.6-284.2	51.1-6815.6	2.9-17.2	16.3-1131.3

Table 2: Statistical analysis of diversity in proximate and micronutrients contents in *Morinda citrifolia* L. genotype

Anti-nutritional factors

Anti-nutrients analysis in fruits of 33 genotypes of *M. citrifolia* showed the highest nitrate content in 'HD-6' (98.8 mg/100 g) and lowest in 'MEM-3' (22.23 mg/100 g). Phytate was ranged from 185.0 (TRA-1) to 967.9 mg/100 g (CHTAP-13) while oxalate content was highest in 'ABF-1' (67.05 mg/100 g). Saponin content was high in *M. citrifolia* genotypes and observed in the ranged of 130 to 440 mg/100 g, lowest in 'LH-1' and highest in 'LH-12'. The genotypes low in anti-nutrients were identified as 'MHP-19', 'FF-8' and 'MEM-2' for nitrate; 'JGH-5', 'MEM-1', 'CHLD-17' and 'TRA-1' for phytate; 'MANJ-9', 'MBAY-16' and 'LH-' for oxalate and 'TRA-1', 'HD-6' and 'TRA-2' for saponin. Though, *M. citrifolia* is rarely consumed as fresh salted fruits, roasted fruits or vegetables but high concentration of anti-nutritive factors in fresh fruits is concern for consumers, thus, genotypes low in anti-nutrients should be used for food purpose. The promising genotypes identified for various phyto-nutrients such as 'GAH-1', 'CHTAP-13', 'TRA1' and 'TRA-2' given in Table 3 could be used in breeding programme or recovery of higher phytochemicals in herbal products.

	Constituents	Promising genotypes
Proximates	Juice (%)	HD-6A (65.5), HD-6 (60.3), HBAY-11 (45.6), ABF-1 (43.3)
	Crude fiber (%)	MEM-1 (10.0), ABH-1 (9.9), WAND-4 (9.8), MEM-3 (9.6)
	Fat (%)	ABF-1 (0.24), ABF-2 (0.23), AHD-1 (0.22), BRJ-19 (0.22)
	Protein (%)	FRG-14 (6.0), ABF-1 (5.5), MEM-1 (5.5), BRJ-19 (5.4)
	Acidity (%)	CHLD-17 (0.4), HD-6A (0.4), CHTAP-13 (0.3), FRG-14 (0.3)
	CHO (mg/100g)	LH-1 (930.4), HD-6A (866.9), ABF-2 (861.8)
	TSS (°Brix)	ABH-1 (9.8), TRA-1 (9.5), MEM-1 (9.2), TRA-2 (9.2)
Micronutrients (mg/100g)	Mn	SPG-2 (284.2), JGH-1 (248.2), ABF-2 (205.4), ABF-1 (185.2)
	Cu	PBAY-7 (17.2), LH-1 (13.4), ABF-1 (12.2), SPG-2 (12.1)
	Ca	MBAY-16 (6815.6), MHP-19 (6722.7), HBAY-11 (6651.6)
	Mg	AHD-1 (1131.3), JGH-1 (1121.2), ABH-1 (1114.1)
Antioxidants (mg/100g)	Polyphenol	TRA-1 (370.3), CHTAP-13 (365.9), HD-6 (365.2), HD-6A (347.0)
	Flavonoid	JGH-5 (656.2), TRA-1 (614.0), PBAY-7 (599.4), TRA-2 ((588.2)
	Anthocyanin	MANJ-1 (340.4), ABF-2 (326.5), ABF-1 (303.9), AHD-1 (290.9)
	Tannin	HD-6 (395.2), TRA-1 (390.6), CHTAP-13 (380.4), HD-6A (361.3)
	Carotenoid	LH-12 (696.3), AHD-1 (678.8), TRA-2 (663.4), MEM-2 (618.9)
	Ascorbic acid	GAH-1 (98.2), TRA-2 (94.9), TRA-1 (93.5), JGH-1 (91.8)
	DPPH activity (%)	FRG-14 (88.6), HBAY-11A (83.0), WAND-4 (82.5), TRA-1 (82.4)

	Constituents	Promising genotypes
Anti-nutrients (mg/100g)	Saponin	LH-12 (440.0), CHLD-17 (435.0), PBAY-7 (415.0)
	Phytate	CHTAP-13 (968.0), ABH-1 (953.8), HD-6A (911.0), JGH-1 (896.8)
	Nitrate	HD-6 (98.8), TRA-1 (97.7), CHTAP-13 (95.1), HD-6A (90.3)
	Oxalate	ABF-1 (67.1), GAH-1 (62.8), CHTAP-13 (62.6), CAL-10 (60.3)

*Value in parentheses are actual concentration values.

Table 3: Phyto-nutrient rich promising genotypes of *Morinda citrifolia* L

Micronutrients

The analyses of micronutrient estimation in 33 genotypes of *M. citrifolia* are presented in Table 2. Manganese content was observed to highest in 'SPG-2' (284.2 ppm) while lowest in 'ABH-1' (4.6 ppm). *M. citrifolia* genotypes showed great variation for calcium content in fruits which ranged from 51.1 ppm in 'GAH-1' to 6815.6 ppm 'MBAY-16'. Similarly, Mg in *M. citrifolia* fruits was ranged from 16.3 ppm ('GAH-2') to 1131.3 ppm ('AHD-1'). However, *M. citrifolia* was observed to be poor in copper content as it ranged from 2.9 to 17.2 ppm, highest in 'PBAY-7' and minimum in 'MHP-19'.

Correlation and regression studies

The results for correlation and regression analysis of phytochemical contents in *M. citrifolia* germplasm are presented in Table 4. The DPPH antioxidant activity of methanol extract of fruits showed good correlation with carotenoids ($r^2 = 0.335$; $p < 0.05$) and flavonoids ($r^2 = 0.249$; $p < 0.05$). Interestingly, the present study revealed strong correlation between antioxidant activity and Cu ($r^2 = 0.953$; $p < 0.05$), Mn ($r^2 = 0.953$; $p < 0.05$) and Mg ($r^2 = 0.582$; $p < 0.05$). However, Ca content also showed good correlation with antioxidant activity ($r^2 = 0.220$; $p < 0.05$). Among phytochemicals, the carotenoids content in *M. citrifolia* germplasm showed strong correlation with ascorbic acid ($r^2 = 0.973$; $p < 0.05$), tannin ($r^2 = 0.598$; $p < 0.05$), flavonoids ($r^2 = 0.691$; $p < 0.05$) and phenol ($r^2 = 0.598$; $p < 0.05$). The correlation analysis between phytochemicals and micronutrients revealed strong positive correlation viz., flavonoids and Mn ($r^2 = 0.902$; $p < 0.05$); tannin and Cu ($r^2 = 0.916$; $p < 0.05$) carotenoids and Mg ($r^2 = 0.553$; $p < 0.05$).

Parameters	DPPH activity	Phenol	Flavonoids	Tannin	Carotenoids	Ascorbic acid	Mn	Ca	Cu	Mg	Eigen value	% variance
DPPH activity	1.00	0.010	0.249	0.012	0.335	0.013	0.912	0.220	0.953	0.982	3.3	33.3
Phenol		1.000	0.220	0.000	0.598	0.001	0.173	0.029	0.458	0.153	2.0	19.7
Flavonoids			1.000	0.197	0.691	0.397	0.902	0.398	0.553	0.249	1.3	12.5
Tannin				1.000	0.809	0.023	0.626	0.194	0.916	0.569	1.0	10.0
Carotenoids					1.000	0.973	0.555	0.437	0.346	0.553	0.8	7.8
Ascorbic acid						1.000	0.110	0.000	0.183	0.310	0.6	5.9
Mn							1.000	0.039	0.001	0.000	0.4	4.0
Ca								1.000	0.035	0.081	0.3	3.3
Cu									1.000	0.013	0.3	2.7
Mg										1.000	0.1	0.7

Table 4: Correlation between antioxidant and phytonutrients *M. citrifolia* L

Conclusion

The present study is an attempt to investigate the genetic influence on phytochemistry of *Morinda citrifolia* using 33 collections from Andaman Islands. The significant ($p < 0.05$) variations in dietary micronutrients, potential antioxidants and antioxidant activity suggest that phytochemical composition affected by the genotypes and also identified potential genotypes for further use in breeding or food schemes. The identified genotypes could help in further breeding programme, study of biochemical pathways in *Morinda citrifolia* or higher recovery of phyto-nutrient in its herbal products.

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