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Phytochemical and Antioxidant Study of *Morinda citrifolia*

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Abstract

The study was designed to investigate the phytochemical screening and antioxidant properties of *Morinda citrifolia.* Aqueous extract obtained from *M. citrifolia* were subjected to phytochemical analysis. It proved that *M. citrifolia* contain large amount of secondary metabolite like steroids, Cardiac glycosides, Tannins, Terpanoids, Carbohydrate, flavonoids and proteins, but resins, Anthraquinones and phyloabtannins were absent. Antioxidant activity is measured by their ability to scavenge the free radicals such as 1). DPPH 2). ABTS 3) FRAP 4). Nitric Oxide Scavenging assay 5). Reducing Power assay and 6). Phosphomolybdenum method. These assays confirmed the presence of antioxidants in the aqueous extract of *Morinda citrifolia.*

Keywords: Phytochemical Screening, *Morinda citrifolia*, Antioxidant Study, DPPH, ABTS, FRAP

Introduction

India is known for its rich diversity of medicinal plants and from ancient times these plants have been utilized as therapeutic agents [1].The medicinal plants possess many bioactive compounds and medicinal power mainly depends on phytochemical constituents that have great pharmacological significance. Researchers identified 119 secondary metabolites isolated from the plant that are being used globally as drugs. It has been estimated that 80%of the world's population still use the traditional medicines for their primary health care needs [2].

Natural products contain different valuable chemical components such as phenolic compounds, phthalides, phenylpropanoids, terpenoids, essential oils, aromatic compounds, alkaloids, alkynes, sterols, polysaccharides, fatty acids, anthocyanin, tannins, etc. They also have significant antioxidant activity [3].

Apart from their role of health benefactors, antioxidants are added in foods to prevent or delay oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature [4]. Aromatic plants are traditionally used as antibacterial agents and are today accepted as source of antioxidants. Antioxidants are the substances that can prevent damage to cell by free radicals.

Morinda citrifolia is also known in different names locally as Nino, Nona, Nono, Nonu, Nuna, etc [5]. *Morinda citrifolia* is an evergreen shrub whose ripe fruit has a strong butyric acid smell and flavour. *Morinda citrifolia* L. fruit contains a number of antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids, beta-sitosterol, carotene, polyphenols such as flavonoids, flavone glycosides, rutin etc. [6]. It can also increase immune system activity. Present study was aimed to evaluate the phytochemical and antioxidant content of juice extract of *Morinda citrifolia* L. prepared from fruits.

Methodology

Collection of the Fruit

The fruits of *Morinda citrifolia* (Noni) were collected from the kitchen garden of my residence, Kadalundi, Kozhikode, Kerala, India.

Method of Extraction

Before starting the extraction of the juice, 10 ripe fruit were washed twice and were introduced in ten transparent individual glass containers for one month. The juice collected was freeze-dried and used to prepare aqueous extracts of 50 mg/ml and 200 mg/ml.

Estimation of Phytochemicals and Antioxidants

The juice extract of the fruits of *Morinda citrifolia* were analyzed for the presence of various phytoconstituents using standard protocols

Phytochemical analysis

The aqueous extracts of *Morinda citrifolia* were screened for the presence of secondary metabolite using the standard procedure.

Evaluation of antioxidant activity

Free Radical scavenging activity

2, 2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging assay

The DPPH assay was performed according [7] [8].

Total Antioxidant Activity by Phosphomoly bdenum Method

The total antioxidant capacity of the extract was evaluated by the phosphor –moly3bdenum method, according to the procedure described by Prieto *et al*. [9].

ABTS Radical Scavenging Activity

ABTS Radical Scavenging Activity capacity of the extract was evaluated by the method, according to the procedure [10].

Nitric Oxide Scavenging Assay

Nitric Oxide Scavenging Assay capacity of the extract was evaluated by the method, according to the procedure [11].

Ferric Reducing Antioxidant Power Assay (FRAP assay)

The FRAP assay was performed according [12].

Reducing Power Assay (RP)

The reducing power was determined according to the method [12].

Results and Discussion

Preliminary phytochemical screening of *Morinda citrifolia* fruit extracts revealed the presence of various bioactive compounds. The phytochemical analysis of *Morinda citrifolia* showed the presence of all major phytochemicals like steroid, cardiac glycosides, terpenoids, carbohydrates and flavanoids in higher amount and. Whereas phlyobatannins, anthraquinones and resins were absent (Table-1). Shixin Deng *et al.* (2007) stated that several classes of compounds have been isolated from *M. citrifolia* L., including amino acids, anthraquinones, coumarins, fatty acids, flavonoids, iridoids, lignans and polysaccharides. Among these, scopoletin, a coumarin derivative, is one of the representative ingredients in *M. citrifolia* L. phytochemical screening for the presence of secondary metabolite was conducted on the Indian *M. citrifolia* fruit aqueous, ethanol and methanol extracts detecting steroids, cardiac glycosides, phenol, tannins, terpenoids, alkaloids, carbohydrates, flavonoids, reducing sugar, lipids and fats in all types of extracts [13].

Table: 1 Phytochemical analysis of *Morinda citrifolia* Fruit Juice Extract

Secondary metabolites	Fruit juice extract
Steroids	++
Cardiac Glycosides	++
Phenol	+
Tannins	++
Terpenoids	++
Alkaloids	+
Resins	-
Carbohydrates	++
Flavanoids	++
Anthraquinones	-
Phylobatannins	-
Saponins	+
Protein	++

The *in vitro* methods for evaluation of antioxidant activity have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. These methods are popular due to their high speed and sensitivity [14].

The radical scavenging capacity of the Noni fruit juice extract was tested using the stable free radical assays (Table-2). In the present study of 2, 2-diphenyl-1-picryhydrazyl (DPPH) scavenging activity of the Noni juice fruit extract, at 200µg concentration exhibited the highest radical scavenging effect which was higher than the other concentrations. The lowest activity was observed at the concentration of 25µg. DPPH scavenging activity of the extracts can be correlated to the presence of flavonoids [15]. In DPPH assay the extract analysed exhibited varying degrees of scavenging capacities with different concentrations. Total antioxidant activity was estimated by the 2, 2, diphenyl-1-picrylhydrazyl (DPPH) assay. According to the method, maximum decrease of DPPH inhibition was observed in 60 and 90 days of fermented noni extracted juice samples [16].

In the present study, the Ferric Reducing Antioxidant Power Assay (FRAP) assay of Noni fruit juice at 200µg concentration showed 16.84mM Fe^{2+} (µg) the reducing potency. FRAP measures the reducing potency of extract and standard antioxidant. Comparable ferric reducing potentials were observed for both unripe (11.90 ± 0.20 mM Fe^{2+} g^{-1} FW) and ripe fruit extracts (11.26 ± 0.33 mM Fe^{2+} g^{-1} FW)[17].

Study of Total antioxidant activity is expressed as ascorbic acid equivalent (AAE) and total antioxidant activity of Noni juice fruit extract is 12.51µg AAE/100µg concentration. 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.The total antioxidant capacity observed in the fruit was 241.66±2.00mg/g ascorbic acid [18].Total antioxidant activity was estimated by phosphomolybdenum (PM) assay. The phosphomolybdenum method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts.

In (ABTS) radical assay of Noni juice fruit extract, highest % inhibition of 33.37% were found in 200µg concentration. Lowest % of inhibition 7.49% was found to be minimum at 25µg concentration but at the same concentration the standard were showed maximum % of inhibition of 56.35%. IC_{50} value is an indicator of antioxidant potential. The scavenging activities of *Morinda citrifolia* fruit and ascorbic acid correlated well with increasing concentrations. Cation is reactive towards most antioxidants including phenolics, thiols and Vitamin C During this reaction, the blue ABTS radical cation is converted back to its colourless neutral form. 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 [19]. .

Nitric oxide (NO) and reactive nitrogen species (RNS) are free radicals that are derived from the interaction of NO with oxygen or reactive oxygen species. Noni juice fruit extract shown maximum % of inhibition of 5.17% at concentration of 200µg. Minimum % of inhibition produced were 1.13% at 25µg concentration.

Table. 2 Free radical scavenging activity of different extracts of *Morinda citrifolia* Fruit Juice extracts.

Free radical scavenging activity of *Morinda citrifolia* fruit juice extracts					
Free Radicals Scavenged	***M. citrifolia* Juice Extract Concentration (µg)**				**Ascorbic Acid**
	25	**50**	**100**	**200**	
DPPH	5.28	6.79	10.5	11.04	46.88
Total antioxidant	2.24	5.67	9.55	12.51	48.53
ABTS	7.49	10.24	22.60	33.37	56.35
Nitric oxide scavenging	1.13	1.94	3.72	5.17	15.37
FRAP	4.13	7.28	15.54	16.84	36.63

The Reducing power (RP) assay showed an increased absorbance of Noni juice fruit extract as the concentration increases. Reducing power is the measure of the reductive ability of antioxidant and it is evaluated by the transformation of $Fe3^{+}$ to $Fe2^{+}$ in the presence of extracts. The reducing power is mainly correlated to the presence of reductones like ascorbic acid [18]. Reducing power indicates compounds that are electron donors, which can act as primary and reducing power of a compound may serve as a significant indicator of its potential antioxidant activity.

Antioxidants are biologically synthesized as defensive mechanism having an important role in preventing or alleviating chronic diseases by reducing the oxidative damage to cellular components caused by free radicals. The present study shows that *Morinda citrifolia* fruit juices extract possess significant amount of phytochemicals and *in vitro* antioxidant activity. A significant linear relationship between antioxidant activity and phytochemicals, that are responsible for the *in vitro* antioxidant property of the fruit extracts. The juice extract extract possess a better antioxidant capacity. Analyzing the results, it is clear that the Juice extract of extract *Morinda citrifolia* account for the plethora of bioactive compounds. However isolation and characterization of the compounds and validating their therapeutic efficacy against different pathologies is required for clinical implementation.

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