



Research Article

Screening of Different Extraction Methods for Quantitative and Qualitative Analysis of Antioxidants and Assessment of Free Radical Scavenging Activity in *Morinda citrifolia* L.

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ABSTRACT

The interest of scientists and medical professionals in herbal medicinal products containing phytochemicals derived from plants is growing daily due to the shift of consumers' preferences from convenience to environmental sustainability. One plant utilized in many medicines is *Morinda citrifolia* L., a tree commonly used in folk medicine, health supplements and immune-boosting products. In this study, Bioactive compounds from *M. citrifolia* were extracted using conventional methods and modern methods (Ultrasound-Assisted, Microwave Assisted, Water bath Assisted, Shaker Assisted, Soxhlet Assisted and Magnetic Stirrer Assisted Extraction). This study screens a variety of extraction methods used to extract active compounds: total phenolic content and antioxidant properties through DPPH, ABTS and FRAP from different parts of *M. citrifolia*. The results indicate that the total phenolic content and FRAP activity were highest in all parts of the plant in Soxhlet Assisted extraction. In contrast, Microwave Assisted extraction showed high DPPH and ABTS activity. This study offered valuable details on the effective phytochemical extraction processes from various parts of *Morinda citrifolia* L.

Keywords: Extraction methods, Antioxidant activity, *Morinda citrifolia*, Phenolic content.

INTRODUCTION

M*orinda cirtifolia* L. (noni) plant belongs to the Rubiaceae family. It is a small evergreen tree growing in open coastal regions and forest areas up to 1300 feet above sea level. From ancient times, this plant has been used by Polynesians for over 2000 years to cure breast cancer and eye problems in broad terms¹. It has many therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, antihelminthic, analgesic, anti-inflammatory, hypotensive and immune-enhancing effects. Its dried fruits have smooth muscles, stimulatory activity, and histaminergic effects². The roots of this plant are reported to be a good source of anthraquinones, which also have antioxidant activities and therapeutic properties³.

Medicals and scientists' professions have shown increased interest in these fields as they recognize the actual health benefits of these remedies. In 2002, *Morinda* juice was accepted as a novel food and in the topics, plants were cultivated for its roots, leaves and fruits due to their medical values⁴. Various parts of the Noni plant have reportedly contained more than 160 phytoconstituents, of which over 120 have verified biological activity and nutraceutical benefits⁵. Micronutrients, non-volatile and volatile substances, ketones, lactones, beta-carotenoids, terpenoids, and proxeronine are all present in fermented fruit extract⁶. According to certain studies, the mature Noni fruit's ethanolic extract has the most antibacterial effects against *Klebsiella pneumonia*. In contrast, the ripe Noni fruit's methanolic extract has the most potent antifungal effects against *Aspergillus flavus*⁷.

Natural antioxidants, mainly phenolics, have higher antioxidant activities than other compounds, such as vitamins C, E and beta-carotene⁸. Therefore, various extraction methods have been used to extract the maximum amount of these bioactive compounds. So, the primary step is the extraction of these bioactive compounds to utilize as antioxidant, anticancer, antimicrobial, and analgesic from *M. cirtifolia* L. by various methods and then identifying them. However, there is no doubt that the different extraction methods are based on other principles, which influence the efficiency of extracted bioactive compounds from medicinal plants. Some methods lead to modifying these bioactive compounds' qualitative and quantitative properties. Hence, the present study evaluated or screened different extraction methods for bioactive compounds, particularly phenolics, antioxidants, and free radical scavenging compounds of *M. cirtifolia* L.

MATERIALS AND METHODS

The root, stem and leaves of *M. citrifolia* L. were selected for the present study, collected in the winter season (January) from the roadside near Victoria Park in Meerut (28° 59' N lat. And 77° 40' E long.), U.P. India. The collected plant parts were washed in tap water to remove dust. All plant part samples were dried in a laboratory oven, ground into a fine uniform powder, and stored in air-tight polybags for further phytochemical analysis.

Phytochemical Analysis**Qualitative analysis**

Prepared powdered samples of *M. citrifolia* plant parts were subjected to qualitative chemical screening to



identify bioactive compounds alkaloids, anthraquinone, flavonoids, saponins, sterols, tannins and terpenoids using a prescribed standard method⁹⁻¹¹.

Extraction of Plant Materials: The dried plant samples (Root, Stem, Leaves) were extracted in ethanol using six different methods. i.e., Ultrasound-Assisted Extraction (UAE), Microwave Assisted Extraction (MAE), Water bath Assisted Extraction (WAE), Shaker Assisted Extraction (SAE), Soxhlet Assisted Extraction (Sx.AE), Magnetic Stirrer Assisted Extraction (Mg.AE).

UAE: 100 ml of absolute ethanol was used to extract 5 g of each powdered plant sample. Sonication was performed for 1 hour in an ultrasonic cleaning bath (Citizen). The extracts were filtered and stored at low temperatures¹².

MAE: 100 ml of absolute ethanol was used to extract 5 g of each powdered plant sample. The microwave was performed for 1 hour at 40°C and 150 W; microwave power was taken as optimum. The extracts were filtered and stored at low temperatures¹².

WAE: 100 ml of absolute ethanol was used to extract 5 g of each powdered plant sample for 1 hour at 55.6°C. The extracts were filtered and stored at low temperatures¹².

SAE.: 100 ml of absolute ethanol was used to extract 5 g of each powdered plant sample for 48 hours at 120 revolutions per minute (rpm). The extracts were filtered and stored at low temperatures¹³.

Sx.AE.: 5 g of each powdered plant sample was extracted with 100 ml of absolute ethanol for 12 hours, 7 hours, and 8 hours. In the case of root, stem and leaves, respectively. The extracts were filtered and stored at low temperatures¹³.

Mg.AE.: Magnetic stirrer Assisted Extraction. 5 g of each powdered plant sample was extracted with 100 ml of absolute ethanol for 8 hours at 120 rpm¹³.

Quantitative Analysis

To estimate the entire phenolic content in different extracts by Bray & Thorpe¹⁴. Folin-Ciocalteu colourimetric method was used. The result was calculated as mg/g, i.e., Gallic acid equivalents (GAE) mg/g dry wt., Catechol equivalents (CE) mg/g dry wt.

Estimation of free radicle scavenging activity was measured by three methods (1) DPPH radical scavenging assay¹⁵, (2) Ferric reducing antioxidant power (FRAP) assay¹⁶ and (3) ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)¹⁷.

Statistical Analysis

Effect of various extraction methods on total phenolic content and antioxidant activity of *Morinda cirtifolia* L. obtained data were statistically analyzed using SPSS 20.

RESULTS AND DISCUSSION

A comparison of extraction methods for qualitative and quantitative analysis of phenolics and antioxidants in *Morinda cirtifolia* L. brought out salient findings. The varieties of phytochemical components are present in plant parts that are beneficial in medical sciences. They are commonly associated with various pharmacological activities of natural products of *M. citrifolia* exhibited several secondary metabolites, including alkaloids, flavonoids, tannins, and saponins, which are known to be responsible for the antioxidant, antimicrobial, antidiabetic, and anticancer activities of the plant¹⁸.

Table 1: Screening for secondary metabolites in different parts of *Morinda cirtifolia* L.

Secondary Metabolites	Root	Stem	Leaf
Alkaloids	+	+	+
Anthraquinones	-	-	-
Flavonoids	+	+	+
Saponins	+	+	+
Sterols	-	-	-
Tannins	+	+	+
Terpenoids	+	+	+

It was noted that among all the secondary metabolites Tannins, Flavonoids, Saponins, Terpenoids, and Alkaloids were present in all parts of the plant (Table 1), so all parts of plants have been shown to fight against infectious bacterial strains such as *Pseudomonas aeruginosa*, *Proteus moegaii*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella spp.* and *Shigella spp.*; these secondary metabolites are responsible for the treatment of skin infections, cold, fevers and bacterial health problems¹⁹. Sterols and anthraquinone were not present in any part of the plant. Given the biological actions of the polyphenols and alkaloids present in *M. citrifolia*, the secondary metabolites' composition clearly explains how this plant has been used traditionally²⁰. Alkaloids' potential correlation with antibacterial properties is vital. This species is so anticipated to have a wide range of medical applications.

In all plant parts, the leaf was recorded to show higher phenolic content (phenolic content was expressed as gallic acid (Fig. 1a) and catechol (Fig.1b) equivalent (mg/g dry extract). Followed by roots and stem, out of six different extraction methods, Sx.AE method was recorded for more outstanding phenolic content than other examined techniques (Fig. 1), suggesting that extracting phenolic compounds from the plant matrix is crucial in determining the extracts' therapeutic qualities²¹. 100% ethanol extract of *Centella asiatica* and other medicinal plants contained significantly higher polyphenols²². *M. cirtifolia* L. has several polyphenol compounds contributing to its nutritional value²³. One of the most significant concerns that food producers encounter is lipid oxidation.

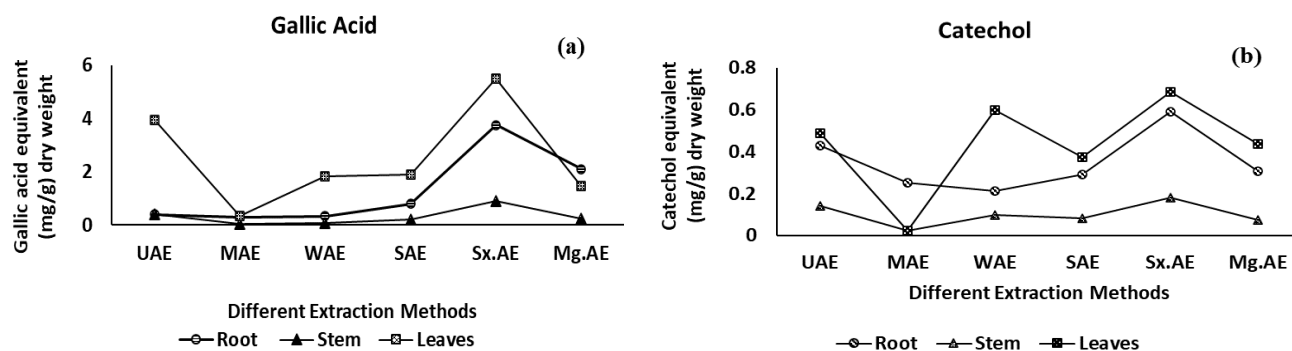


Figure 1: Phenolic content (a) Gallic acid and (b) Catechol in different parts of *M. citrifolia* by different extraction methods

Table 2: Antioxidant Activity (a) DPPH, (b) ABTS and (c) FRAP in different parts of *Morinda citrifolia* L. by different extraction methods. Mean values in the same column differ by means of ANOVA compared to the DMRT test at 0.05 probability level.

S.No.	Extraction Methods	Plant Parts								
		Root (Mean ± S.E.)			Stem (Mean ± S.E.)			Leaves (Mean ± S.E.)		
		DPPH (%)	ABTS (%)	FRAP (µg/ml)	DPPH (%)	ABTS (%)	FRAP (µg/ml)	DPPH (%)	ABTS (%)	FRAP (µg/ml)
1.	UAE	68.13±0.85	18.56±0.94	0.103±0.02	23.91±0.5	57±0.28	0.04±0.01	11.22±0.58	17.95±0.35	0.25±0.01
2.	MAE	90.69±0.51	28.47±0.73	0.053±0.001	77.32±0.51	58.28±0.8	0.012±0.001	62.35±0.39	20.61±0.33	0.114±0.001
3.	WAE	73.91±0.19	25.37±0.29	0.045±0.001	41.94±0.39	56.61±0.5	0.014±0.008	18.02±0.34	15.61±0.08	0.15±0.002
4.	SAE	70.25±0.87	15.89±0.33	0.061±0.004	30.95±0.58	36.14±0.37	0.015±0.001	10.65±0.03	14.8±0.33	0.126±0.004
5.	Sx.AE	62.58±0.34	21.04±0.93	0.151±0.002	39.56±0.5	22.04±0.16	0.053±0.001	28.91±0.58	14.66±0.08	0.262±0.001
6.	Mg.AE	88.65±0.5	16.13±0.28	0.126±0.002	37.86±0.85	32.85±0.37	0.016±0.003	27.77±0.39	17.85±0.2	0.142±0.001

It decreases the food's quality and nutritional value resulting in a loss of profit. Food manufacturers are now using extracts of plants high in phenolic compounds to solve the oxidation issues²⁴.

DPPH radical scavenging activity was recorded maximum in roots compared to the other parts of the plant (Table 2). Therefore, noni roots extract is an inhibitor of Ras function. The Ras oncogene is thought to be connected to signal transduction in many human malignancies, including leukaemia, colon, pancreatic, and pulmonary cancers²⁵. According to Bramorski et al.²⁶, the antioxidant activity of plants is strongly correlated with their phenolic content; therefore, plants containing phenolic compounds have antioxidant activity. Out of six different extraction methods, the MAE method showed maximum DPPH and ABTS activity in all plant parts (Table 2); It is a practical alternative to a standard approach for many samples, as several studies have previously demonstrated. This revolutionary extraction technique that operates at high temperatures and in an oxygen-rich environment, known as microwave-assisted extraction (MAE), has recently acquired popularity since it uses less solvent and takes less time to extract materials²⁷. Microwaves are used in MAE to raise the internal pressure of the solid medium, increasing extraction efficiency and slowing down phenolic compounds' degradation²⁸.

FRAP activity was maximum shown by the leaves of the plant compared to other parts. Therefore, the leaves were

used traditionally in tea and contained health-promoting nutrients, including antioxidants and bioflavonoids; they are also rich in several vitamins and minerals, viz. phosphorus, iron, calcium, magnesium, vitamin E, vitamin K1, niacin and more²⁹. Sx.AE method was noted to show the maximum activity of FRAP in all parts of the plant (Table 2). Comparing 100% methanolic extract to Ultrasonic-Assisted Extraction, FRAP Assay results revealed that Soxhlet-Assisted Extraction had the maximum antioxidant activity³⁰.

CONCLUSION

From the study, plant parts are a great source of phytochemicals that could be utilized in curing various ailments. Alkaloids, Flavonoids, Saponins, Tannins and Terpenoids were the phytoconstituents present abundantly in plants. These results showed that extraction methods varied significantly in their capacity for phytochemical contents and biological activities. The screening of different extraction methods suggests that Sx.AE can provide more phenolic content and FRAP activity in extraction. FRAP assay is sensitive to phenolics richly available in the leaf. MAE method showed maximum activity for DPPH and ABTS scavenging activity. All three assays for free radical scavenging activity were found to be most susceptible to specific parts of *Morinda citrifolia* L., meaning, thereby, the antioxidant compounds are different in the root, stem and leaf portion of the plant.

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