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Research Article

ANTIOXIDANTS AND FREE RADICAL SCAVENGING ACTIVITY OF *MORINGA CONCANENSIS* ROOT BARK EXTRACTS

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ABSTRACT

Moringa concanensis, Nimmo. (Moringaceae) tree commonly known as Horseradish tree, Drumstick tree, Never Die tree, West Indian Ben tree, and Radish tree. Moringaceae tree is native through the sub- Himalayan tracts of India. *Moringa concanensis*, Nimmo. is widely used in India, since the Ayurveda and Unani medicinal systems use it for the treatment of several ailments. The antioxidative properties and total phenolic contents of methanol and acetone root bark extracts of *M. concanensis* were analyzed. Total phenolic content of methanol and acetone extract of 16.78 mg/g and 13.45 mg/g extract. Antioxidant capacities were examined by different chemical assays including, free radical scavenging (DPPH and ABTS), reducing power, metal chelating, inhibition of bleaching, nitric oxide, superoxide anion and hydroxyl radical scavenging activity. In general, the methanol extract were the most potent antioxidant suppliers and free radical scavengers. The present studies may be of importance in varietal improvement, nutraceutical and bio-pharmaceuticals as possible sources of cost-effective natural antioxidants.

Keywords: Antioxidant activity, *M. concanensis*, Free radical scavenging activity, Phenolics, Food composition.

INTRODUCTION

The genus Moringa Adans. (Family: Moringaceae) has more than 13 species (Verdcourt, 1985), of which two species viz. *M. oleifera* Lam. (syn. M. pterygospermaGaertn.) and *M. concanensis* Nimmo grow in India. *M. concanensis*, a small tree that resembles *M. oleifera* grows wild in India (Rajasthan, Madhya Pradesh, Gujarat, Maharashtra, Goa, Andhra Pradesh and Tamil Nadu). This little known species differs from the former in bipinnately compound longer leaves and yellow flowers streaked with pink or red. It is locally used for edible fruits and medicinal purpose (Singh *et al.*, 2000).

Antioxidants are of great importance in preventing stress that may cause several degenerative diseases (Helen *et al.*, 2000). On the other hand, antioxidants are molecules capable of stabilizing or deactivating free radicals before they attack cells (Kaliora *et al.*, 2006). Food antioxidants are important for human nutrition, decreasing oxidative damage to lipids, proteins and nucleic acids induced by free radicals (Soler-Rivas *et al.*, 2000). The frequent consumption of fruits and vegetables is associated with a lowered risk of cancer, heart disease, hypertension and stroke; the effect has been attributed to the presence of phytochemicals and antioxidants present in foods including flavonoids and anthocyanins (Lako *et al.*, 2007).There is an extended interest in using natural antioxidant compounds, as the consumer's pressure on food industry augments, to avoid chemical preservatives, due to the increasing evidence implies that synthetic antioxidant produce toxicity.

Many herbal medicines and food stuff are believed to have preventive effects on chronic diseases due to their radical scavenging or antioxidant properties (Potterat, 1997). In herbal products, phenolic compounds have been shown to be effective antioxidant constituents. Many polyphenolics exert more powerful antioxidant effect than vitaminE *in vitro* and inhibit lipid peroxidation by chain-breaking peroxyl-radical scavenging. They can also directly scavenge reactive oxygen species (ROS), such as hydroxyl, superoxide and peroxynitrite radicals (Tsao and Akhtar, 2005). Therefore in recent years considerable attention has been directed towards identification of plants with antioxidant ability that may be used for human consumption. In view of the several ethno botanical uses of *Moringa concanensis* described above, it was proposed to screen its successive extracts for the in vitro antioxidant activity using standard procedures.

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MATERIALS AND METHODS

Collection of plant materials

The plant material selected especially for the present study is the root bark of *Moringa concanensis*. The root barks were collected from the Villamuthur village, Perambalur Tk, Tamil Nadu state. After that the plant materials were dried under shade condition. After optimum drying, the bark materials were coarsely powdered separately and stored in well closed containers for further laboratory analysis.

Solvent Extraction

After defatting by petroleum ether, the root bark samples of *Moringa concanensis*(15 g) were extracted by stirring with 105 ml 80:20 (methanol: H_2O) at 25°C for 48 h and filtering through Whatman No. 4 filter paper. The residues were re-extracted with an additional 75 ml of methanol, as described above, for 3 h. The solvent of the combined extract was evaporated under low temperature at 40°C in incubator respectively. The remaining residues, after methanol extraction and air drying, were extracted by stirring with 105 ml 70:30 (acetone:H_2O) (v/v) at 25°C for 48 h and filtering through Whatman No. 4 filter paper. The solvent of extract was evaporated under low temperature at 40°C in an incubator (NSW make, New Delhi). The extract thus obtained was used directly for total phenolic and also for the assessment of antioxidant activity through various *in vitro* assays.

Estimation of total phenolics

The total phenolic content was determined according to Folin-Ciocalteau method (FCM) described by Siddhuraju and Becker (2003). FCM actually measures a sample's reducing capacity and can be considered as another antioxidant (electron transfer) capacity assay. For the assay, aliquots (100 µl) of extracts were taken in test tubes and the volume was made upto1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteau reagent (1N) and 2.5 ml of sodium carbonate solution (20 %) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 minutes and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents.

Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of phenolic extracts of root bark extracts of *M. concanensis* samples were estimated according to the procedure described by Benzie and Strain (1996) and as modified by Pulido *et al.* (2000). FRAP reagent (900 µl), prepared freshly and incubated at 37 ºC, was mixed with 90 µl of distilled water and 30 µl of test sample, or acetone (for the reagent blank). The test samples and reagent blank were incubated at 37 ºC for 30 minutes in a water bath. The FRAP reagent contained 2.5 ml of 20 mmol/l TPTZ solution in 40 mmol/l HCl plus 2.5 ml of 20 mmol/l $FeCl_3.6H_2O$ and 25 ml of 0.3 mol/l acetate buffer, pH 3.6 (Benzie and Strain, 1996). At the end of incubation period the absorbance readings were recorded immediately at 593 nm using a spectrophotometer. The known Fe (II) concentration ranging between 100 and 2000 µmol/l ($FeSO_4.7H_2O$) was used for the preparation of the calibration curve. The parameter Equivalent Concentration (EC_1) was defined as the concentration of antioxidant has a ferric- TPTZ reducing ability equivalent to that of 1 mmol/l $FeSO_4.7H_2O$. EC_1 was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution determined using the corresponding regression equation.

Phosphomolybdenum assay

The antioxidant activity of samples were evaluated by the green phosphomolybdenum complex according to the method of Prieto *et al.* (1999). An aliquot of 100 µl of sample solution was combined with 1ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 ml vial. The vials were capped and incubated in a water bath at 95^{o}C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported are mean values expressed as µmol of ascorbic acid equivalents/g extract (ascorbic acid equivalent antioxidant activity) using the calibration curve of ascorbic acid. Linearity range of the calibration curve was 0.05-0.25 µmol.

Metal chelating activity

The extracts (100 µl) were added to a solution of 2 mmol/l $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mmol/l ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 minutes. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The results were expressed as mg EDTA equivalents/g extract using the calibration curve of EDTA. Linearity range of the calibration curve was 0.5-2.5 µg (Dinis *et al*., 1994).

STABLE Free Radical Scavenging Activity Using DPPH Method

The antioxidant activity of the root bark extracts of *M.* concanensis sample extracts, ASC and BHA were measured in terms of hydrogen donating or radical scavenging ability, using the DPPH method (Brand-Williams *et al*., 1995) modified by Sanchez-Moreno *et al*. (1998). A methanolic solution (0.1 ml) of the sample extracts at various concentrations was added to 3.9 ml (0.025 g/l) of DPPH solution. The solution was incubated at room temperature for 60 minutes and the decrease in absorbance at 515 nm was determined at the end of incubation period with a spectrophotometer. The antioxidant activity of the extract was as expressed as g extract/g DPPH hydrogen donating ability.

Total antioxidant activity by radical cation ($ABTS^{\bullet+}$)

ABTS was dissolved in water to a 7 mM concentration, ABTS radical cation (ABTS) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark for 12-16 hours before use. Prior to the assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to 30 ^{o}C to give an absorbance at 734 nm of 0.700 ± 0.02 in a 1cm cuvette. The stock solution of the sample extracts in ethanol were diluted such that, after introduction of a 10 µl aliquot of each dilution into the assay, they produced between 20-80 % inhibition of the blank absorbance. After the addition of 1 ml diluted ABTS solution to 10µl of samples or trolox (final concentration 0-15 µmol) in ethanol OD (Optical density) was taken at 30 ^{o}C exactly 30 minutes after the initial mixing. Appropriate solvent blanks were also maintained in each assay. Triplicate determinations were made at each dilution of standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of trolox concentration (Re *et al*., 1999) described by Siddhuraju and Becker (2003). The unit of total antioxidant activity (TAA) is defined as the concentration of trolox having equivalent antioxidant activity expressed as µmol/g sample extracts using the calibration curve of trolox. Linearity range of the calibration curve was 0.25-1.25 mmol/l. The total antioxidant activity of ASC and BHA were also measured by ABTS method for comparison.

Nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside (SNP) and measured by the Griess reaction. Nitric oxide interacts with oxygen to produce nitrite ions that can be observed by Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci*et al*., 1994). Different concentrations of samples and sodium nitroprusside (5 mM final concentration) in phosphate buffer saline, pH 7.4, in a final volume of 1 ml were incubated at 25 °C for 150 minutes. A control experiment without samples but with equivalent amount of vehicles was conducted in an identical manner of the sample. After incubation, the reaction mixtures were mixed with Griess reagent (1 % sulfanilamide and 0.1 % naphthylethylene diamine dihydrochloride in 5 % H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 540 nm. The total antioxidant activity of ASC (500 µg) are also measured by nitric oxide scavenging method for comparison. The % nitric oxide scavenging activity was calculated by the following equation;

(%) Nitric oxide scavenging activity = (Control OD-Sample OD)/Control OD × 100.

Superoxide anion radical scavenging activity

The method used by Martinez *et al*. (2001) for determination of the superoxide dismutase was followed with modification (Dasgupta and De, 2004) in the riboflavin-light-nitroblue tetra zolium (NBT) system (Beauchamp and Fridovich, 1971). Each 3 ml of reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM EDTA, 75 µM NBT and 1 ml of sample solution. The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm after 10 minutes of illumination from a fluorescent lamp. The total antioxidant activity of BHA (150 µg) are also measured by superoxide anion radical scavenging method for comparison. The % superoxide anion radical scavenging activity was calculated by the following equation;

(%) Superoxide anion radical scavenging activity =

$$\frac{\text{Control OD-Sample OD}}{\text{Control OD}} \times 100.$$

Antihemolytic Activity

In order to induce free radical chain oxidation in erythrocytes, peroxyl radicals were generated by thermal decomposition of AAPH (dissolved in PBS; final concentration 50 mM). To study the protective effects of root bark extracts of *M. concanensis* samples against AAPH-induced hemolysis, an erythrocyte suspension at 2 % hematocrit was pre-incubated with the extracts of samples at 37 °C for 30 minutes, followed by incubation with and without 50 mM AAPH. This reaction mixture was shaken gently while being incubated at 37°C for 4 hours. In all experiments, a negative control (erythrocytes in PBS), as well as extract controls (erythrocytes in PBS with each extract) were used. The extent of hemolysis was determined spectrophotometrically according to a method reported earlier (Ko *et al*., 1997; Costa *et al*., 2009; Magalhaes *et al*., 2009; Carvalho *et al*., 2010). Briefly, aliquots of the reaction mixture were taken out at each hour of the 4 hours of incubation, diluted with saline, and centrifuged at 4000 rpm for 10 minutes to separate the erythrocytes. The percentage of hemolysis was determined by measuring the absorbance of the supernatant at 545 nm and compared with that of complete hemolysis by treating an aliquot with the same volume of the reaction mixture with distilled water.

(%) Antihemolytic activity = (Control OD-Sample OD)/Control OD × 100.

Hydroxyl radical scavenging activity

The scavenging activity of the extracts of root bark extracts of *M. concanensis* samples on hydroxyl radical were measured according to the method of Klein *et al.* (1991). The extracts were added to 1.0 ml of iron-EDTA solution (0.13 % ferrous ammonium sulphate in 0.26 % EDTA), 0.5 ml of EDTA solution (0.018 %) and 1.0 ml of DMSO (0.85 % v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22 %) and incubated at 80-90 ^{o}C for 15 minutes in a water bath. After incubation the reaction was terminated by the addition of 1.0 ml of ice-cold TCA (17.5 % w/v). 3 ml of Nash reagent (75 g ammonium acetate, 3 ml glacial acetic acid, 2 ml acetyl acetone per litre of

reagent) was added and left at room temperature for 15 minutes. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectrophotometrically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated by the following equation;

(%) HRSA = 1-(difference in absorbance of sample / difference in absorbance of blank) × 100

Inhibition of β-carotene bleaching

Two milliliters of a solution of β-carotene in chloroform (2 mg/10 ml) was pipetted into a flask containing 40 mg of linoleic acid and 400 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator at 45 °C for 4 minutes, and 100 ml of distilled water was added slowly to the semisolid residue with vigorous agitation to form an emulsion. 4.8 ml aliquot of the emulsion was added to a tube containing 0.2 ml of the antioxidant solution at 500 mg/l, and the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without β-carotene (Taga *et al*., 1984). The tubes were placed in a water bath at 50 °C, and the absorbance measurements were conducted again at 15 minutes intervals up to 120 minutes. All determinations were carried out in triplicate. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of β-carotene using the following formula: AA = [1- (A_0 - A_t)/(A'_0 - A'_t)] × 100, where A_0 and A'_0 are the absorbance of values measured at zero time of the incubation for test sample and control and A_t and A'_t are the absorbances measured in the test sample and control, respectively, after incubation for 120 minutes. The total antioxidant activity of BHA (50 µg) are also measured by inhibition of β-carotene bleaching method for comparison.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple-range test ($P < 0.05$) using SPSS 16. Values expressed are means of triplicate determinations ± standard deviation.

RESULTS AND DISCUSSION

Total phenolic contents

Phenolics or polyphenolsare plant secondary metabolites and are very important byvirtue of their antioxidant activity by chelating redox-active metalions, inactivating lipid free radical chains and preventing hydroperoxide conversions into reactive oxyradicals. The results of total phenolic contents investigated in this study were presented in Table-1. The amounts of phenols in methanol and acetone extract were found to be 16.78 mg/g and 13.45 mg/g. The findings of the present study result are good agreement with Hassan *et al.* (2013), Yen *et al.* (1996) and Hertog *et al.* (1993) which suggested that methanol is a widely used and effective solvent for extraction of antioxidants.

Free radical scavenging assays

The scavenging effects of methanol and acetone extracts on the DPPH and ABTS radical are illustrated in Table-2. The amount of sample needed to decrease the initial DPPH concentration by 50% is a parameter widely used to measure antioxidant activity. *Moringa concanensis* root bark methanol extracts significantly reduced DPPH radicals. The DPPH scavenging ability of the extract may be attributed to its hydrogen donating ability and root bark extract showed significant scavenging activity. Our present findings are similar to Sreelatha and Padma (2009) demonstrated that methanol extract of *M. oleifera* leaves significantly reduced DPPH radicals.

ABTS radical scavenging ability of both the samples can be ranked as BHA>MCA>MCM. The results obtained clearly imply that all the tested samples inhibit or scavenge the radical in a concentration manner. The acetone extracts of *M. concanensis* exhibited highest radical scavenging activity compared to methanol. The acetone extract had strong activity to quench ABTS radical which may be ascribed to the presence of phenolic compounds with hydroxyl group attached to the aromatic ring structures (Vinson *et al.,* 1998). Our present findings are similar to Moyo *et al.* (2012) who reported that acetone extract showed higher radical scavenging activity in *M. oleifera* leaves.

Reducing power by FRAP

In the reducing power assay, the presence of reductants (antioxidants) in the fractions would result in the reduction of Fe^{+3}/ferriccyanide complex to the ferrous form by donating an electron. The sequence for reducing power was BHA>MCM>MCA. Siddhuraju and Becker (2003) reported that the reducing power of bioactive compounds was associated with antioxidant activity. The findings obtained from this study agreed with Siddhuraju and Becker (2003) who showed that antioxidant properties were concomitant with the development of reducing power. Therefore, phenolic compounds present in *Moringa concanensis* root bark extracts are good electron donors and could terminate the radical chain reaction by converting free radicals to stable product.

Metal chelating and phosphomolybdenum

An important mechanism of antioxidant activity is the ability tochelate/deactivates transition metals, which possess the ability to catalyze hydro peroxide decomposition and Fenton-type reactions. Therefore, it was considered of importance to screen the iron (II) chelating ability of extracts (Manian *et al.,* 2008).The sequencefor chelating power of *M. concanensis* extracts can be ranked as BHA>MCA>MCM. Our present findings are similar to Hassan *et al.* (2013) reported that methanol extract had high chelating activity compared to the acetone extract. This general chelating abilityof phenolics is probably

related to the high nucleophiliccharacter of the aromatic rings rather than to specificchelating groups within the molecule.

The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) bythe antioxidant compounds and the formation of greenphosphate/Mo(V) complex with the maximal absorption at 695 nm (Prieto *et al.* 1999). The antioxidant capacity of different extracts of *M. concanensis* can be ranked in the order of BHA>MCA>MCM. The results obtained imply that the acetone extracts has a strong ability to act as antioxidant as compared to other extracts.

Nitric oxide scavenging assay

Nitric oxide is a key signaling in the physiological and pathologicalconditions and when react with macromolecules may induce inflammatory.It has been reported to play an important role in various inflammatoryprocesses such as carcinomas, muscle sclerosis, arthritis and ulcerative colitis (Hazra *et al.*, 2009). The effect of both solvent extracts of *M. concanensis* root bark against nitric oxide was evaluated (Fig.1). Both solvent extracts appreciably reduced the release of nitric oxide radicals. Our present findings are similar to Moyo *et al.* (2012) who reported that the percentage inhibition of NO radical by *M. oleifera* acetone and aqueous extract are 65.77 and 59.4.

Superoxide anion radical scavenging assay

Superoxide anion radical is a precursor to active free radicals that have the potential of reacting with biological macromolecules and there by inducing tissue damage (Pardini, 1995). Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non enzymatic reactions such as autooxidation by catecholamines. Fig. 2 shows the percentage inhibition of superoxide radical generation by methanol and acetone extracts of *M. concanensis* with BHA. Among the samples, MCM (82.3 %) exhibited a strong radical scavenging activity when compared with positive controls. Our present findings are similar to Nithiyanantham *et al.* (2012) who reported that methanol extract of *S. torvum* (85 %) exhibited a strong radical scavenging activity. Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals.

Hemolysis inhibition

Erythrocytes, which are the most abundant cells in the human body and possess desirable physiological and morphological characteristics, are exploited extensively in drug delivery (Hamidi and Tajerzadeh, 2003). The AAPH-induced oxidative damage on human erythrocytes has been extensively studied as a model for the peroxidative injury in biological membranes (Carvalho *et al.*, 2010). Among the samples, MCM (76 %) showed high haemolytic inhibition activity followed by MCA (69 %). Our results are in agreement with other studies showing that polyphenolics are able to protect erythrocytes from oxidative stress or increase their resistance to damage caused by oxidants (Youdim *et al.*, 2000; Costa *et al.*, 2009; Magalhaes *et al.*, 2009; Carvalho *et al.*, 2010).

Inhibition of β -carotene bleaching

The bleaching of β-carotene could be inhibited by antioxidants, which are capable of reducing the rate of chain reaction initiated during lipid peroxidation and transforming the reactive end product to a more stable form. The bleaching inhibition, measured by the peroxidation of β-carotene, is presented in Fig. 5. Bleaching inhibition of MCM (78 %) showed high antioxidant activity compared to the positive control BHA (76 %). The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralization the linoleate-free radical and other free radical formed in the system (Jayaprakasha *et al.*, 2001).

Hydroxyl radical scavenging assay

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage. The hydroxyl radical scavenging abilities of *M. concanensis* are shown in Fig.4. Among the various samples, MCA (76 %) showed high radical scavenging activity compared to methanol extract. The ability of extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and they seem to be good scavengers of active oxygen species, thus reducing the rate of reaction.

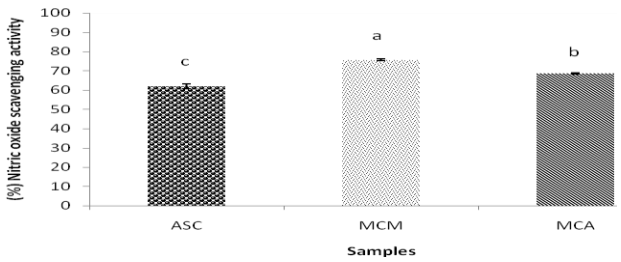

Figure 1. Nitric oxide scavenging activity of methanol and acetone extracts of *M. concanensis* (Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($P < 0.05$) different.MCM, *M. concanensis* methanol extract; MCA, *M. concanensis* acetone extract; ASC, Ascorbic acid).

Table 1. Total phenolics, frap, phosphomolybedenum and metal chelating activity of methanol and acetone extracts of *M. concanensis.*

S.No	Samples	Phenolics (mg/g)[a]	FRAP (mmol/g)[b]	Phosphomolybdenum (µmol/g)[c]	Metal chelating (mg/g)[d]
1.	MCM	16.78[a]±1.34	1568.83[b]±5.67	345.67[c]±5.87	3.67[c]±0.54
2.	MCA	13.45[b]±0.98	1348.56[c]±2.23	567.7[b]±1.34	7.86[b]±0.65
3.	BHA	-	11354[a]±1.34	3615.37[a]±7.88	13.45[a]±0.43

Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($P < 0.05$) different. MCM, *M. concanensis* methanol extract; MCA, *M. concanensis* acetone extract; BHA, Butylated hydroxyl anisole.

[a]mg tannic acid equivalents/g extract

[b]Concentration of substance having ferric-TPTZ reducing ability expressed as mmol Fe(II) equivalents/g extract.

[c]Ascorbic acid equivalent antioxidant capacity (µmol equivalent of ascorbic acid/g extract) through the formation of phosphomolybdenum complex.

[d] mg EDTA equivalents/g extract

Table 2. $DPPH^{\cdot}$ radical and $ABTS^{\cdot+}$ cation radical scavenging activities of methanol and acetone extracts of *M. concanensis.*

S.No	Samples	IC_{50} of DPPH (g extract/g DPPH)[a]	ABTS (mmol/g)[b]
1.	MCM	5.26[b]±0.47	6576[c]±0.56
2.	MCA	7.82[c]±0.56	7435[b]±0.67
3.	BHA	0.34[a]±0.01	7687[a]±0.56

Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($P < 0.05$) different. MCM, *M. concanensis*methanol extract; MCA, *M. concanensis* acetone extract; BHA, Butylated hydroxyl anisole.

[a]g of sample required to decrease one g of the initial DPPH concentration by 50%.

[b]Total antioxidant activity (mmol equivalent Trolox performed by using ABTS radical cation).

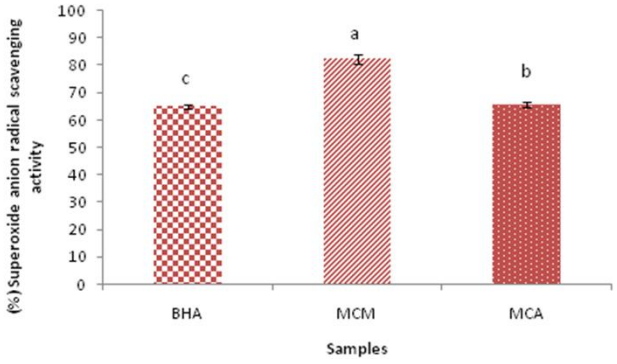

Figure 2. Superoxide anion radical scavenging activityof methanol and acetone extracts of *M. concanensis* (Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($P < 0.05$) different. MCM, *M. concanensis* methanol extract; MCA, *M. concanensis* acetone extract; BHA, Butylated hydroxyl anisole).

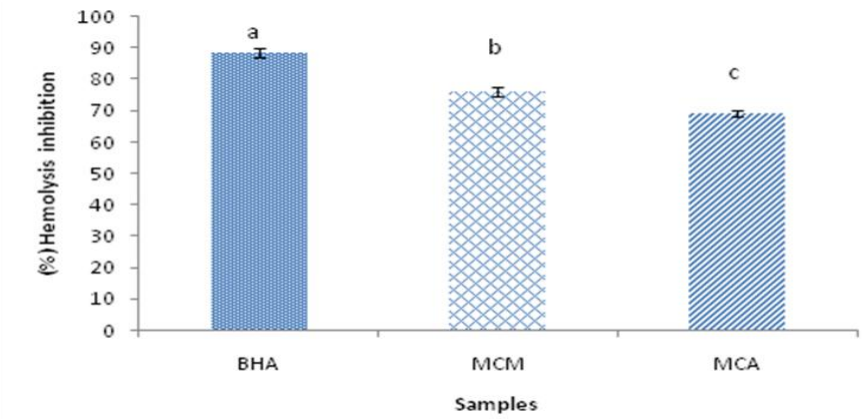

Figure 3. Hemolytic inhibition of methanol and acetone extracts of *M. concanensis* (Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($P < 0.05$) different. MCM, *M. concanensis* methanol extract; MCA, *M. concanensis* acetone extract; BHA, Butylated hydroxyl anisole).

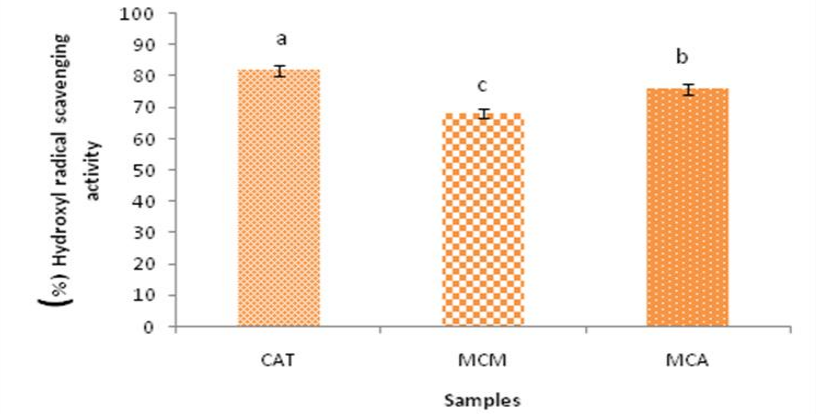

Figure 4. Hydroxyl radical scavenging activity of methanol and acetone extracts of *M. concanensis* (Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($P < 0.05$) different. MCM, *M. concanensis* methanol extract; MCA, *M. concanensis* acetone extract; CAT, Catalase).

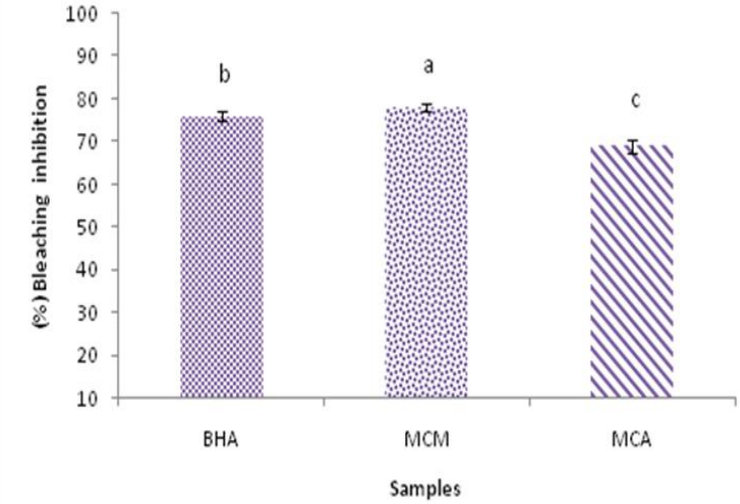

Figure 5. Inhibition of β -carotene bleaching activity of methanol and acetone extracts of *M. concanensis* (Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($P < 0.05$) different. MCM, M. concanensis methanol extract; MCA, M. concanensis acetone extract; BHA, Butylated hydroxyl anisole).

CONCLUSION

The results of the present work indicate that the methanol extracts possess high antioxidant activity and free radical scavenging activity. These assays are useful for establishing the ability of phenolics to chelate and reduce Fe^{3+} and have important applications for the pharmaceutical and food industries. However, further investigation of individual phenolic compounds, there in vivo antioxidant activity and the different antioxidant mechanisms is warranted.

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