Evaluation of Antioxidant Potential of Morinda citrifolia L. Root Extract

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Abstract

This work attempts to determine the antioxidant activity of methanolic extract from roots of Morinda citrifolia using in-vitro model using several methods such as 1,1-Diphenyl-2-Picryl-hydrazyl (DPPH) radical, hydrogen peroxide (H2O2), superoxide radical, nitric oxide (NO) scavenging assay and FRAP (Ferric ion reducing antioxidant power) assay. The IC50 value of methanolic extract from roots of Morinda citrifolia L. for DPPH radical scavenging and Ferric ion reducing antioxidant power assay, was 25µg/ml, while in hydrogen peroxide scavenging and nitric oxide (NO) assay it was greater than 50µg/ml. It was more than 25µg/ml for superoxide scavenging assay. The results showed high antioxidant activities in all the methods tested for the extracts. This investigation suggested that Morinda citrifolia L. might serve as a potent source of natural antioxidant activity. The results provide useful information on the pharmacological activities associated with this traditional folk remedy.

Keywords: Morinda citrifolia, Antioxidant Activity, DPPH Radical, Free Radicals, Superoxide Radical

1. Introduction

An edible tropical medicinal plant-Morinda citrifolia (Noni) is a traditional medicinal plant belonging to the family Rubiaceae. It is a small fruit-bearing shrub mostly found in Southeast Asian region and believed to have spread across Australia and Pacific Islands (Brown, 2012). The Noni plant is a small evergreen tree, usually 1300 ft above the sea level and spotted growing along lava strewn formed by effusive eruption (Wang et al, 2002a). It is ascribable by its elongated trunk with 8-10 inches long bright green leaves (Rivera et al, 2012). It bears peculiar “grenade-like” unripe green to ripe yellowish gawky fruit which softens to lucid an overly ripen form. Due to its lousy taste and odour, it is not preferred to eat by the people (Su et al, 2005). The ancestors of Polynesians, who are believed to have brought Noni from south Asia, have utilized the whole Noni plant in various combinations (Brown, 2012). The roots, leaves, bark and the fruit are widely used for its medicinal values to treat various ailments including breast cancer and eye problems (Su et al, 2005). The fruit juice is of high demand in unconventional medicine for the treatment of muscle aches, gastric problems, treat blood pressure, headaches, mental depression and in menstrual difficulties (Brown, 2012). Mouse treated with Noni juice exhibits augmented immune system activity by stimulating the production of macrophages and lymphocytes (Kirti, 2007). Bushnell et al (1950) reported that Noni was a traditional remedy used to treat broken bones, deep cuts, bruises, sores, and wounds. In addition, Joseph Betza a chemist in FDA stated “Morinda citrifolia has been tested for a number of biological activities in animal and anti-microbial studies” (Wang et al, 2002a). Today, in United States, both leaves and fruit are sold in health centres and grocery as juice, tablets and as a tea (Su et al, 2005). The roots of Noni contain natural sedative and anti-malarial activity (Kirti, 2007).

Therefore, the present investigation was designed to study the antioxidant activity of Morinda citrifolia root extracts and the results have been discussed in detail.
2. Materials and Methods

2.1. Chemical Reagents

The standards Butylated Hydroxytoluene (BHT), L-Ascorbic acid were obtained from Hi-Media, Mumbai, India. (DPPH) 1,1-diphenyl-2-picryl-hydrazyl radical was purchased from Sigma Chemical Co, St. Louis, MO, USA. All other chemicals and solvents were of analytical grade (Sigma-Aldrich). The solvents used were of HPLC grade.

2.2. Plant Material

Fresh plant materials were collected from Kundapura hills, Mangalore. The dried specimen was deposited in the herbarium of CAS in Botany, University of Madras, Chennai 25.

2.3. Extract Preparation

The plant extracts were prepared as described by Aqil & Ahmad (2007) with modification. The roots of Morinda citrifolia L. was collected and dried at room temperature (26 ± 2° C) for of 2 weeks. The dried material was coarsely powdered and 100 gm of the same was soaked in a 1 litre of methanol for 48 hours with occasional stirring and the extract was passed through Whatman (UK) filter paper No. 1. It was concentrated by distillation over boiling water bath and the traces of solvent were removed under vacuum. One gram of the extract was weighed in a dry clean bottle and diluted using 10% solution of dimethylsulfoxide (DMSO) to make the known concentration of 100 mg/ml. The diluted solution was used for further works.

2.4. Estimation of Radical Scavenging Activity (RSA) using DPPH Assay

The RSA of different extracts was determined by using DPPH assay according to the standard method with small modification (Nenadis & Tsimidou, 2002) (Table 1). The decrease of the absorbance at 517 nm of the DPPH (1,1-Dipheny-2-picryl-hydrazyl) solution after the addition of the extract (100 µg/100 µl) was measured in a glass cuvette (1 cm width) containing 2960 µl of 0.1 mM methanolic DPPH solution of 100 µl (0.1%). The extract was dissolved in the concentration of 1 mg/ml, which was then used to determine the antioxidant assay. The DPPH \(^{-} [100 µl (0.1%)] \) with 100 µl (0.16%) BHT was used as standard, DPPH \(^{-} (0.1%) \) was used as control. The mixture was shaken vigorously and covered with aluminium foil left to stand at room temperature for 30 minutes in the dark. The absorbance was then measured after 30 minutes in 5 minutes interval at 517 nm against a blank by using spectrophotometer (Shimadzu, Tokyo, Japan). The DPPH solution was freshly prepared. The percentage Free radical-scavenging activities of test samples and the positive control were expressed in terms of IC\(_{50}\) values, which is the concentration of a sample required to decrease the absorbance at 517 nm by 50% compared to the control response. The percentage inhibition of DPPH radical was calculated by using the following equation. (% of DPPH Radical Scavenging Activity (% RSA) = \(A_o - A_1/A_0 \times 100\). Where \(A_o\) is the absorbance of DPPH radical + methanol; \(A_1\) is the absorbance of DPPH radical + plant extract. Measurements were performed in triplicate.

2.5. Estimation of Hydrogen Peroxide (H\(_2\)O\(_2\)) Radical Scavenging Assay

The ability of the extracts to scavenge hydrogen peroxide was determined (Nabavi et al, 2008). A solution of hydrogen peroxide (20 mM) was prepared in Phosphate Buffer Saline (PBS). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Sample extracts (100 µl, 0.1-1 mg/ml) in distilled water were added to a 2 ml hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing only phosphate buffer without hydrogen peroxide.

The percentage of hydrogen peroxide scavenging by the extracts and standard compounds were calculated according to the following equation. (% Scavenge\(d \)H\(_2\)O\(_2\) = \(A_o - A_t/A_o \times 100\). Where \(A_o\) was the absorbance of the control and \(A_t\) was the absorbance in the presence of the extract & standard (Ascorbic acid). Measurements were performed in triplicate.

2.6. Estimation of Superoxide Anion Radical Scavenging Assay

Measurement of superoxide anion radical scavenging activity was done by using standard method of Nishikimi et al (1972) with small modification. Superoxide anion radical scavenging assay is based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide-adenine-dinucleotide-phosphate reduced (NADH) and Phenazonium Methosulphate (PMS) under aerobic condition. The superoxide anions generated by Phenazonium Methosulphate (PMS)/ nicotinamide-adenine-dinucleotide-phosphate reduced (NADPH) system, were detected by the reaction with 2,2'-di-p-nitrophenyl-5,5'-diphenyl-3,3'-dimethoxy-4,4'-diphenylenditerazolium chloride (nitro blue tetrazolium - NBT) (Wang et al, 2002b). The reaction mixture contained 1 ml of Nitro blue tetrazolium (NBT) solution (312 µM prepared in phosphate buffer of pH 7.4), (100 µl NBT 0.6 M), 1 ml of Nicotinamide adenine dinucleotide (NAD+) solution (936 µM prepared in phosphate buffer of pH 7.4) or (50 µl NADPH 0.5 mM) and 2.7 ml, 50 mM PBS of pH 7.8, 50µl
EDTA (0.1M). Finally the reaction was accelerated by adding 100 µl phenazonium methosulphate (PMS) solution (120 µM prepared in PBS of pH-7.4). The reaction was incubated at 25°C for 5 minutes and absorbance at 560 nm was measured against quercitin as control. Quercitin (1 M/ml) was used as a standard.

Percent inhibition was calculated against a control without the extract according to the following equation. (% Inhibition = A0–A1/A0 x 100). Where A0 was the absorbance of the control and A1 was the absorbance in the presence of extract & standard. Measurements were performed in triplicate.

2.7 Estimation of Nitric Oxide Radical (NO) Scavenging Assay

Nitric Oxide radical inhibition was studied by the use of Griess Illosvoy reaction, based on the standard method with small modification (Sreejayan & Rao, 1997). The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of each extracts and incubated at 25°C for 15 minutes. After the incubation period, 0.5 ml of reaction mixture was mixed with 1 ml of sulphanilamide (1%). The reaction was allowed to stand for 5 minutes for completing diazotization, and then 2% phosphoric acid and 1 ml of N-(1-naphthyl) ethylene diamine dihydrochloride (0.1 %) was added, mixed well and allowed to stand for 30 minutes at 25°C. A pink coloured chromophore was formed. The absorbance of these solutions was measured at 540nm against the corresponding blank solution according to the following equation. (% Inhibition = A0–A1/A0 x 100). Where A0 was the absorbance of the control and A1 was the absorbance in the presence of extract & standard (Ascorbic acid). Measurements were performed in triplicate.

2.8 Estimation of Total Antioxidant Activity of Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric Reducing Antioxidant Power (FRAP) assay was determined according to the standard method of Benzie & Strain (1996) with small modification FRAP assay uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. At low pH, reduction of ferric tripyridyltriazine (FeIII TPTZ) complex to ferrous form (which has intense blue colour) can be monitored by measuring the change in absorption at 593 nm. The change in absorbance is therefore, directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture. The stock solutions included 300 mM/L acetate buffer (3.1g CH3COONa x 3H2O and 16 ml conc. CH3COOH/L of buffer solution at pH 3.6), 10 mM/L TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM/L HCl and 20 mmol/L FeCl3 x 6H2O in distilled water (d.w.). The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, 2.5 ml FeCl3 x 6H2O solution and standard ferrous sulphate (1mM). (The FRAP reagent was prepared by mixing 1, 2 and 3 in the ratio of 10:1:1) The working solution must be always freshly prepared. The temperature of the solution was raised to 37°C before using. Plant extract (100µl) was mixed with 1.5 ml of working FRAP reagent and allowed to react with them for 30 minutes in the dark condition and then incubated at 37°C for 4 minutes. After incubation, the absorbance at 593 nm was measured. The relative activities of samples were assessed by comparing their activities with that of Trolox®, (Hoffman-LaRoche) or L- ascorbic acid. The standard curve was linear between 200 and 1000 µM FeSO4. Ferrous sulphate standard was processed in the same way and the FRAP value was calculated from the standard graph. Results are expressed in µM Fe (II)/g dry mass and compared with that of BHT and ascorbic acid.

3. Results and Discussion

Various concentrations (10-100 µg/ml) of methanol extract of M. citrifolia roots were tested for their antioxidant activity using different in-vitro models. It was observed that free radicals were scavenged by the test compounds in a dose-dependent manner.

3.1. DPPH Radical Scavenging Activity

The model of scavenging, the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh et al, 2008). This method is also very convenient for the screening of large numbers of samples with different polarity. The effect of antioxidant on DPPH radical scavenging was thought to be their hydrogen donating ability or radical scavenging. Assessed samples were able to reduce the stable violet DPPH radical to the yellow DPPH. DPPH is a stable nitrogen-centred free radical. The colour changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances, which are able to perform this reaction, can be considered as antioxidants and hence they are radical scavengers. (Dehpour et al, 2009) Figure 1 shows that DPPH radical-scavenging activity results for methanol extract of M. citrifolia and synthetic antioxidants BHT. Discolouration of the stable free DPPH radicals by antioxidants in the sample was measured spectrophotometrically. The IC50...
value and annihilation of DPPH radical was expressed in % inhibition after 30 minutes. The result showed maximum free radical scavenging activity was observed in 25 µg/ml and the scavenging effect of synthetic antioxidants for BHT was 96 µg/ml. The effect of antioxidants on DPPH might to be due to their hydrogen donating ability (Baumann et al, 1979). The DPPH radical scavenging ability of the methanolic extracts were significant when exposed to that of BHT. It was evident that the extract did show proton donating ability and could serve as a free radical inhibitor or with scavenging activity possibly as a primary antioxidant. Usually higher total phenol and flavonoids contents lead to better DPPH-scavenging activity. (Ebrahimzadeh & Bahramian, 2009). Morinda citrifolia possessed high amount of phenolics and flavonoids, which could be a possible explanation for its high anti-oxidant activity.

3.2. Hydrogen Peroxide (H$_2$O$_2$) Scavenging Activity

Scavenging of H$_2$O$_2$ by extracts may be attributed to their phenolics, which might have donated electrons to H$_2$O$_2$, thus neutralizing it to water. The ability of the extracts, to effectively scavenge hydrogen peroxide, was determined and was compared to that of quercetin. The extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner. Figure 2 shows that IC$_{50}$ value for scavenging activity of H$_2$O$_2$ was greater than 50 µg/ml for methanol extract when compared with standard quercetin (140 µg/ml). Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by the production of to hydroxyl radicals in the cell. Thus, removal of H$_2$O$_2$ is very important in the food chain.

3.3. Superoxide Anions Scavenging Activity

In this system superoxide anion derived from dissolved PMS-NADH coupling reaction reduces NBT. The decrease of absorbance at 540 nm with antioxidants indicated the consumption of superoxide anion in the reaction mixture. Which showed that IC$_{50}$ value for scavenging activity of superoxide is greater than 25 µg/ml for methanol extract of strong superoxide radical scavenging activity, when compared with standard quercetin (58 µg/ml). Figure 3 shows results obtained emphasized the capacity of M. citrifolia extracts to annihilate the superoxide anions generated through PMS-NADPH-NBT system.

3.4. Nitric Oxide Radical Scavenging Activity

The percentage inhibition increased with an increase in the concentration of Morinda citrifolia L. extract. Figure 4 shows that the IC$_{50}$ value of methanolic extract in the nitric oxide scavenging activity were greater than 50 µg/ml. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other associated pathological stress.

3.5. Ferric Reducing Antioxidant Property (FRAP)

The antioxidant potentials of methanol extracts of Morinda citrifolia were estimated from their ability to reduce
TPRZ-Fe (III) complex to TPTZ-Fe (II). Increase in antioxidant activity is directly correlated to the polyphenol content. According to recent reports, a highly positive relationship existed between total phenols and antioxidant activity appears to be the trend in many plant species. Figure 5 shows that the reducing ability of the extracts was in the range of 320-400 mm Fe (II)/g. FRAP method is sensitive for the measurement of total antioxidant power of fresh biological fluids, such as plant homogenate and pharmacological plant products. Antioxidant activities of residues from Morinda citrifolia L. were excellent natural sources of phenolic antioxidants for the preparative antioxidant nutraceuticals were confirmed through this study.

Figure 2. Graph Representing the Percentage of Hydroxyl Radical Scavenging Activity – IC<sub>50</sub> at >50µg

Figure 3. Graph Representing the Percentage of Superoxide Anion Scavenging Activity– IC<sub>50</sub> Value was Determined at >25µg

4. Conclusion

It was confirmed that Morinda citrifolia extracts could act as hydrogen and/or electron donors and react with free radicals, converting them into more stable products and in this way terminating radical chain reactions. The methanol extract exhibited high level of antioxidant activity due to the presence of high amount of phenolics. The type of solvent used in the extraction plays a key role in assessing the impact of antioxidant activity and total phenolic content of whole plant extracts.

Figure 4. Graph Representing the Percentage of Nitric Oxide Scavenging Activity–IC<sub>50</sub> Value was Determined at >50µg

Figure 5. Graph Representing the Percentage of Ferric Reducing Antioxidant Power (FRAP)–IC<sub>50</sub> Value was Determined at 25 µg

In conclusion, Morinda citrifolia could be developed as an effective antioxidant against several kinds of oxidative degenerative diseases such as cancer and liver diseases. These results provide information on the pharmacological activities associated with free radicals of this traditional folk remedy. Utilization of this plant product will be of advantage to humanity and increased consumption will help in prevention of chronic life style diseases.

References

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