ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 10, Issue, 06(F), pp. 33107-33113, June, 2019

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT, VITAMIN C AND ANTIBACTERIAL PROPERTIES OF *Morinda citrifolia* – USED TRADITIONALLY IN AYURVEDIC TREATMENTS IN INDIA

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DOI: http://dx.doi.org/10.24327/ijrsr.2019.1006.3607

ARTICLE INFO

Article History:
Received 6th March, 2019
Received in revised form 15th April, 2019
Accepted 12th May, 2019
Published online 28th June, 2019

Key Words:

Morinda citrifoila, Phytochemical analysis, Vitamin C, antioxidant and antibacterial activities.

ABSTRACT

Morinda citrifolia, commonly referred as 'Noni' belongs to Rubiaceae and is known for its high medicinal values. The present work aimed to determine the phytochemical studies, the total phenolics, flavonoid, tannin and Vitamin C estimation and to assess the antioxidant properties and antibacterial activities from various extracts of flower, matured and ripened fruits of *M. citrifolia*. Among all the extracts methanol extracts of ripened fruit noticed phytochemicals such as carbohydrates, proteins, alkaloids, steroids, terpenoids, saponins total phenols, flavonoids and tannins. Highest content of ascorbic acid (Vitamin C) was observed in the ripened fruit extract. All the extracts showed moderate to potent antioxidant activities, among which methanol extract of ripened fruit exhibited 78% and IC_{50} with 0.204 mg/mL. Thus, revealed to be the strongest antioxidant activity almost nearer to standard ascorbic acid and thus could be a potential rich source of natural antioxidant. In antimicrobial screening, methanol extracts of ripened fruit showed the highest mean zone of inhibition ranging from 9.0 to 13 mm against tested microorganisms. All these experimental finding suggests that the ripened fruit can be the best source to develop pharmaceutical products for the benefit of human health.

INTRODUCTION

Morinda citrifolia has been known to mankind since great antiquity due to its broad range of medicinal value in roots, leaves, fruits, bark, flower and seeds which has been exploited in traditional medicine across India and Pacific Islands. In *M. citrifolia* a number of phytochemical constituents have been reported. According to Inada *et al*., 2017, phytochemicals such as saponins, phenols, flavonoids, steroids and alkaloids have showed anti-inflammatory effects. Saponins possess hypocholesterolemic and antidiabetic properties (Nguyen *et al*., 2013). The terpenoids have also been shown to decrease blood sugar level in animal studies. The steroids and saponins are responsible for central nervous system and also used in the treatments of boils and carbuncles, fungal infections, poor digestion, atherosclerosis, blood vessel problems, drug addiction, relief of pain, constipation as well as diarrhoea (Heinicke, *et al*., 1985; Dixon *et al*., 1999; Nerurkar *et al*., 2015). *Morinda citrifolia* L. belongs to the family Rubiaceace. The tropical climate zone is the home for about 80 different species of Morinda (Mortan, 1992) *M. citrifolia* is an evergreen tree with 3 to 6 m height. The flowers are white, fruits are usually ovoid in shape and outer surface showing polygonal shape commonly called as "Indian mulberry" 'Noni' or 'Nonu'. The immature fruit is hard and green in color. Ripening fruits are very soft and looks translucent yellow or white in color while the ripened fruit has unpleasant butyric and cheesy odour and soapy taste. In Australia and New Zealand fruits are used as staple foods. However, consumption is limited in India because of its unpleasant taste and flavour of ripened fruit, but the products derived from fruit noni have gained considerable popularity and is traded worldwide as food supplements (Potterat, and Hamburger, 2007).

Many researchers reported phytochemical studies on *M. citrifolia* on secondary metabolites in leaves, roots and bark. Several classes of metabolites have been reported including polysaccharides, fatty acid, glycosides, and a range of volatile constituents including monoterpernes and short chain fatty acids and esters (Sun *et al*., 2016). Therefore, it is essential to investigate traditional medicine for safe and effective remedies for many diseases.

Antioxidants are the common compounds present in low concentrations which can prevent biomolecules from

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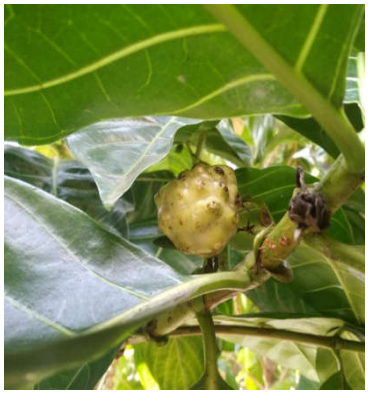
undergoing oxidative damage through free radical mediated reactions (Rajasekhar and Venkata Raju, 2016). The fruits rich in vitamin c exhibit high content of antioxidant. These fruits and its products are considered to be active against human pathogenic microorganisms. The present work aimed to determine the phytochemical studies, the total phenolics, flavonoid, tannin and Vitamin C estimation and to assess the antioxidant properties and antibacterial activities from various extracts of flower, matured and ripened fruits of *M. citrifolia*.

MATERIALS AND METHODS

Collection of flowers and fruits: Flowers and fruits were collected from Bhudevi farm, Mysore, Karnataka India. The plant was identified from the Taxonomist experts from Maharani's Science College for women, Mysore. After identifying plant leaves and fruits were maintained and was confirmed by comparing with authentic specimen maintained in Department of Botany, University of Mysore, Mysore.

A



B

C

Fig 1 Plant of *M. citrifolia* showing (A) flowers (B) Mature fruit (c) Matured and Ripened fruit

Phytochemical studies: Flower, matured fruit and ripened fruit were dried thoroughly in shade, powdered (100g) and successively extracted with ethanol, acetone, petroleum ether, methanol, chloroform and distilled water using Soxhlet apparatus. The extracts were reduced to dryness and subjected to phytochemical studies (Samoylenko, *et al*., 2006).

Preliminary Phytochemical Investigations

The presence of both primary metabolites and secondary metabolites such as proteins, carbohydrates, alkaloids, saponins, phenols, tannins, flavonoids, steroids, and terpenoids were assessed according to the standard procedure (Harborne *et al.,*1998).

Total Phenolic Content (TPC)

Total phenolic content was determined by using standard Folin–Ciocalteu (FC). A 100 µL of extract was mixed with 250 µL of Folin- Ciocalteu's reagent and kept for 5 min at 25 ^{0}C. This was followed by the addition of 750 µL of 15% Na_2CO_3 and 3.4 mL of water to the reaction mixture and kept for 90 min at room temperature and the absorbance was measured at 765 nm. The same procedure was repeated for standard Gallic acid solutions and total phenolic content was calculated using a calibration curve of gallic acid equivalent (GAE) (1–10 mg/mL, y = 0.0151x - 0.2675, R2 = 0.9965, y is the absorbance of sample, x is the solution concentration) according to the standard method (Singleton, *et al*., 1999). The results are expressed as 'milligram' of gallic acid equivalents GAE/g of extract.

Total Flavonoid Content (TFC)

Total flavonoid content was determined by colorimetric method. 500 µL of extract, 100 µL of aluminium chloride (10%), 100 µL of potassium acetate (1 M) and 2.8 mL of distilled water were mixed thoroughly for 5 min by vortexing. The reaction mixture was kept at room temperature for 30 min and the absorbance was measured at 415 nm against blank. The same procedure was repeated for standard rutin solutions and total flavonoid content was calculated using a calibration curve of Rutin Equivalent (RE) (1–10 mg/mL, y = 0.0053x- 0.3452, R2 = 0.9916, y is the absorbance of sample, x is the solution concentration). The results were expressed as mg of rutin equivalents RE/g of extract (Woisky and Salatino, 1998).

Total Condensed Tannins (TCT)

Condensed tannins were determined according to the method. To 50µL of diluted sample, 3 mL vanillin (4%) solution and 1.5 mL of Concentrated HCl was added. The reaction mixture was allowed to stand for 15 min at room temperature and absorption was measured at 500 nm. Same procedure was followed for standard catechin solutions using a calibration curve of catechin equivalent, the total tannin content was calculated (CE) (1–10 mg/mL, y = 0.0199x - 0.1873, R2 = 0.9979, y is the absorbance of sample, x is the solution concentration). The results were expressed as mg of catechin equivalents CE/g of extract (Sun, *et al*., 1998).

Estimation of Vitamin C

This method of using metaphosphoric acid-acetic acid extraction solution has been reported to efficiently extract 99% of ascorbic acid from fruit samples (Hernandez *et al*., 2006). The principle behind this method is the reduction power of oxidation-reduction indicator dye, 2,6-dichlorophenol to a colourless solution where the end point pink colour is reached when there is absence of ascorbic acid for reaction and hence the dye shows up. Ascorbic acid standards at various concentrations prepared in the same meta-phosphoric acid-acetic acid extraction solution was also analysed to construct the standard curve as well as to verify the results by sample spiking method.

One gram of matured and ripened fruit pulp and flower sample (in triplicate) from *M. citrifolia* was crushed using mortar and pestle in an aliquot of extraction solution and the sample was completely recovered from mortar-pestle to make the final volume 10 ml taken in capped-centrifuge tubes. All such samples were centrifuged at 10000 rpm for 10 min and the supernatant was used for estimation. Titration was repeated thrice for each sample as well as spiked samples (with known aliquot of ascorbic acid standard solution) and quantified as suggested in the procedure. Ascorbic acid content was calculated (AA) (1–10 mg/mL, y = 0.0884x + 0.0419, R2 = 0.9989, y is the absorbance of sample, x is the solution concentration).

Determination of Antioxidant Activities

Determination of free-Radical Scavenging Activity (DPPH Assay)*:* While there are several methods to determine radical scavenging efficacy, the present study chose to analyze this by using the vastly accepted DPPH radical scavenging by photometric method, which is simple, rapid and reasonably accurate (Brand-Williams *et al*., 1995). The precision of this method has also been verified and confirmed through a collaborative study by a group of laboratories involving many countries (Plank *et al*., 2012).

The reagent used in the assay 2, 2-diphenyl-1- picrylhydrazyl solution (Sigma Aldrich GmbdH, Germany) was mixed with different solvent extracts at various concentrations (50µg/mL). The reaction solution was covered with aluminium foil to protect from light and incubated at 37 ^{0}C, allowing the reaction mixture with stirring for 30 min in a dark place and absorbance was recorded at 517 nm. In every experiment, methanol was considered as blank and pure L-ascorbic acid (AA) was used as a comparative standard. The DPPH activity of plant extracts was expressed as IC_{50}, the concentration of extract (µg/mL) required to scavenge 50% of DPPH radicals. IC_{50} values were estimated by a linear regression analysis. This IC_{50} values were used for remaining antioxidant assays for access the percentage of inhibition. Scavenged DPPH radical was calculated by the following equation

$$\frac{Ac - As \times 100}{Ac}$$

Where, Ac = absorbance of the blank sample, and As = absorbance of the *M. Cirtifolia* samples.

Nitric Oxide Radical Scavenging Assay

Greiss reaction was used to measure the nitric oxide which was generated from sodium nitroprusside. This assay was done according to the method described by Noguchi, *et al*., 1997. 320 µL of extract, 360 µL of sodium nitroprusside, 216 µL of Greiss reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% napthyl ethylene diamine dihydro chloride) were mixed and incubated at 25 ^{0}C for 1 h. Finally, 2 mL of distilled water was added, and absorbance was measured at 546 nm. Nitric oxide radical scavenging activities were measured using the formula.

$$\frac{Ac - As \times 100}{Ac}$$

Where, Ac = absorbance of the blank sample, and As = absorbance of the *M. cirtifolia* samples.

Antibacterial Activities

Microorganisms

Antibacterial potency of each extract was evaluated using four bacterial strains which includes two strains of gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (E. coli and P. aeruginosa) bacteria which causes food poisoning. The bacterial strains were provided from Department of Studies in Microbiology, University of Mysore, Mysore, Karnataka, India.

Preliminary Experiments

Preliminary experiments were carried out using ethanol, methanol, Petroleum ether, acetone, chloroform and distilled water at 100µL concentration against all the tested bacterial strains for flower, M. fruit and R. fruit samples of *M. citrifolia* were carried out by Agar well diffusion method. In all the tested extracts methanol extracts showed the highest zone of inhibition against four bacterial strains. Based on these experiments, methanol extractions with different concentrations (50, 100 and 200 µL) of flower, M. fruit and R. Fruit samples were carried out against four bacterial strains and chloramphenicol was used as the positive reference for all bacterial strains using agar well diffusion method (Yildrim, *et al*., 2001). After incubation the zone of inhibition was measured.

Statistical Analysis

Statistical analysis was done using Microsoft Excel and SPSS 15.0 version. One-way ANOVA a post hoc test were conducted and P value less than 0.05 was considered as significantly different

RESULTS AND DISCUSSION

Phytochemical Screening

Results obtained for qualitative screening of phytochemicals in flowers, matured fruit and ripened fruit of *M. citrifolia* is presented in Table 1. Of the eight phytochemicals screened, six were found to be present in various solvent extracts. They are proteins, carbohydrates, alkaloids, saponins, steroids, and terpenoid compounds are present in matured fruit and ripened fruit. In all, more phytochemicals were found in extracts prepared with distilled water and Methanol. Among the extracts tested, maximum result was observed in methanol extract and lesser in Pet. ether extract. According to (Tiwari *et al*.,) the fluctuation of biochemical content in the different solvent extracts is based on a number of intrinsic and extrinsic factors and specific metabolic activities as well as endogenous

physiological changes in the plants. The chemical composition of *M. citrifolia indicates* that noni is a promising source of pharmaceutically. Phytochemicals are essential in order to enhance potential and valuable biological activities of human health benefits. Among plant secondary metabolites, phenolic compounds were shown to exhibit important antioxidant, anti-inflammatory, antihyperglycemic, immunomodulatory and anticancer activity which is also confirmed by (Heinicke, 1985; Chan-Blanco *et al*., 2006; Motshakeri, and Ghazali, 2015) respectively.

Table 1 Phytochemical analysis of *M. citrifolia*

Phytochemicals	Plant part	Extracts					
		Ethanol	Acetone	Pet. Ether	Methanol	Chloroform	D. water
Carbohydrates	Flower	-	-	-	+	-	-
	M. Fruit	-	-	-	+	+	+
	R. Fruit	+	+	+	+	+	+
Proteins	Flower	-	-	-	+	-	-
	M. Fruit	+	-	-	+	+	-
	R. Fruit	+	+	-	+	+	+
Alkaloids	Flower	-	-	-	+	-	-
	M. Fruit	-	-	-	+	-	+
	R. Fruit	+	-	-	+	+	+
Steroids	Flower	-	-	-	+	-	-
	M. Fruit	-	-	-	+	-	+
	R. Fruit	-	-	-	+	+	+
Sapoins	Flower	-	-	+	+	-	-
	M. Fruit	-	-	-	+	-	-
	R. Fruit	-	+	-	+	+	+
Terpenoids	Flower	-	-	-	-	+	-
	M. Fruit	-	-	+	-	-	+
	R. Fruit	+	+	-	+	+	+

Total Phenolics, Flavonoids and Tannin Contents

The results showed that extracts of flower samples have lower levels of phenolic substances compared to matured and ripened fruits as shown in (Table 2). The concentration of phenol was higher in methanolic extracts of ripened fruit showed the highest phenolic content (32.25±1.8 mg GAE/g FW) when compared to matured fruit extracts and flower extracts which showed very low phenol content (29.89±1.8 and 10.12±0.4 GAE/g FW**)** respectively. (Yang *et al*., 2011) reported that extracts of fruits yielded higher values of phenolic compounds compared to stem and leaves in different solvents. The flavonoid content was higher in the methanolic ripened fruit extracts (101.13 ± 2.5 mg RE/g FW) followed by chloroform (98.97± 2.1 mg RE/g FW) and lowest in (58.50±1.5 mg RE/g FW) in pet. ether extracts. Whereas matured fruit showed highest in methanol extract with 89.49± 1.2 mg RE/g FW followed by 76.87± 1.0mg RE/g FW in chloroform extract and lowest in pet. ether with 39.08± 1.5 mg RE/g FW. In different extracts of flowers, highest flavonoid was recorded in chloroform extracts with 29.02± 2.5 mg RE/g FW and lowest in ethanol extract 14.23±1.6 mg RE/g FW as shown in table 2. This shows that flavonoids are more in ripened fruit when compare to matured and flower. These reports are in accordance with the results obtained by other researchers (Hsu, and Yen, 2007; Ramesh*, et al*., 2012)

Apart from phenolics and flavonoids, tannins are also widely distributed and very important plant phytochemical. Tannins show an effective antibacterial (Scalbert, 1991) and high antioxidant activity (Hagerman, *et al*., 1998). Apart from these effects, tannins also have anti-nutritional effect (Serrano, *et al*., 2009). In *M. citrifolia*, the tannin content was higher in methanolic extracts of ripened fruit showing (28.32 ± 1.3 CE/g FW) followed by methanol extract (26.52± 1.3 CE/g FW). Whereas, matured fruit showed highest tannin content of (15.89± 1.3 CE/g FW) in methanolic extract followed by (14.25± 1.3 CE/g FW) in chloroform extract and very less content of tannin is recorded in chloroform extract of flower showed (0.8± 1.3 CE/g FW) as shown in table 2. Although tannins in plants function as the electron supplier for the antioxidative enzymes, they act as a backup defence mechanism of plants (Sakihama, *et al*., 2002). Similarly, the difference of phenol and flavonoid contents within the plant parts was reported in the family Rubiaceace (Motshakeri and Ghazali, 2015). The present report has shown the total phenolic, flavonoid and condensed tannin contents were higher in ripened fruit.

Table 2 Total phenolics, flavonoid and tannin contents in flower, M. fruit and R. fruits extracts of *M. citrifolia*

Sl. No.	Plant part used	Solvents	Total phenolic content (mg GAE/g FW)[a]	Total flavonoids (mg RE/g Fw)[b]	Total tannins content (mg CE/g FW)[c]
1	Flower	Ethanol	7.51±1.3i	14.23±1.6	2.1±2.1
2		Acetone	9.30±1.2	21.42±1.5	3.3±0.8
3		Pet. Ether	9.34±0.5	25.33±1.0	5.23±0.4
4		Methanol	9.02±0.6	26.41±1.2	4.56±0.6
5		Chloroform	10.12±0.4	29.02±1.3	0.8±0.7
6		D. water	09.45±1.0	17.68±1.1	3.28±1.0
1	M. Fruit	Ethanol	18.10±1.1	58.25±1.2	08.2±1.0
2		Acetone	22.32±1.4	49.45±1.8	10.23±1.1
3		Pet. Ether	20.00±1.5	39.08±1.5	11.25±1.2
4		Methanol	25.25±1.6	89.49±2.5	15.89±1.3
5		Chloroform	21.25±1.4	76.87±1.0	14.25±1.3
6		D. water	29.89±1.8	48.06±1.8	10.25±1.1
1	R. Fruit	Ethanol	23.71±1.1	85.33±1.2	15.07±1.0
2		Acetone	26.45±1.2	68.55±1.8	19.54±1.1
3		Pet. Ether	22.14±1.0	58.50±1.5	17.36±1.2
4		Methanol	32.25±1.8	101.13±2.5	28.32±1.3
5		Chloroform	25.25±1.2	98.97±2.1	26.52±1.3
6		D. water	29.19±2.0	92.25±2.1	17.52±1.1

Bold value indicates the highest amount of phenolics, flavonoids and tannin contents in each samples and extracts

1. Measurements are mean ± SE of three replicates, and expressed as tannic acid equivalent per gram fresh weight
2. Measurements are mean ± SE of three replicates, and expressed as rutin equivalent per gram fresh weight
3. Measurements are mean ± SE of three replicates, and expressed as catechin equivalent per gram fresh weight

Estimation of vitamin C

It is clear from the results obtained for different concentrations of AA by the dye titration method were linear with a regression value of 0.996. This data is almost similar to that reported on the basis of spectrophotometric method (Kapur *et al* 2012) indicating the method is acceptable and the values are in the comparable range as in other studies. This is further supported by the AA concentration obtained for vastly reported ripened fruit and unripened fruit of *M. citrifolia*, showed, noni ripened fruits were richer in vitamin C (76.24 mg/100 g) than the matured fruit sample (53.19 mg/100 g) followed by 65 mg/100 g in methanol extract, and lesser in ethanol extract with 44 mg/100 g. Matured fruit showed 54 mg/100 g in methanol extract followed by chloroform extract 55 mg and lesser in ethanol extract with 38 mg/100 g of vitamin C. Flower showed highest vitamin C content in methanol extract with 28 mg/100 g followed by acetone extract 27 mg/100 g and lesser in Pet.

ether extract with 22 mg/100 g as shown in figure 2. Ruhomally, *et al*., 2015 reported that noni ripe fruits were richer in vitamin C than the unripe sample. They demonstrated that vitamin C is the most prominent vitamin in ripened noni fruit with 100 g provides 251% of the vitamin C requirement for adults. With all these experiments we noted that there was an increase in ascorbic acid as the fruits ripened.

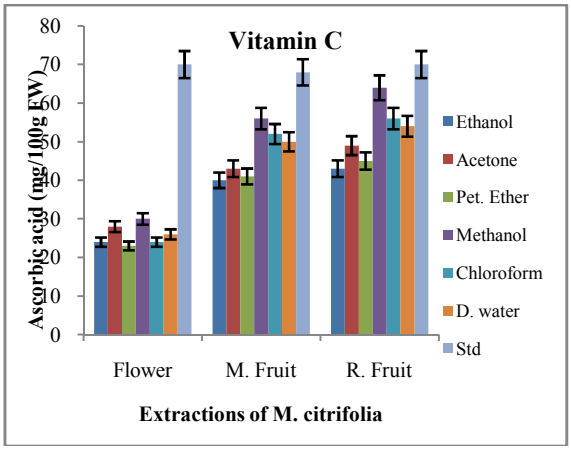


Figure 2 Graph of concentration of Vitamin C in flower, matured fruit and ripened fruit (concentrations mg/100g FW) with different extracts. Data presented as an average of 3 replicates

DPPH Radical Scavenging Activity and IC_{50}

DDPH radical scavenging is specifically used to determine chain breaking activity in the proliferation phase of protein and lipid oxidation. Antioxidant effects on DPPH scavenging was may be due to their hydrogen donation capacity (Mao, *et al*., 2006). All the extracts showed dose-dependent increase in DPPH scavenging activities. Ripened fruit extracts had showed a higher activity of radical scavenging. In particular all the solvent extracts of ripened fruit had 78% activity which is almost equal to standard ascorbic acid (79%). Flower extracts showed very lesser activities when compared to fruits. Methanol extracts of flower showed highest DPPH activities of 40% followed by 38% in chloroform extract and least in water extract 31%. Matured fruit showed highest in chloroform with 62% and lowest was observed in water extract. Table 3 shows antioxidant activity with IC_{50} values of flowers, matured fruit and ripened fruit measured by DPPH radical-scavenging assays. Overall, methanol ripened fruit extract is showing the best antioxidant properties when compare to all extracts (significantly lower IC_{50} values = 0.204 ± 0.31 mg/mL) and the matured fruit methanol extracts possess moderate radical scavenging activity (0.425 ± 0.33 mg/mL). Methanol flower extracts revealed a poor antioxidant activity (significantly higher IC_{50} values = 0.956 ± 0.45 mg/mL). This data proves that the ripened fruit methanol extract exhibits a strong scavenging activity than all the extracts of matured fruit and flower parts used. Similar reports showed the presence of solvent extracts and the compounds present will increase the scavenging activity may be due to the presence of phenols, vitamin and flavonoids and ripened fruit extracts of *M. citrifolia* possessed the strong effects on reducing DPPH radical scavenging 65% comparing with standards (Kumar *et al*., 2014).

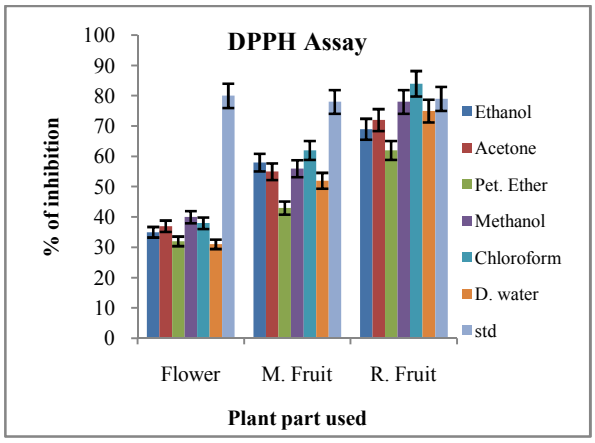


Figure 3 Antioxidant activity measured by DPPH radical- scavenging activity of flower, M. fruit and ripened fruit of *M. citrifolia*

Table 3 The antioxidant activity with IC_{50} values of flower M. fruit and R, fruit measured by DPPH radical-scavenging assays

Solvents	Plant part used		
	Flower (µg/ml)	M. Fruit (µg/ml)	R. Fruit (µg/ml)
Methanol	0.956±0.45	0.425±0.33	0.204±0.31
Ethanol	2.038±0.30	0.874±0.45	0.356±0.33
Pet. Ether	1.259±0.45	0.945±0.45	0.658±0.45
Acetone	2.780±0.75	1.235±1.10	0.296±0.31
Chloroform	4.470±1.20	0.865±0.45	0.250±0.31
D. Water	3.564±1.20	1.568±1.21	0.548±0.45

Measurements are mean ± SE of triplicate determinations, IC_{50} concentration required for inhibit the radical formation by 50%.

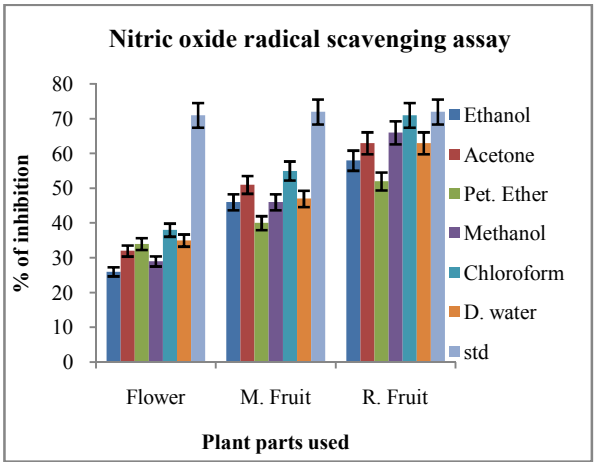


Figure 4 Antioxidant activity measured by nitric oxide radical- scavenging activity of flower, M. fruit and ripened fruit of *M. citrifolia*

Nitric Oxide Radical Scavenging Assay

Nitrogen radicals react with other reactive species to produce more toxic reactive nitrogen species (RNS) and reactive oxygen species (ROS) under oxidative stress (Dastmalchi, *et al*., 2008). In this study, chloroform extracts of ripened fruit (71mg/mL) scavenged hydroxyl radicals showed higher which is similar to standard ascorbic acid (73%) while matured fruit of chloroform extracts showed moderate scavenging activity of 55mg/mL and chloroform extract of flower showed 38mg/mL of scavenging activity as shown in figure 4. This result expressed that the different solvent extracts of ripened fruit of *M. citrifolia* had high scavenging activity than compare to flower and matured fruit. Antioxidants present in noni fruit increase from the green to ripened stage (Millonig, *et al*.,

2005). Similar reports on higher phenol content and higher vitamin C content, antioxidants in noni fruit was also reported by (Yan *et al*., 2011; Motshakeri *et al*., 2015)

Antibacterial activity of M. citrifolia

Methanol extracts showed highest zone of inhibition against all the microorganisms (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis*) used. Based on all the results, the ripened fruit showed the highest zone of inhibition compared flower and matured fruit against all the tested microorganisms. Ripened fruit showed 13mm against *S. aureus*, 10mm against *B. subtilis*, 8mm against *E. coli* and 10mm against *P. aeruginosa*. Matured fruit also showed zone of inhibition against all the pathogenic bacteria but lesser activity than ripened fruit but showed more activity than flower. In the other hand methanol (control) did not exhibit any effect on the tested microorganisms. In all the test samples antibiotic (chloromphenical) showed highest zone of inhibition of 24 mm as shown in table 4.

Table 4 Antibacterial activity (zone of inhibition) of flower, matured fruit and ripened fruit) of *M. citrifolia* methanol extract

Microorganisms	Concentration of solvent	Flower	M. Fruit	R. Fruit
Staphylococcus aureus	50 µL	5±0.5	7±0.7	10±0.7
	100 µL	5±0.5	8±0.6	12±0.8
	200 µL	5±0.5	8±0.6	13±0.8
	Std	24±0.6	24±0.6	24±0.6
	control	0.0±0.0	0.0±0.0	0.0±0.0
Bacillus subtilis	50 µL	2±0.4	5±0.5	9±0.5
	100 µL	1±0.3	6±0.5	10±0.7
	200 µL	1±0.3	7±0.5	10±0.7
	Std	24±0.6	24±0.6	24±0.6
	control	0.0±0.0	0.0±0.0	0.0±0.0
Escherichia coli	50 µL	1±0.4	5±0.5	8±0.6
	100 µL	1±0.4	6±0.5	9±0.7
	200 µL	1±0.5	5±0.5	9±0.6
	Std	24±0.6	24±0.6	24±0.6
	control	0±0.0	0.0±0.0	0.0±0.0
Pseudomonas aeruginosa	50 µL	2±0.5	6±0.5	8±0.6
	100 µL	2±0.5	6±0.5	10±0.7
	200 µL	2±0.5	6±0.5	10±0.7
	Std	24±0.6	24±0.6	24±0.6
	control	0.0±0.0	0.0±0.0	0.0±0.0

From these results methanol extracts were found to be the most effective, with a broad antimicrobial spectrum against both Gram-positive bacteria *Staphylococcus* and Gram-negative bacteria *Pseudomonas* as shown in figure 5. These finding are found to be more effective, because these bacteria are resistant to a number of antibiotics and produce toxins causing septicaemia in human health which may lead to death finally. Phenols, flavonoids, tannins and antioxidants have previously been reported to have a wide spectrum of biological activity, including anti-thrombotic, cardioprotective, vasodilator and antimicrobial activities (Cushnie and Lamb, 2005) *M. citrifolia* contains alkaloids, phenolic compounds, terpenoids and anthraquinones which possess good antibacterial properties against different microorganisms (Zhang, *et al*., 2016). Rivera *et al*., 2011 reported the cold and hot aqueous extracts of the *M. citrifolia* fruit does not exhibit any zone of inhibition against *S. aureus*, *E. coli*, *B. cereus*, *P. aeruginosa* and *H. Pylori* through well diffusion assay. The study showed that helps in stomach ulcer through inhibition of the bacteria *H. pylori*. (Kakad, *et al*., 2015) also reported that *M. citrifolia* antibacterial activity against certain infection bacterial strains such as *P.aeroginosa, P. morgaii, S. aureus , B. subtilis, E. coli, Salmonella* and *Shigella* stated that the observed antibacterial activity may be due to the presence of phenolic compounds, such as acubin, L-asperuloside and alizarin in the fruits. Our findings are closely related to all the published data except using flowers and comparison among matured fruit and ripened fruit.

Fig 5 Antimicrobial activity of ripened and mature fruit and flower methanol extract against various pathogenic bacterial strains.

CONCLUSION

The present work revealed that ripened fruit of *Morinda citrifolia* has rich and diversified phytochemical compounds like alkaloids, saponins, flavonoids, phenols, vitamins tannins and other compounds than matured ones. It also indicates the potential antioxidant activity and free radical scavenging properties of *M. citrifolia* fruit extract. These antimicrobial agents with its significant inhibition activity against various clinical isolates suggest to conduct further studies for isolation and characterization of active principles. Thus, *M. citrifolia* fruits can be encouraged for consumption as fresh produce or processed functional products from ripened fruit for management of health in view of their therapeutic benefits in the management of health and disease.

Acknowledgement

The authors would like to thank Dr. Bhagyalakshmi Neelwarne, Director, Neelgen GreenTech, Mysore, for providing flowers and fruits of *M. citrifolia*

Conflict of interest

No conflict of interest.

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