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Original Article

ANTIOXIDANT AND FREE RADICAL SCAVENGING EFFECT OF *MORINDA CITRIFOLIA* FRUIT EXTRACT

VENNILA SRINIVASAHAN[1*] AND BRINDHA DURAIRAJ[2]

[1*]Department of Biochemistry, PSG College Of Arts and Science, Coimbatore, Tamil Nadu, India, [2]Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India.

Email: vennisrini@yahoo.in, brindhavenkatesh@ymail.com

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ABSTRACT

Objective: *Morinda citrifolia* [Noni] has been extensively used in Folk medicine by Polynesians for over 2000 years. It has been reported to have broad therapeutic effects, including anticancer activity, in both medicinal practice and laboratory animal models. The present investigation was undertaken to evaluate the radical scavenging and antioxidant potential of *Morinda citrifolia*.

Methods: Enzymic antioxidants such as superoxide dismutase [SOD], catalase [CAT], glutathione reductase, glutathione peroxidase [GPx], glutathione-S-transferase [GST] were estimated by standard methods. Free radical scavenging potential was evaluated by reducing power assay, ABTS assay, FRAP assay, Phosphomolybdenum assay using 50% ethanolic extract of *Morinda citrifolia* fruit.

Results: The total antioxidant effects of *Morinda citrifolia* fruit extract was comparable with that of the reference antioxidants. Activity of the SOD, CAT, Glutathione reductase, GPx, and GST was observed to be 12.74, 73.08, 12.37, 36.94 and 7.21 respectively. This plant is rich in non-enzymic antioxidants such as ascorbic acid, α-tocopherol, carotenoids, flavanoids, phenols, tannins and carbohydrates. FRAP assay revealed the maximum antioxidant capacity of 0.186±0.0004mg/ml. ABTS radical scavenging activity was 85.893+1.655 mg/ml. Likewise, reducing power was noticed to be 0.199±0.004mg/ml. Phosphomolybdenum assay exhibited the antiradical activity of 241.66 +2 mg/g normalized with ascorbic acid.

Conclusion: The data obtained in the present study suggest that the fruit extract of M*orinda citrifolia* has potent antioxidant activity against the free radicals, prevents oxidative damage to major bio-molecules and affords significant protection against oxidative damage.

Keywords: Antioxidant, Free radical scavengers, *In vitro*, *Morinda citrifolia*, Noni.

INTRODUCTION

Plants synthesize potent biochemicals which constitute the components of phytomedicine since times immemorial [1]. Medicinal plants typically contain several different pharmacologically active compounds that may act individually, additively or in synergy to improve health. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical induced tissue damage [2]. Since ancient times, many herbs have been potentially used as an alternative remedies for treatment of many infections, diseases, and also as food preservatives suggesting the presence of antimicrobial and antioxidant constituents [3]. Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Current research is now focused towards natural antioxidants originated from plants due to safe therapeutics [4]. Natural antioxidants can protect the human body from free radicals by retarding the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods [5]. Many oxidative stress related diseases are as a result of accumulation of free radicals in the body [6]. Free radicals are implicated for more than 80 diseases including Diabetes Mellitus, Arthritis, Cancer, and Aging etc. In the treatment of these diseases, antioxidant therapy has gained an utmost importance [7]. Plants are endowed with free radicals scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity [6]. The many number of medicinal plants are used in the treatment of cellular and metabolic disease. There are some speculations that the generation of free radicals inside the body in some physiological conditions is resulted in the cellular changes and development of cancer etc and this would be neutralized by the antioxidants from different medicinal plants [8]. *Morinda citrifolia* belonging to the family Rubiaceae, commonly known as noni and it is a most popular drug in ayurvedic medicine. It is tropical and subtropical plant grown in the pacific islands and has been used to treat a broad range of diseases for over 2000 diseases [9]. Commercial noni juice and encapsulated noni powder have become popular in Asia, North America and Europe. Various unsubstantiated claims are currently made from noni products such as immune system stimulant, anticancer agent, menstrual cycle regulator and blood cleanser[10]. Various biological compounds such as glycosides, polysaccharides, alkaloids, lignans, fattyacid esters, anthroquinones, scopoletin, morindin, vitamins and minerals have been isolated from noni fruits, roots and leaves[11]. The present study was carried out to evaluate the radical scavenging and antioxidant potential of 50% ethanolic extract of *Morinda citrifolia* fruit extract.

MATERIALS AND METHODS

PLANT MATERIAL

The fruits of *Morinda citrifolia* were collected from the local areas around Chennai and Coimbatore and further authenticated by Botanical Survey of India (BSI) in Tamil Nadu Agriculture University, Coimbatore. A voucher specimen (No:BSI/SRC/5/23/2012-13/Tech 44) has been deposited at the herbarium of the Botany Department. The samples were washed with running tap water and separated before being chopped into pieces. They were oven dried at 45°C for 2 days and ground to powder.

PREPARATION OF EXTRACTS

The powdered material of *Morinda citrifolia* was extracted with four different solvents like petroleum ether, 50% ethanol, water and chloroform in four different Soxhlet extractors exhaustively for 20-24 hours. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50°C). The dried extracts obtained were used in this study.

Chemicals: All chemicals used were of analytical grade.

Estimation Of Phytochemicals And Antioxidants

The extracts of the fruits of *Morinda citrifolia* were analyzed for the presence of various phytoconstituents using standard phytochemical constituents. The presence of secondary metabolites from the fruit of *Morinda citrifolia* was quantitatively estimated by adopting standard protocols. SOD by Kakkar *et al*[12] method, catalase by Sinha method[13], Peroxidase by Reddy et al.,[14] method, Glutathione –s-transferase by Habig et al[15] method, Glutathione reductase by David method[16], Carbohydrates by Anthrone method[17], Total phenols by Singleton and Rossi method[18], Flavonoids by Jia *et al*[19] method, Tannins by Robert method[20], Ascorbic acid by Roe and Keuther method[21], Tocopherol by Rosenberg method[22] and Vitamin A by Bayfield and Cole method [23].

Total Antioxidant Assays

1. Reducing power assay

The reducing power of the extract was evaluated according to Oyaizu method[24]. 1ml of the different concentrations of various extracts of the sample was mixed with 2.5ml potassium ferricyanide and 2.5ml phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 minutes. After the incubation, 2.5ml of TCA (10%) was added to it and centrifuged at 3000rpm for 10 minutes. 2.5ml of the supernatant was taken. 2.5ml water and 0.5ml of ferric chloride (0.1%) were added to it. The absorbance of the colour was measured spectrophotometrically at 700nm. Increase in absorbance of the reaction mixture indicated increased reducing power. The experiment was conducted in triplicates and the reducing power was expressed as equivalents of ascorbic acid (µg)/mg of extract.

2. Abts radical scavenging assay

2,2'-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid] or ABTS radical scavenging activity of the extract was measured by Rice-Evans *et al* [25] method. ABTS radical cation (ABTS+) was produced by reacting ABTS solution (7mm) with 2.45mM ammonium persulphate and the mixture were allowed to stand in dark at room temperature for 12-16 hours before use. Different concentrations of the 50% ethanolic extract and standard (0.5ml) were added to 0.3ml of ABTS solution and 50% ethanol to make 1ml. Ascorbic acid was used as standard. The absorbance was read at 745nm. The experiment was performed in triplicates.

3. Frap assay

The FRAP assay was used to estimate the reducing capacity of fruit extract according to the method of Oyaizu[26]. The FRAP reagent contained 2.5ml of TPTZ in dilute hydrochloric acid, 20ml of ferric chloride and 25ml acetate buffer was prepared freshly and warmed to 35ºC. FRAP reagent 1.5ml and sample solution 50µl at different concentration was incubated at 37ºC for 10mins and absorbance was recorded at 593nm. Ferrous sulphate was used as a standard. FRAP value was expressed as mmoles/100 on dry weight basis using the calibration curve of Fe^{2+}.

4. Phosphomolybdenum assay

The total antioxidant capacity was measured by spectrophotometric method of Prieto et al [27]. The tubes containing extract (at different concentration) and reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate) were incubated at 95°C for 90 min. After the mixture cooled to room temperature, the absorbance of each solution was measured at 695 nm against blank. Methanol (0.3 ml) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Statistical Analysis

Experimental results are expressed as mean ± SD. All measurements were replicated in three times. The IC50 values were calculated from linear regression analysis.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening Of *Morinda Citrifolia*

Medicinal plants have been recognized as a potential source of natural antioxidants throughout the world. Among the various medicinal plants, few endemic species are of particular interest as they are commonly being used for producing raw materials or preparation containing phytochemical with significant antioxidant capacities[28].

Table 1: Phytochemical screening of *Morinda citrifolia* fruits in different solvent extracts

Phytochemical constituents	50% ethanol	Water	Chloroform	Petroleum ether
Carbohydrates	+	+	+	+
Proteins	+	+	+	+
Oils and fats	+	–	–	–
Steroids	+	+	–	–
Thiols	+	–	–	–
Alkaloids	+	+	+	+
Flavonoid	+	+	–	–
Phenols	+	+	+	–
Saponins	+	+	–	–
Glycosides	+	+	–	–
Tannins	+	+	+	+

+ Present – Absent

The preliminary phytochemical analysis of different solvent extracts revealed the presence of carbohydrates, alkaloids, tannins, flavonoids, saponins, glycosides, steroids, proteins and thiols. The results are shown in Table 1. Besides, carbohydrates, alkaloids, proteins and tannins were uniformly detected in all solvent extracts. When compared with different solvent extracts, 50% ethanolic extract was found to contain all the active phytoconstituents screened in *Morinda citrifolia* extract. Our results are in good agreement with that of Gul Rahim *et al* [29] who suggested that the plant of *Teucrium Stocksianum* was found to possess strong antioxidant activity due to the presence of reducing sugar, alkaloids and tannins. Promising antioxidant potential of hydroethanolic extract of *Moringa oleifera* pods was proven due to the presence of phenolic compounds[30].

Quantification of selected antioxidants in *morinda citrifolia* fruits

Individuals from developed countries use traditional medicine, which as compound derived from medicinal plants. Therefore such plants should be investigated to better understand their properties, safety and efficacy. Plants produce a diverse range of bioactive molecules, makes them a rich source of different types of medicinal compound have continued to play a dominant role in the maintenance of human health[31]. Complex antioxidant systems are very important for protecting cellular membranes and organelles

from the damaging effects of active oxygen species. These include both enzymatic and non enzymatic antioxidants[32].

Enzymic antioxidants

The levels of antioxidant enzymes assessed in *Morinda citrifolia* fruits are collectively represented in Table 2. Superoxide dismutase is the first antioxidant enzyme to deal with oxyradicals by accelerating the dismutation of superoxide (O_2^-) to hydrogen peroxide (H_2O_2). Catalase is a peroxisomal heme protein that catalyses the removal of hydrogen peroxide formed during the reaction catalysed by SOD. Thus, SOD and CAT acts mutually supportive antioxidative enzymes which provide protective defense against reactive oxygen species[33].

In the present study, the superoxide dismutase (SOD) activity of *Morinda citrifolia* fruit extract was found to be 12.74 ± 0.27 U/g whereas the catalase (CAT) activity of *Morinda citrifolia* fruit extract was observed to possess 73.08 ± 3.01 U/g.

Peroxidases are referring to heme containing enzymes which are able to oxidize organic and inorganic compounds using hydrogen peroxide as co-substrate. The non-specificity of peroxidase makes the enzyme suitable to the broad range of electron donor substrates[34]. Peroxidase activity for *Morinda citrifolia* fruit extract was found to be 36.94 ± 2.13µmoles of pyrogallol oxidized/min.

The activity of glutathione reductase in *Morinda citrifolia* fruit extract was observed as 12.37 ± 0.33µmoles of NADPH oxidized/ min/g sample. Badrul Alum *et al.,* 2011[35] reported that the methanol extract of *Oxalis corniculata* was found to have higher activity of glutathione reductase. Glutathione S-transferases are multifunctional proteins involved in diverse intracellular events such as primary and secondary metabolisms, stress metabolism, herbicide detoxification and plant protection against ozone damages, heavy metals and xenobiotics[36]. From the result it is revealed that the *Morinda citrifolia* fruit extract found to possess effective GST activity 7.21±0.17 µ moles of CDNB-GSH conjugate/min/g.

Table 2: Enzymatic antioxidants

Enzymatic antioxidants	*U/g
Superoxide dismutase	12.74 ± 0.27
Catalase	73.08 ± 3.01
Peroxidase	36.94 ± 2.13
Glutathione reductase	12.37 ± 0.33
Glutathione –s- transferase	7.21±0.17

*** Mean for three observations.**

Superoxide dismutase- Amount that causes 50% reduction in the extent of NBT oxidation

Catalase - Amount of enzyme required to decrease the optical density by 0.05 units

Peroxidase- µmoles of pyrogallol oxidized/min

Glutathione reductase- µmoles of NADPH oxidized/ min/g sample

Glutathione -S-transferase - µmoles of CDNB-GSH conjugate/min/g sample

Similar trend was noticed by Vijayakumari *et al*., 2012[37] who reported that *E.officinalis* was found to be rich in enzymatic antioxidant such as catalase, peroxidase, superoxide dismutase, glutathione-s-transferase and glutathione reductase.

NON ENZYMIC ANTIOXIDANTS

The levels of non-enzymatic antioxidants analysed in fruits of *Morinda citrifolia* and are presented in Table 3. The fruits of *Morinda citrifolia* were found to be good source of non enzymatic antioxidants. Ascorbic acid level was found to be 23.6±1.0mg/g. Ascorbic acid is a water soluble vitamin and is widely required in the metabolism of living being. Ascorbic acid is used as a medicine and also added in manufacturing food to conserve the product for long time[38].

α-tocopherol content of *Morinda citrifolia* fruit was found to be 83.5±0.5mg/g. Vitamin E, a major lipid soluble antioxidant belonging to tocopherols, is the most effective chain breaking antioxidant with in cell membrane. It is able to repair oxidizing radicals directly by preventing the chain propagation step during lipid peroxidation[39].

The vitamin A content in *Morinda citrifolia* fruit was found to be 3.347±0.006mg/g. Carotenoids are efficient antioxidants, quenching excited state molecules and scavenging other reactive oxygen species including peroxide radicals. Dietary intake of carotenoids has been associated with a decreased risk of cancer, cardiovascular events, ophthalmological disorders and age related conginitive diseases[40].

Flavanoids are the phenolic substances which are the largest group of phenols. They generally occur as a C_6-C_3 unit linked to an aromatic ring. They are other plant constituents with antibacterial and antifungal properties[41]. The flavonoid content of *Morinda citrifolia* fruit was found to be 8.0±0.866mg/g.

Phenols are the most widespread secondary metabolites in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers[42]. In the present study, the total phenolic content of *Morinda citrifolia* fruit was found to be 3.2±0.1mg/g.

Tannin level in Noni fruit was found to be 9.4±0.2mg/g. Tannins are complex moieties produced by majority of plants as protective substances having wide pharmacological activities. Several reviews on dietary antioxidants were indicated that they have been used since past as tannin agents and reported for astringent, anti-inflammatory, anti-diarrhoeal antioxidant and antimicrobial activities[43].

Carbohydrate level in *Morinda citrifolia* was depicted as 133.83±1.04mg/g. Polysaccharides are important natural products in traditional Chinese medicine, possess antioxidant property by which protect cells against reactive oxygen species, chronic and degenerative diseases[44]. Ashafa AOT *et al*.,[45] reported that the presence of various secondary metabolites such as alkaloids, flavonoids, phenols etc from *Felicia Muricata* leaves showed significant antioxidant property.

Table 3: Non enzymatic antioxidants

Non enzymatic antioxidants	*Mg/g
Ascorbic acid	23.6±1.0
α-tocopherol	83.5±0.5
Carotenoids(vit A)	3.347±0.006
Flavonoids	8.0±0.866
Phenols	3.2±0.1
Tannins	9.4±0.2
Carbohydrates	133.83±1.04

* Mean for three observations.

Our results are in accordance with Deepika Gupta *et al*.,[46] who reported that the root of *Doronicum hookeri f* are rich in phytochemicals such as phenols and flavonoids which possess significant free radical scavenging activity. The preliminary quantitative screening of selected phytoconstituents revealed that *Morinda citrifolia* fruits might have rich medicinal property in curing disease caused by oxidative stress.

Determination of Total Antioxidant Capacity

Following the observations made in *Morinda citrifolia* fruit with regard to various antioxidants, it was felt imperative to study the total antioxidant activity.

1. Ferric reducing antioxidant power (frap) assay

FRAP assays are widely used to determine the efficiency of antioxidant compounds in plants to compete with the FRAP reagent and reduce the ferric to ferrous. Antioxidant compounds that are able to function in this approach are categorized as secondary antioxidants where they suppress the radical formation and prevent oxidative damage[47]. In table 4, FRAP assay of *Morinda citrifolia* fruit was measured by FRAP method. The maximum antioxidant capacity of *Morinda citrifolia* fruit extract was found to be 0.186±0.0004mg at 50mg/ml extract concentration.

Table 4: FRAP Assay

Concentration (µg/ml)	*Morinda citrifolia* extract(mg)
10	0.149±0.0001
20	0.157±0.0001
30	0.166±0.0002
40	0.177±0.0002
50	0.186±0.0004

The FRAP assay is widely used in the evaluation of the antioxidant component in dietary polyphenols. Antioxidant activity is found to be linearly proportional with phenolic contents. Katyayani Dutta Choudhury *et al* [47] have observed that the leaf extracts of *Lasianthus Lucidus* showed good antioxidant potential which supported our observations.

2. Abts radical scavenging activity

ABTS radical assay can be used in both organic and aqueous solvent system. Therefore is often used in evaluating total antioxidant power of single compounds and complex mixtures of various plants[48]. In this, ascorbic acid acted as the comparison standard. The scavenging ability of *Morinda citrifolia* fruit on ABTS free radical was shown in Table5. The scavenging activities of *Morinda citrifolia* fruit and ascorbic acid correlated well with increasing concentrations. The maximum ABTS radical scavenging activity of *Morinda citrifolia* fruit and ascorbic acid were found to be 85.893 ±1.655 and 96.363±0.935µg/ml extract concentration.

Table 5: **ABTS radical scavenging activity**

Concentration (µg/ml)	*Morinda citrifolia* extract (mg)	Standard ascorbic acid (mg)
10	56.140±1.040	60.613±1.675
20	60.227±0.830	66.087±1.465
30	68.353±1.255	76.070±1.350
40	75.450±1.750	86.493±1.854
50	85.893±1.655	96.363±0.935

In this assay, vitamin C acted as the comparison standard. The scavenging activities of *Morinda citrifolia* extract and ascorbic acid correlated well with increasing concentrations. At the low dose, the scavenging activities of both samples were poor. On the contrary, *Morinda citrifolia* extract exhibited high scavenging power in the higher doses indicated that *Morinda citrifolia* extract had strong scavenging power on ABTS radicals, and should be explored as novel potential antioxidants.

Table 6: **Reducing power assay.**

Concentration (µg/ml)	*Morinda citrifolia* extract (mg)	Standard ascorbic acid (mg)
350	0.053±0.002	0.011±0.001
700	0.088±0.001	0.013±0.001
1050	0.125±0.002	0.016±0.002
1400	0.166±0.001	0.020±0.001
1750	0.199±0.004	0.058±0.002

3. Reducing power

Reducing power indicates compounds that are electron donors, which can act as primary and secondary antioxidants. Reducing power of a compound may serve as a significant indicator of its potential antioxidant activity [49].

In table 6, the reducing power increased with increasing concentration of plant extract. Maximum reducing power of *Morinda citrifolia* fruit and ascorbic acid were found to be 0.199±0.58 absorbance regularly. The reducing sugar increased with an increase in extract concentration. The data show that all the samples increased their reducing ability when the concentration of extracts was increased. The ability to reduce Fe (III) may be attributed to hydrogen donation from phenolic compound which is also related to the presence of reducing agent. In addition the number and position of hydroxyl group of phenolic compounds also rule their antioxidant activity [50].

4. Phosphomolybdenum assay

The phosphomolybdenum method is based on the reduction of molybdenum by the antioxidants and the formation of a green molybdenum(V) complex, which has an absorption at 695nm[3].The total antioxidant capacity observed in the fruit was 241.66±2.00mg/g in ascorbic acid.

The assay is successfully used to quantify vitamin E in seeds. This method is simple and independent on other antioxidant measurements and is commonly employed for plant extracts [41].Similar trend was noticed by Aswini M et al., [51] who reported that *Coccicia Grandis* showed good antioxidant potential which supported our observations.

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that 50% ethanolic extract of *Morinda citrifolia* fruit extract exhibits high antioxidant activitity. These *in vitro* assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activity of this extract also has to be assessed prior to clinical use.

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