African Journal of Pharmacy and Pharmacology Vol. 6(5), pp. 278-282, 8 February, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP10.310
ISSN 1996-0816 ©2012 Academic Journals

Full Length Research Paper

Antioxidant and free radical scavenging activity of ethanolic extract of the root of *Morinda citrifolia* (Rubiaceae)

Ranju Pal[1]*, Kundlik Girhepunje[1], Ashutosh Upadhayay[1] and Thirumoorthy N.[2]

[1]NIMS University, Jaipur (Rajasthan), India.
[2]Cherraan's College of Pharmacy, Coimbatore (Tamilnadu), India.

Accepted 21 April, 2011

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) which belongs to the family Rubiaceae. The raw, dry root powder was extracted with 99.9% of ethanol. Phytochemical test shows that the extract contains higher level of total phenol and flavonoids. Total phenolic compound in ethanolic root extract of *M. citrifolia* was found to be 92 mg/g of extract calculated as gallic acid equivalent (r^2=0.9976) and total flavonoids compound was found to be 36 mg/g of extract calculated as rutin equivalent (r^2=0.9985). 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 that MCREt posses potent antioxidant activity when compared with reference compound butylated hydroxytoluene (BHT). MCREt could be useful for preparation of neutraceuticals as potent antioxidants, to treat various human diseases and its complications.

Key words: Rubiaceae, 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. A 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 is involved in many pathological conditions (Madhavi et al., 1996). 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 (Stanner et al., 2000). Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in foods have side effect and are carcinogenic (Branen, 1975). Plant polyphenolic compounds, such as flavonoids are described as scavengers of reactive oxygen species (Chen et al., 1993). Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants has been extensively investigated (Rice-Evans and Miller, 1994). 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 been reported (Elizabeth and Williamson, 2002).

Among the medicinal plants discovered by the ancestors of the Polynesians, *Morinda citrifolia* L (Noni) is one of the traditional folk medicinal plants that has been used for over 2000 years in Polynesia. *M. citrifolia* L is also called Indian Mulberry, Ba Ji Tian, Nono or Nonu and Nhau in various cultures throughout the world. Roots of *M. citrifolia* are the source of important compounds, phenols and flavonoids, which have been proven to have anti-viral, anti-bacterial and anti-cancer activities (Wang et al., 2002). Thus, the present study was undertaken to evaluate the *in vitro* antioxidant effect of ethanolic extract

*Corresponding author. E-mail: pal.ranju@gmail.com.

of *M. citrifolia* seeds. The main constituent present in the roots are isoflavonoids, flavonoids, proteins, alkaloids, carbohydrates and anthraquinones (Wang et al., 2002).

MATERIALS AND METHODS

Phytochemical evaluations

Plant material

The roots of *M. citrifolia* (Rubiaceae) were collected from the place, Quereshi Bagh Nursery, Jamnagar (Gujrat) and authenticated in the senior Faculty of Agriculture University, Nagpur, Maharashtra.

Extraction procedure

The roots were shade dried, broken into small pieces and powered coarsely. About 1000 g (1 kg) of air dried powdered material was extracted with 99.9% of ethanol in a Soxhlet extractor for 15 cycles. The extract was concentrated to dryness under reduced pressure and controlled temperature (40 to 50°C) using rotary evaporator. The ethanolic extract yielded a brown sticky mass weighing 32 g 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 (Kokate et al., 1999; Mehta, 1999).

Phytochemical evaluation

The ethanolic extract of *M. citrifolia* root (MCREt) was subjected to the following chemical tests for the identification of various active constituents (Table 4).

Qualitative phytochemical analysis of ethanolic root extract of *M. citrifolia* (MCREt): The ethanolic extract of *M. citrifolia* root was subjected to the following chemical tests for the identification of various active constituents.

(a) Test for carbohydrates: The following tests were carried out for carbohydrates.

i. Molisch test: It consists of treating the compounds with a-napthol and concentrated sulphuric acid along the sides of the test tube. Purple color or reddish violet color at the junction between two liquids is obtained.

ii. Fehling's test: Equal quantity of Fehling's solution A and B was added; heated gently, and brick red precipitate was obtained.

iii. Benedict's test: To 5 ml of Benedict's reagent, 8 drops of solution was added under examination and properly mixed; the mixture was boiled vigorously for 2 min and then cools. Red precipitate was obtained.

(b) Test for alkaloids:

i. Dragendroff's test: To the extract, 1 ml of Dragendroff's reagent was added; orange red precipitate was produced.

ii. Wagner's test: To the extract, Wagner's reagent was added. Reddish brown precipitate was produced.

iii. Mayer's test: To the extract, 1 or 2 ml of Mayer's reagent was added. Dull white precipitate was produced.
iv. Hager's test: To the extract, 3 ml of Hager's reagent was added; yellow precipitate was produced.

(c) Test for steroids and sterols:

i. Libermann Burchard test: The test sample was dissolve in 2 ml of chloroform in a dry test tube. 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid was then added. The solution becomes red, then blue and finally bluish green in color.

ii. Salkowski test: The sample of test solution in chloroform was dissolve and equal volume of concentrated sulphuric acid added. Bluish red, cherry red and purple color was noted in chloroform layer, whereas acid assumes marked green flourescense.

(d) Test for glycosides

i. Legal's test: Sample was dissolved in pyridine, sodium nitropruside solution was added to it and made alkaline. Pink red colour was produced.

ii. Baljet test: To the drug sample, sodium picrate solution was added, yellow to orange colour was produced.

iii. Borntrager test: A few millilitres of dilute sulphuric acid was added to the test solution; the mixture was boiled, filtered and the filtrate was extracted with ether or chloroform. Then the organic layer was separated to which ammonia was added. Pink, red or violet colour was produced in the organic layer.

(e) Test for saponins

1. Foam test: About 1 ml of alcoholic sample was diluted separately with distilled water to 20 ml and shaken in graduated cylinder for 15 min. 1 cm layer of foam indicates the presence of saponins.

(f) Test for flavonoids

(i) The drug was taken in alcohol or water along with a few millilitres of ammonia and viewed in UV and visible lights; formation of fluorescence indicates the presence of flavonoids.
(ii) A little quantity of the extract was treated with amyl alcohol, sodium acetate and ferric chloride. Solution turns yellow and disappears on addition of an acid. This indicates the presence of flavonoids.
(iii) Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl gives intense cherry red colour which indicates the presence of flavonones. Orange red colour indicates the presence of flavonols.
(iv) The extract was treated with sodium hydroxide; formation of yellow colour indicates the presence of flavones.
(v) The extract was treated with concentrated H_2SO_4; formation of yellow or orange colour indicates flavones.
(vi) The alcoholic and aqueous extract was treated with 10% sodium chloride; formation of yellow colour indicates the presence of coumarins.

(g) Test for tannins

i. To a sample of the extract, ferric chloride solution was added, a dark blue or greenish black colour was produced.
ii. To a sample of the extract, potassium cyanide was added; a deep red colour was produced.
iii. To a sample of the extract, potassium dichromate solution was added, and a precipitate was produced.

h) Test for triterpenoids

In a test tube, 2 or 3 granules of tin was added and dissolved in 2 ml of thionyl chloride solution and the test solution was added. Pink

colour was produced which indicates the presence of triterpenoids.

(i) Test for protein and amino acid

(a) Biuret test: 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate was added to the extract, a violet colour indicates the presence of proteins.

(b) Ninhydrin test: 2 drops of freshly prepared 0.2% Ninhydrin reagent was added to the extract and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids.

(c) Xanthoprotein test: To the extract, 20% of sodium hydroxide or ammonia was added. An orange colour indicates the presence of an aromatic amino acid.

Estimation of total phenolic content

The total phenolic content of MCREt was estimated according to the method of Makkar et al. (1997). Aliquots of the extract was taken in a test tube and made up to a 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 overtaxing 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 to10 µg/ml using the standard curve; the total phenolic content was calculated and expressed as gallic acid equivalent in mg/g of extract.

Estimation of total flavonoid content

Flavones and flavonols in the ethanolic extracts of *M. 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 of 95% ethanol (v/v), 0.1 ml of 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 substituted 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.

Evaluation of *in vitro* antioxidant activity

Hydroxyl radical-scavenging activity

Hydroxyl radical scavenging activity of the extract was measured according to the method of Halliwell et al. (1987). 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 at 532 nm against a blank containing phosphate buffer.

Reducing power activity

The reducing power of the extract was determined by the method of Yen and Duh (1993). 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.

Hydrogen peroxide-scavenging activity

The hydrogen peroxide-scavenging activity of the extract was determined by the method of Ruch et al. (1984). The extract was dissolved in 3.4 ml of 0.1 M 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 subtraction.

RESULTS AND DISCUSSION

Total phenol content

Total phenolic compound in ethanolic extract of *M. citrifolia* root was found to be 92 mg/g of extract, calculated as gallic acid equivalent (r^2=0.9976).

Total flavonoid content

Total flavonoids compound in ethanolic extract of *M. citrifolia* root was found to be 36 mg/g of extract calculated as rutin equivalent (r^2=0.9985).

Hydroxyl radical scavenging activity

BHT and MCREt showed hydroxyl radical scavenging activity with about 34.18 to 68.13% and 43.62 to 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. Fenton's reagent is a solution of hydrogen peroxide and an iron catalyst that is used to oxidize contaminants or waste waters. Iron(II) is oxidized by hydrogen peroxide to ferric iron(III), a hydroxyl radical and a hydroxyl anion. Iron(III) is then reduced back to iron(II), a peroxide radical and a proton by the same hydrogen peroxide (disproportionation). This process can be utilised for antioxidant activity.

Hydrogen peroxide-scavenging activity

Scavenging activity of hydrogen peroxide in MCREt (10 µg) and BHT (10 µg) as reference compound was not

Table 1. Hydroxyl radical-scavenging activity of ethanolic seeds extract of *M. 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.

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.

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

Table 4. The ethanolic extract of *M. citrifolia* root (MCREt) was subjected to the following chemical tests for the identification of various active constituents.

S/ N	Phytochemical constituents	Ethanolic extract of *M. citrifolia* root *(MCREt)*
1	Carbohydrates	+ve
2	Alkaloids	+ve
3	Steroids and sterols	+ve
4	Glycosides	+ve
5	Saponins	+ve
6	Flavonoids	+ve
7	Tannins and phenolic compound	+ve
8	Proteins and Amino acids	+ve
9	Anthraquinone	+ve

+ve, presence of compound.

remarkably different and was shown to be 82 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.

Reducing power activity

At a concentration of 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.

Conclusion

Free radicals play a positive role *in vivo* such as energy production, phagocytosis, regulation of cell growth and intercellular signaling, or synthesis of biologically important compounds. However, free radicals are very detrimental in attacking lipids in cell membranes and also DNA, inducing oxidations that cause membrane damage such as membrane lipid peroxidation and a decrease in membrane fluidity and also cause DNA mutation leading to cancer.

Based on the results obtained, MCREt showed antioxidant and free radical scavenging activity not remarkably different from the reference compound butylated hydroxyl toluene (BHT) and the major antioxidative component seems to be phenolic and flavonoids. Therefore, it can be concluded that the ethanolic extract of *M. citrifolia* root could be considered for prevention (chemopreventive) and treatment of human diseases and its complications as potent antioxidant Table 4.

REFERENCES

Branen AL (1975). Toxicological and biochemistry of butylated hydroxyl anisole and butylated hydroxytoluene. J. Am. Oil Chem. Soc., 52: 59-63.

Chen S, Hwang J, Deng PSK (1993). Inhibition of NAD(P)H: Quinone acceptor oxidoreductase by flavones: a structural activity study, Arch. Biochem. Biophys., 303: 72-77.

Elizabeth M, Williamson (2002). Textbook of Major herbs of Ayurveda, I: 7-11.

Halliwell B, Guttridge JMC, Aruoma OI (1987). The deoxyribose method: a simple 'test tube' assay for determination of rate constant for reaction of hydroxyl radicals, Anal. Biochem., 165: 215-219.

Kokate CK, Purohit AP, Gokhle SB (1999). Textbook of Pharmacognosy, (XII): 81-82.

Madhavi DL, Deshpande SS, Sulunkhe DK (1996). Food antioxidants: technological, toxicological and health perspectives, New York, Marcel dekker. p. 6

Makkar HPS, Becker K, Abel H, Pawelzik E (1997). Nutrient contents, rumen protein degradability and antinutritional factors in some colour and white flowering cultivars of vicia faba beans. J. Sci. Food Agric., 75: 511-520.

Mehta RM (1999). Textbook of pharmaceutics, (II): 146-14.

Rice-Evans C, Miller NJ (1994). Total antioxidant status in plasma and body fluids, Methods. Enzymol., 234: 279-293.

Ruch RT, Cheng SJ, Klaunig JE (1984). Spin trapping of superoxide and hydroxyl radicals, Methods Enzymol., 105: 198-209.

Stanner SA, Hughes J, Kelly CN, Buttriss JA (2000). Review of the epidemiological evidence for the 'antioxidant hypothesis, Public Health Nutr., 7: 401-422.

Wang MY, Brett J, West C, Jarakae Jensen (2002). *Morinda Citrifolia* (Noni): A literature reviewand recent advances in noni research Immunomodulatory. Act. Pharmacologica Si nica,, 23, (12): 1127-1141.

Yen DPD (1993). Antioxidative properties of methanolic extracts from peanut hulls. J. Am. Oil Chem. Soc., 70: 383-386.