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Antioxidant and free radical scavenging activity of ethanolic extract of *Morinda citrifolia*

[*]Ranju Pal[1], Kundlik Girhepunje[2], Nidhi Shrivastav[1], Mohammed Misbah Hussain[1] and Thirumoorthy N[3]

[1]Sagar Institute of Research and Technology, Bhopal (M.P.)
[2]Radharaman College of Pharmacy, Bhopal (M.P.)
[3]Patlolla Ramkrishna Reddy College of pharmacy, Hyderabad (A.P.)

ABSTRACT

The aim of this work was to estimate the total phenolic and flavonoid content, and to evaluate in-vitro antioxidant activity of ethanolic root extract of Morinda Citrifolia (MCREt). The raw, dry root powder was extracted with 99.9% of ethanol. Phytochemical test shows that extract contains higher level of total phenol and flavonoids. Total phenolic compound in ethanolic root extract of Morinda Citrifolia was found to be 41.89 mg/g of extract calculated as gallic acid equivalent (r2=0.9971) and total flavonoids compound was found to be 17.27 mg/g of extract calculated as rutin equivalent (r2=0.9992). The extract was screened for its potential antioxidant activities using tests such as hydroxyl radical-scavenging activity, reducing power activity, and hydrogen peroxide-scavenging activity. The in-vitro antioxidant assay showed MCREt posses potent antioxidant activity when compared with reference compound butylated hydroxytoluene (BHT). MCREt could be useful for preparation of neutraceuticals as potent antioxidant to treat various human diseases and its complications.

Key words: *In vitro* antioxidant activity, Phenol, Flavonoid, Reducing power activity, Hydrogen peroxide-scavenging activity, *Morinda Citrifolia*

INTRODUCTION

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electrons and involved in many pathological conditions [1]. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defences or by supplementing with proven dietary antioxidants [2]. Synthetic antioxidants like butylated

hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in foods have side effect and are carcinogenic [3]. Plant polyphenolic compounds, such as flavonoids are described as scavengers of reactive oxygen species [4]. Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants has been extensively investigated[5]. Most sources of natural antioxidants originate from plant materials, but the content of polyphenolic compounds in the roots and pericarp of tropical and sub-tropical flora have sparsely reported [6].

Among the medicinal plants discovered by the ancestors of Polynesians, *Morinda citrifolia* L (Noni) is one of the traditional folk medicinal plants that has been used for over 2000 years in Polynesia. *Morinda citrifolia* L is also called *Indian Mulberry, Ba Ji Tian, Nono* or *Nonu* and *Nhau* in various cultures throughout the world. Roots of *Morinda citrifolia* are the source of important compounds, phenols and flavonoids, which have been proven to have anti-viral, anti-bacterial, anti-cancer activities. Thus, present study was undertaken to evaluate the *in vitro* antioxidant effect of ethanolic extract of *Morinda citrifolia* root.

The main constituent present in the roots are isoflavonoids, flavonoids, proteins, alkaloids, carbohydrates and anthraquinones [7].

MATERIALS AND METHODS

1. Phytochemical evaluations:

1.1 Plant material:

The roots of *Morinda Citrifolia* were collected from the place, Quereshi bagh nursery, Jamnagar (Gujrat) and authenticated by senior Faculty of Agriculture university, Nagpur, Maharashtra.

Sr. No.	Phytochemical constituents	Ethanolic extract of *Morinda citrifolia* root *(MCREt)*
1	Carbohydrates	+ve
2	Alkaloids	+ve
3	Steroids and sterols	+ve
4	Glycosides	+ve
5	Saponins	+ve
6	Flavanoids	+ve
7	Tannins and phenolic compound	+ve
8	Proteins and Amino acids	+ve
9	Anthraquinone	+ve

1.2 Extraction procedure:

The roots were shade dried, broken into small pieces and powered coarsely. About 1000 gm of air dried powdered material was extracted with 99.9% of ethanol in a soxhlet extractor for 15 cycle. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50^{o} C) using rotary evaporator. The ethanolic extract yielded an brown sticky mass weighing 32g and extractive value was found to be 8.431% w/w. the extract was

used directly for total phenol and flavonoid content and also for assessment of antioxidant capacity through various chemical assays [8-9].

1.3 Phytochemical evaluation:

The ethanolic extract of *Morinda Citrifolia* root (MCREt) was subjected to the following chemical tests for the identification of various active constituents.

1.4 Estimation of total phenolic content:

The total phenolic content of MCREt was estimated according to the method of Makkar et.al (1997) [10]. The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after overtexing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm against the reagent blank. Using gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 µg/ml. using the standard curve, the total phenolic content was calculated and expressed as gallic acid equivalent in mg/g of extract.

1.5 Estimation of total flavonoid content:

Flavones and flavonols in the ethanolic extracts of *Morinda Citrifolia* root were estimated as rutin equivalent. Rutin was used to make the calibration curve [10, 20, 30, 40, 50, 60, 70, 80, 90, 100 in 99.9% ethanol (v/v)]. The standard solutions or extracts (0.5 ml) were mixed with 1.5 ml 95% ethanol (v/v), 0.1 ml 10% aluminum chloride 42(w/v), 0.1 ml of 1 mol/l sodium acetate and 2.8 ml water. The volume of 10% aluminum chloride was substitued by the same volume of distilled water in blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm.

2. Evaluation of in vitro antioxidant activity:

2.1. Hydroxyl radical-scavenging activity:

Hydroxyl radical scavenging activity of extract was measured according to the method of Halliwell et al. (1987) [11]. One milliliter of the final reaction solution consisted of aliquots (500 µL) of various concentrations of the extract, 1 mM $FeCl_3$, 1 mM EDTA, 20 mM H_2O_2, 1 mM L-ascorbic acid, and 30 mM deoxyribose in potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1 h at 37 °C, and further heated in a boiling water bath for 15 min after addition of 1 mL of 2.8% (w/v) trichloroacetic acid and 1 mL of 1% (w/w) 2-thiobarbituric acid. The color development was measured of 532 nm against a blank containing phosphate buffer.

2.2. Reducing power activity:

The reducing power of extract was determined by the method of Yen and Duh (1993) [12]. Different concentrations of extracts were mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixtures were incubated for 20 min at 50 °C. After incubation, 2.5 mL of 10% trichloroacetic acid were added to the mixtures, followed by centrifugation at 650×*g* for 10 min. The upper layer (5 mL) was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700 nm.

2.3. Hydrogen peroxide-scavenging activity:

The Hydrogen peroxide-scavenging activity of extract was determined by the method of Ruch et al., (1989) [13]. The extract was dissolved in 3.4 mL of 0.1M phosphate buffer (pH 7.4) and mixed with 600 µL of 43 mM solution of hydrogen peroxide. The absorbance value

(at 230 nm) of the reaction mixture was recorded at 10 min intervals between zero and 40 min. for each concentration, a separate blank sample was used for background substraction.

RESULTS AND DISCUSSION

Total phenol content:

Total phenolic compound in ethanolic extract of *Morinda Citrifolia* root was found to be 41.89 mg/g of extract calculated as gallic acid equivalent. (r^2=0. 0.9971).

Total flavonoid content

Total flavonoids compound in ethanolic extract of *Morinda Citrifolia* root was found to be 17.27 mg/g of extract calculated as rutin equivalent. (r^2=0.9992).

Hydroxyl radical scavenging activity:

BHT and MCREt showed hydroxyl radical scavenging activity with about 34.18-68.13% and 43.62-72.69% at concentration of 500 µg/mL (Table 1). A concentration dependent inhibition against hydroxyl radical induced deoxyribose degradation was observed in the deoxyribose assay. Because the MCREt was high in its phenol and flavonoid content, its antioxidant compounds may well act as antioxidant and scavenge hydroxyl radical generated from the Fenton reagent.

Table 1. Shows hydroxyl radical-scavenging activity of ethanolic root extract of *Morinda Citrifolia*

Sample	Concentration (µg/mL)		
	50	500	1000
BHT	34.18	68.13	84.31
MCREt	43.62	72.69	76.61

All the values are means of three independent determinations, n=3, analyzed in triplicate.

Hydrogen peroxide-scavenging activity:

Scavenging activity of hydrogen peroxide in MCREt (10 µg) and BHT (10 µg) as reference compound was not remarkably different and shown to be 79 % and 92 % at initial time respectively (Table 2). The composition of hydrogen peroxide into water may occur according to the antioxidant compounds as the antioxidant component present in the extract are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O.

Table 2. Shows hydrogen peroxide-scavenging activity of MCREt (10 µg/mL)

Sample	Time (min)		
	0	10	20
BHT	92	84	78
MCREt	82	76	72

All the values are means of three independent determinations, n=3, analyzed in triplicate.

Reducing power activity:
At concentration 500 µg/mL, BHT (Reference) and showed absorbance with about 0.32 and 0.54 respectively (Table 3). Thus MCREt exhibited reducing activity. The reducing power might be due to hydrogen donating ability.

Table 3. Shows reducing power activity of ethanolic root extract of *Morinda Citrifolia*

Sample	Concentration (µg/mL)		
	50	500	1000
BHT	0.127	0.321	0.719
MCREt	0.267	0.548	1.341

All the values are means of three independent determinations, n=3, analyzed in triplicate.

CONCLUSION

Based on the results obtained, MCREt showed antioxidant and free radical scavenging activity not remarkably different than reference compound butylated hydroxyl toluene (BHT) and major antioxidative component seems to be phenolic and flavonoids. Therefore, it can be concluded that the ethanolic extract of *Morinda Citrifolia* root could be considered for prevention and treatment of human diseases and its complications as potent antioxidant.

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