PHYTOCHEMICAL SCREENING AND FREE RADICAL SCAVENGING ACTIVITY OF MORGINGA CONCANENSIS NIMMO LEAF EXTRACTS

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ABSTRACT

The objective of the present study was aimed to evaluate the qualitative analysis of phytochemical constituents and free radical scavenging activity of methanol extract of Moringa concanensis leaves. Phytochemicals are responsible for medicinal activity of plants. In the present study, an attempt has been made to document the presence of phytochemicals and radical scavenging activity of the medicinal plant Moringa concanensis leaves which is used in the indigenous system of medicine for therapeutic purposes. A plant contains many phytochemicals constituents including alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, steroids, carbohydrates, glycloses, amino acids and proteins. 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. The extract showed strong antioxidant capacities and free radical scavenging activity due to the presence of phytochemicals.

KEYWORDS: Moringa concanensis, Phytochemicals, antioxidant, DPPH.

INTRODUCTION

Moringa concanensis, a small tree that resembles Moringa oleifera grows wild in India. 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 that implies that synthetic antioxidant produce toxicity. Many herbal medicines and food staff are believed to have preventive effects on chronic diseases due to their radical scavenging or antioxidant properties (Potterat, 1997).

MATERIALS AND METHODS

Plant material

Plant material free from infection was collected from Esanai village, perambalur district, Tamilnadu, India. The plant sample was identified and authenticated by Dr. G. Murugan, Scientist D, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification No. BSI /SRC/5/23/2016/Tech-152. The plant leaf was used to prepare extracts for the study. The plants collected were washed with water to remove the soil and dust particles. Then they were dried in thoroughly shaded place and blended to form a fine powder and stored in airtight containers.

Preparation of Extract

The Moringa concanensis leaves were washed, shade dried and powdered using mixer grinder. The powdered material (10 g) was extracted with 100 ml of selected organic solvents (water, methanol, ethanol, chloroform...
and ethyl acetate) using soxlet apparatus and filtered through Whatmann No 1 filter paper. The filtrate was concentrated and dried under reduced pressure and controlled temperature. The concentrated extracts of the leaves were stored in small vials at -20° C and used for further analysis (Peach and Tracey, 1995).

Phytochemical Screening
Phytochemical evaluation for major phytochemicals was done using standard qualitative methods (Sowofora, 1993; Tiwari et al., 2011). Tests for presence of reducing sugars, alkaloids, anthraquinones, tannins, terpenoids, saponins, oils and fats, flavonoids and cardiac glycosides were carried out on both extracts. The methods used are briefly described below.

Alkaloids (Wagner’s test)
To 5 ml of the extract was added Wagner’s Reagent. A reddish-brown precipitate indicates presence of alkaloids.

Carbohydrates In a test tube, 5 ml of the filtrate was treated with 5 ml of Fehling’s solutions (A & B) and was heated; the appearance of a red precipitate indicates the presence of reducing sugars.

Cardiac glycosides (Keller-Killiani test)
0.5 ml of extract was diluted in 5 ml of water. 2 ml of glacial acetic acid was added with a drop of ferric chloride solution. 1 ml of concentrated sulfuric acid was used to underplay this. A brown ring at interface shows presence of cardenolides.

Saponins (Foam test)
5 ml distilled water were added to 0.5 ml of the extract in a test tube and shaken vigorously. The solution was observed for the formation of a persistent froth. Emulsion formation on mixture of the froth with olive oil indicates saponin presence.

Flavonoids
5 ml of sodium hydroxide were added to 5 ml of the extract. Formation of a deep yellow color that lessens when a few drops of dilute sulphuric acid are added indicates presence of flavonoids.

Amino acid
2 ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Reducing sugars (Fehling’s test)
1 ml of extract in 10 ml of water was mixed with 5 ml of boiling Fehling’s solution (A and B). A brick-red, orange or yellow precipitate showed presence of reducing sugars.

Steroids
The powder was dissolved in 2 ml of chloroform in a dry test tube. Ten drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution became red, then blue and finally became bluish which indicates the presence of steroids. Terpenoids (Salkowski’s test)
To 0.5 ml extract was added to 2 ml of chloroform. 3 ml of conc. Sulfuric acid was carefully added to the sides of the test tube to form a layer. Reddish-brown colour at the interface shows presence of terpenoids.

Phenolic compounds
To 1 ml of extract were added 2 ml of distilled water and a few drops of 10% ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

Tannins (Ferric chloride test)
0.5 ml of the extract was diluted with 10 ml water and boiled in a test tube. After boiling it was filtered and a few drops of 0.1% ferric chloride added. A yellow to red precipitate indicated presence of tannins.

DPPH radical scavenging activity
The DPPH scavenging effect was assayed according to the method of Makris et al., 2007. Various concentrations (100 - 500 µg / ml) of a leaf extract (4.0 ml) were mixed with 1.0 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken vigorously and left for 30 minutes at room temperature and the absorbance was measured at 517 nm. Ascorbic acid was used as a control. The DPPH radical scavenging activity was calculated as follows.

\[ I = \frac{A_o - A_1}{A_o} \times 100 \]

Where, \( A_o \) is absorbance of control reaction, \( A_1 \) is absorbance of test compound.

ABTS Radical Scavenging Activity
The ABTS+ radical scavenging effect was assayed according to the method of Re et al., 1999. The reaction was initiated by the addition of 1.0 ml of diluted ABTS+ to 10 µl of different concentrations (20 - 250 µg / ml) of leaf extract and also to 10 µl of ethanol as a control. Ascorbic acid was used as positive control. The absorbance was read at 734 nm after 6 minutes and the percentage inhibitions were calculated. The inhibition was calculated according to the equation,

\[ I = \frac{A_o - A_1}{A_o} \times 100 \]

Where, \( A_o \) is absorbance of control reaction, \( A_1 \) is absorbance of test compound.

Hydroxyl Radical Scavenging Activity
The Hydroxyl Radical scavenging effect was assayed according to the method of Rajeshwar et al., 2005. A reaction mixture of 3.0 ml volume contained, 1.0 ml of 1.5 mM FeSO₄, 0.7 ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and 1.0 ml of different concentrations (100 - 500 mg / ml) of leaf extract. After incubation for an hour at 37 °C, the absorbance of the hydroxylated salicylate complex was measured at 562
nm. Vitamin E was used as positive control. The percentage scavenging effect was calculated as,
\[
\text{Scavenging activity} = \frac{1 - (A1 - A2)}{A0} \times 100
\]
Where, \(A0\) is absorbance of the control, \(A1\) is absorbance in the presence of the extract; \(A2\) is absorbance without sodium salicylate.

**Nitric Oxide Scavenging Assay**

The interaction of Ethanolic leaf extract of Solanum lycopersicum with nitric oxide was assessed by the nitrate detection method by Madan et al., 2005. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured at 540 nm. The reaction mixture (6.0 ml) containing sodium nitroprusside (4.0 ml), phosphate buffer saline (PBS, 1.0 ml) and different concentrations (100 - 500 µg / ml) of a leaf extract (1.0 ml) in DMSO was incubated at 25°C for 15 minutes after incubation, 0.5 ml of the reaction mixture containing nitrite was removed, 1.0 ml of sulphonic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization and 1.0 ml of naphthyl ethylene diamine dihydrochloride was added, mixed well and allowed to stand for 30 minutes in diffused light. A pink coloured chromophore was formed. The absorbance of these solutions was measured at 540 nm against corresponding blank solutions. Rutin was used as a standard. The inhibition was calculated according to the equation,\[
I = \frac{A0 - A1}{A0} \times 100
\]
Where, \(A0\) is absorbance of control reaction, \(A1\) is absorbance of test compound.

**RESULTS**

**Phytochemical Screening**

The result of the preliminary phytochemical screening was carried out on the five different extracts of *Moringa concanensis* leaves and revealed the presence of a wide range of phytoconstituents including alkaloids, flavonoids, saponins, terpenoids, steroids, phenols, tannins, carbohydrates, glycosides, amino acids and proteins. The primary metabolites and secondary metabolites like carbohydrates, amino acids, proteins and alkaloids, flavonoids, terpenoids, tannins and glycosides are most commonly present in all the type of extracts. But phenol and flavonoids are absent in chloroform and ethyl acetate extract. It was represented in Table 1.

**Table 1. Qualitative analysis of phytoconstituents present in different solvent extracts of *Moringa concanensis* leaves.**

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Aqueous</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Proteins</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ → present in small concentration; ++ → present in moderately high concentration; +++ → present in very high concentration; -- → absent.

**DPPH Scavenging Activity**

The radical scavenging activity of the *Moringa concanensis* leaves extract estimated by DPPH is shown in (Fig 1 and Table 2). The scavenging effect of the plant extract of *Moringa concanensis* on the DPPH radical was 64.20 % at a concentration of 500 µg/ml and the scavenging activity also increased in a dose dependent manner. These results indicate that the extract has noticeable effects on scavenging the free radicals. The standard antioxidant vitamin C showed the maximum value of 70.10 % at a concentration range from 100 to 500 µg / ml, the scavenging effect also increased in a dose dependent manner. The inhibition value was found to be high in standard ascorbic acid when compared to the *Moringa concanensis* leaf extract. The IC50 of the extract was found to be 375 for *Moringa concanensis* leaf extract and for the standard antioxidant vitamin C was found to be 322.

**Table 2. DPPH Scavenging Activity.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>EEMC</td>
</tr>
<tr>
<td></td>
<td>11.30 ± 0.24</td>
</tr>
<tr>
<td>2</td>
<td>Standard (Vitamin C)</td>
</tr>
<tr>
<td></td>
<td>15.10 ± 0.50</td>
</tr>
</tbody>
</table>

The experiment was conducted in triplicates (n=3)
IC_{50} value of EEMC: 375.17 µg/ml
IC_{50} value of Vitamin C (standard): 332.46 µg/ml

Figure 1: DPPH Scavenging Activity.

Table 3. Abts Scavenging Activity.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>EEMC</td>
</tr>
<tr>
<td>2</td>
<td>Standard (Vitamin C)</td>
</tr>
</tbody>
</table>

The experiment was conducted in triplicates (n=3).

Figure 2: ABTS Scavenging Activity.

IC_{50} value of EEMC: 363.06 µg/ml
IC_{50} value of Vitamin C (standard): 346.91 µg/ml

Figure 3: ABTS Scavenging Activity.

Table 4. Hydroxyl Radical Scavenging Assay.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>EEMC</td>
</tr>
<tr>
<td>2</td>
<td>Standard (Vitamin C)</td>
</tr>
</tbody>
</table>

The experiment was conducted in triplicates (n=3).

Hydroxyl Radical Scavenging Assay

Hydroxyl radicals were generated from the substrate deoxyribose by the reaction of ferric-EDTA together with H_2O_2 and ascorbic acid. When the leaves extract was incubated with the above reaction mixture, it can prevent the damage against sugar. The results are shown in the (table 4 Fig 3). The scavenging activity of the leaves extract of M. concanensis against hydroxyl radical was found to be 66.30 %. Standard vitamin C for hydroxyl radical showed the inhibition value of 72.40 % at 500 µg/ml concentration. The inhibition value of standard vitamin C was high compared to the M. concanensis. IC_{50} value of the extract was found to be 338.64 µg/ml for M. concanensis. For the hydroxyl radical test, vitamin C was used as a standard where the IC_{50} values was found to be 310.83 µg/ml.

ABTS Scavenging Activity

The activity was found to be increased in a dose-dependent manner from 13.20 % to 60.44 % at a concentration of 50 - 250 µg/ml. The extract and vitamin C exhibited an IC_{50} value of 363.06 and 346.91 µg/ml respectively figure 2 and Table 3. Therefore, the ABTS radical scavenging activity of ethanolic extract of M. concanensis indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction.
IC\textsubscript{50} value of EEMC : 338.64 µg/ml  
IC\textsubscript{50} value of Vitamin C (standard) : 310.83 µg/ml

**Figure 3: Hydroxyl radical scavenging assay.**

Nitric Oxide Scavenging Assay

The scavenging of nitric oxide by leaf extract increased in a dose dependent manner as illustrated in (table 5 and Fig 4). The results were expressed as IC50 values. All the tested extracts showed nitric oxide scavenging activity. M. concanensis exhibited inhibition of 58.20 % at a concentration of 500 µg/ml. Rutin, a standard nitric oxide scavenging agent showed an inhibition of 62.10 % at the concentration of 500 µg/ml. The inhibition value of Vitamin C was found to be high when compared to the M. concanensis. IC50 value of the extract was found to be a 380.85 µg/ml for M. concanensis. For the nitric oxide test, Vitamin C was used as a standard were the IC50 values was found to be 417.01 µg/ml.

**Table 5: Nitric Oxide Scavenging Assay.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>EEMC</td>
<td>11.20 ± 0.50</td>
</tr>
<tr>
<td></td>
<td>23.60 ± 0.50</td>
</tr>
<tr>
<td></td>
<td>33.25 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>45.16 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>58.20 ± 0.44</td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Standard (Vitamin C)</td>
<td>16.50 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>25.70 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>35.90 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>50.10 ± 0.50</td>
</tr>
<tr>
<td></td>
<td>62.10 ± 0.40</td>
</tr>
</tbody>
</table>

The experiment was conducted in triplicates (n=3)

**Figure 4: Nitric Oxide Scavenging Assay.**

IC\textsubscript{50} value of EEMC : 380.85 µg/ml  
IC\textsubscript{50} value of Vitamin C (standard) : 417.01 µg/ml

**DISCUSSION**

Phytochemicals are responsible for medicinal activity of plants. These are non-nutritive chemicals that have protected human from various diseases. Phytochemicals are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. Primary metabolites comprises common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, Saponins, steroids, flavonoids, tannins and so on (Kumar et al., 2009; Vasu et al., 2009). Phytochemical analysis of *Moringa concanensis* leaf showed the existence of Terpenoids, flavonoids, tannins, reducing sugars and alkaloids. Flavonoids belong to the group of polyphenolic compounds and are typically known for health promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties (Aiyelaagbe and Osamudiamen, 2009). The plants of *Moringa concanensis* have the flavonoids in methanol, ethanol and aqueous extract of root. Antimicrobial activity is often attributed to phytochemicals such as terpenoids, flavonoids, tannins, phenolic compounds or presence of free hydroxyl groups (Rojas et al., 1992). Flavonoid based antimicrobial activity is thought to be a result of their capacity to disrupt enzymatic action in cell division, platelet aggregation and immunological responses and complex formation in the bacterial cell wall as well as extracellular and soluble proteins (Yadav and Agarwala, 2011). Flavonoids are also used by plants in their own defense against microbial agents. Terpenoid activity is said to be from their ability to disrupt membranes while tannins act by interfering with protein synthesis through binding to proline rich areas (Cowan, 1999).

Correspondingly, *Moringa concanensis* extracts also tested positive for phenolic compounds. The phenolic compounds are aromatic secondary metabolites that impart colour, flavour and associated with health benefits such as reduced risk of heart and cardiovascular diseases (Alothman et al., 2009; Bhat et al., 2011). According to (Aliyu et al. 2009) phenolic compounds account for most of the antioxidant activities in plants. Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities (Okwu and Okwu, 2004; Oomah, 2003). Terpenoids such as triterpenes, sesquiterpenes and diterpenes have been referred to as antibiotics, insecticidal, anthelmintic and antiseptic in pharmaceutical industry (Duke, 1992;
Proteins are the huge group of macromolecules and act as antibiotic and antimicrobial agents. Plants defend themselves against microbial pathogens by various defence responses including production of antimicrobial proteins which are small molecular mass antimicrobial peptides (Walter, 2012).

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radical was usually used as a substrate to evaluate antioxidant activity. It involves reactions of specific antioxidant with stable free radical 2, 2-diphenyl-1-picryl-hydrazyl (DPPH). As a result, there is reduction of DPPH concentration by the antioxidant, which decreases the optical absorbance of DPPH, and it is detected at 517 nm (Dhalwal et al., 2005). ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants. The extract efficiently scavenged ABTS radicals generated by the reaction between 2,2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABT Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases. β cells are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes (Ray and Hussain, 2002). In conclusion the methanol leaf extract of Moringa concanensis is a potential source of natural phytochemicals exhibiting antioxidant and radical scavenging activity that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and oxidative stress related degenerative diseases. The strong antioxidant capacities and free radical scavenging activity may be due to the presence of phytochemicals.

REFERENCES


