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The antioxidant activity of the methanolic leaf extract of Moringa concanensis Nimmo

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Abstract

The methanolic crude extract *Moringa concanensis* leaves were screened for their free radical scavenging properties such as 1,1-Diphenyl-2-Picryl-hydrazyl (DPPH) radical, hydroxyl radical scavenging activity, reducing power assay and superoxide radical scavenging activity. The IC_{50} value of methanolic extract from leaves of *M. concanensis* for DPPH radical scavenging, hydroxyl radical scavenging activity, reducing power assay and superoxide radical scavenging activity were 97.46, 45.3, 61.37 and 94.55 µg/ml respectively. The results showed high antioxidant activities in all the methods tested for the extracts. This examination recommended that *M. concanensis* may serve as a strong wellspring of natural antioxidant activity. The outcomes give valuable data on the pharmacological activities connected with alternate therapy.

Keywords *Moringa concanensis,* antioxidants, DPPH, free radical scavenging, reducing power.

INTRODUCTION

In both ancient and modern times, therapeutic plants have assumed an imperative part in healthcare. The Indian system of medicine, Ayurveda, exploits the therapeutic value of plants in making medications to treat different human sicknesses [1]. Likewise, these medications which utilize plants are essential as they are easily available, sensibly less expensive and non-harmful when contrasted with modern medicine [2].

One vital method for delivering free radicals in foods, drugs and even in living systems is through the oxidative process [3]. Oxidative anxiety, contributed by Reactive oxygen species (ROS, for example, superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxynitrite radicals, are identified with the pathogenesis of numerous illnesses [4]. Anti-oxidative defense mechanisms are the best way to wipe out and reduce the activity of free radicals. In healthy individuals, the production of free radicals is checked by the anti-oxidative defense system; be that as it may, when there is a depletion of antioxidant levels, oxidative stress is produced when equilibrium.

DNA mutation or/and damage target cells or tissues bringing about cell senescence and death may happen as a result of oxidation of lipid, DNA, protein, starch, and other natural particles by dangerous ROS. Oxidative stress has been accounted for to be the major causative variables for chronic and degenerative diseases, including atherosclerosis, ischemic coronary illness, maturing, diabetes mellitus, growth, immune suppression, neurodegenerative ailments and others [5]. Numerous restorative plants contain chemical compounds uncovering antioxidant agent properties.

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Substances which possess free radical chain reaction breaking properties are called antioxidants. As of late an expansion of interest has been seen using the helpful potential medicinal plants as antioxidants to diminish oxidative stress-induced tissue damage [6]. Ascorbic acid, carotenoids and phenolic compounds among the various normally occurring antioxidants are more effective [7]. They inhibit lipid peroxidation, scavenge free radicals and dynamic oxygen species and chelate heavy metal particles [8]. *In vitro* investigates these compounds in higher plants have indicated how they secure against oxidation harm by obstructing free radicals and reactive oxygen species [9]. Their part as potential cell antioxidants can be comprehended by their closeness to synthetic antioxidive agents of related structures.

The family Moringaceae has a single genus *Moringa* with two species recorded in India viz., *M.concanensis* and *M. oleifera*. *M. concanensisis* has been generally utilized as a part of India for the treatment of a several sicknesses . *M. concanensis* leaves have the prospective to act as a source of useful drugs because of presence of various phytochemical constituents such as alkaloids, flavonoids, phenol, terpenoids, saponin and carbohydrates [10]. *M. concanensis* leaves decrease blood pressure, menstrual agony, jaundice, constipation, skin tumor, diabetes and spleenomegaly. *M. concanensis* flowers additionally help in premature birth and leucorrhea [11]. Dried seeds of *M. concanensis* have been accounted for to have been used in ophthalmic preparation, venereal affection; ingoitre, glycosuria and lipid issue [12]. Ethanolic extract of *M. concanensis* have likewise been accounted for to have pain relieving and anti-inflammatory properties. In the present study, the plant *M. concanensis* has been considered for exploring the antioxidant activity of methanol extract of leaf utilizing four different assays.

MATERIALS AND METHODS

Selection of Plant Species

The leaves of *M. concanensis* were collected from the Kunnam of Perambalur District, Tamil Nadu. The plant materials were washed thoroughly 2-3 times with running tap water. Then the plant parts were a shade dried and powdered with a mechanical grinder and stored in an air-tight container for further analysis in the laboratory.

Authentication of Plant Materials

The plant was authenticated at Rapinet Herbarium, St.Joseph s College, Tiruchirappalli, Tamil Nadu and Botanical Survey of India [BSI], Southern Circle, Coimbatore. India. The specimen was labeled, numbered and annotated with the date of collection and locality.

Extraction of the Plant Materials

The powdered samples (25g) were then subjected to successive extraction in 250ml of methanol solvent by using a Soxhlet apparatus. The collected extracts were stored and then used for further analysis.

DPPH activity

DPPH radical scavenging activity was carried out by the method of Molyneux [13] To 1.0 mL of 100.0 µM DPPH solution in methanol, equal volume of the test sample in methanol of different concentration was added and incubated in dark for 30 min. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1.0 ml of methanol instead of test sample was added to the control tube. The different concentration of ascorbic acid was used as a reference compound. Percentage of inhibition was calculated from the equation [(Absorbance of control - Absorbance of test) / Absorbance of control)] × 100.

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of the test sample was estimated by following the method of Halliwell *et al.* [14]. The hydroxyl radical was generated by a fenton-type reaction. The reaction mixture contained 0.2 ml of sample in varied concentrations to which, 0.1 ml EDTA (1 mM)-FeCl3 (10 mM) mixture, 0.1 ml H_2O_2 (10 mM), 0.36 ml deoxyribose (10 mM), 0.33 ml phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid (1 mM) was added in sequence. The mixture was incubated at 37°C for 1 h followed by the addition of 1 ml of each of TCA (10 %) and TBA (0.67 %) and kept in boiling water bath for 20 min.The colour developed was read at 532 nm. The control tube contains phosphate buffer, instead of samples. Different concentration of ascorbic acid was used as a reference compound.

Reducing power assay

The reducing power of the methanolic crude extract of *M. concanensis* leaves was determined by the method of Oyaizu [15]. One ml of test sample solution was mixed with 2.5ml phosphate buffer (0.2 M, pH 6.6) and 1% of potassium ferricyanide (2.5 ml). The mixture was then kept in a 50°C water-bath for 20min. The resulting solution was then cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid, and centrifuged at 3000rpm for 10 min. The supernatant (5ml) was then mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride. The absorbance at 700nm was then detected after reaction for 10min. The higher the absorbance represents the stronger the reducing power. The reducing power assay was expressed in terms of Ascorbic acid equivalent per gram dry weight basis.

Superoxide Radical Scavenging Activity

The superoxide radical scavenging activity of the test sample was studied using the method of Liu *et al.* [16] with slight modifications. Superoxide radicals are generated in phenazine methosulphate (PMS) - (Nicotinamide adenine dinucletide (NADH) systems by oxidation of NADH and assayed by the reduction of Nitro Blue Tetrazolium (NBT). About 200 µl of methanol extract of different concentrations were taken in a series of test tube. Superoxide radicals were generated by 1.0 ml of Tris-HCl buffer (16.0 mM, pH-8.0), 1.0 ml of NBT (50.0 µM), 1.0 ml NADH (78.0 µM) solution and 1.0 ml of PMS (10 µM). The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured. A control tube containing Tris-HCl buffer was also processed in the same way without test sample. Different concentration of ascorbic acid was used as a reference compound.

RESULTS AND DISCUSSION

The free radicals are produced in aerobic cells because of utilization of oxygen in cell growth and development [17]. Free radicals cause diminishes in membrane fluidity, loss of enzyme receptor action and harm to membrane protein, leading to death [18]. These free radicals are included in various disorders like ageing, tumor, cardiovascular disease, diabetes, rheumatoid joint pain, epilepsy and degradation of unsaturated fats [17]. So the medicinal plants can be used as an alternative medication source to alleviate the ailments associated with oxidative stress [19]. Antioxidants from plant materials terminate the action of free radicals, thereby protecting the body from various diseases [20]. The antioxidant function by the inhibition of chain initiation, transition metal ion catalyst binding, peroxides deterioration, prevention of continued proton deliberation, and radical scavenging [21].

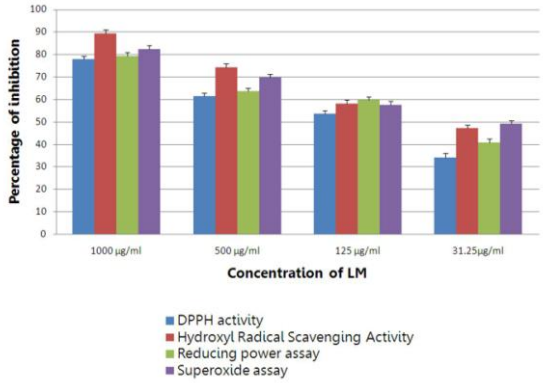

Figure.1: Antioxidant activity of the leaf extract of *M. concanensis*

The antioxidant properties of methanol concentrate of *M. concanensis* leaves were observed to be concentration dependent. DPPH antioxidant assay depends on the capacity of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a steady free radical, to decolorize within the sight of antioxidants. The DPPH radical contains an odd electron, which is in charge of the absorbance at 514 nm furthermore for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance [22]. The dose response curve of DPPH radical scavenging activity of methanol extract of leaves was observed and shown in Figure 1. The IC_{50} of methanol extract of leaves of *M. concanensis* for DPPH radical scavenging activity was 97.46 µg/ml concentrations (Figure-2) as opposed to that of ascorbic acid (IC_{50} 42.64 µg/ml) which is a well known antioxidant.

Hydroxyl radical can be framed by the Fenton response in the presence of reduced transition metals (such as Fe2+) and H2O2, which is known not the most receptive of all the reduced types of dioxygen and is thought to start cell harm in vivo [23] and harm to the cell by responding with polyunsaturated fatty acid moieties of cell membrane [24]. Searching of hydroxyl radical is an essential antioxidant activity as a result of the high reactivity of the OH radical, empowering it to respond with an extensive variety of atoms found in living cells, such as sugars, amino acids, lipids, and nucleotides [25] . Along these lines, evacuating OH* is essential for the defense of living systems. The hydroxyl radical scavenging potential of methanolic extracts of M.concanensis leaves is shown in figure-1. Hydroxyl radical scavenging activity was increased with increasing concentration of sample extracts. In the present investigation, the hydroxyl radical scavenging activity observed was in the range of 47.03–89.15 % at the concentration of 1000 - 31.25 µg/ml respectively. While scavenging hydroxyl radical, the IC50 value obtained was 45.3 µg/ml of the leaf methanol extracts (Figure-2) and for standard ascorbic acid, it was found to be 58.2 µg/ml.

A reducing power is a characteristic of reducing agent having the accessibility of molecules which can give electron and respond with free radicals and after that change over them into more stable metabolites and end the radical chain reaction [26]. Accordingly, methanolic extracts of *M.concanensis* leaves might contain a sizable amount of reductants (Figure-1) that can act as antioxidants. The IC_{50} value of reducing power assay in the leaf methanol extract was 61.37 µg/ml (Figure-2). The IC_{50} value of leaf methanol extract compared with the standard antioxidant ascorbic acid is 43.42µg/ml.

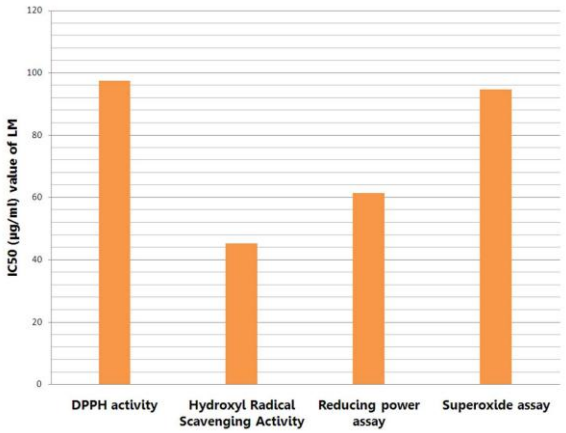

Figure.2: IC_{50} value LM using several methods of antioxidant assay

Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are created after the oxygen is taken into living cells. Superoxide anion changes to other harmful ROS and free radicals, for example, hydrogen peroxide and hydroxyl radical, which instigate oxidative harm [27]. When the concentration of the plant extract is from 31.25 to 1000 µg/ml, the superoxide radical scavenging activity ranged from 49.15 to 82.32% (Figure-1). Leaf extract of *M. concanensis* showed the IC_{50} value of 94.55 µg/ml which was presented in figure-2. The IC50 value of Leaf extract of *M. concanensis* compared with the standard antioxidant ascorbic acid (38.65 µg/ml). It was revealed that free radicals were scavenged by extracts in a concentration-dependent manner due presence of a free hydroxyl group of phenolic compounds in plants [28].

CONCLUSION

In the previous couple of years, enthusiasm for the hunt of new natural antioxidants has developed on the grounds that receptive oxygen species (ROS) generation and oxidative stress is connected to numerous diseases. The utilization of synthetic antioxidants generally leads to problems of toxicity. The results of this study demonstrate that the

methanolic extract of *M. concanensis* leaves could go about as hydrogen and/or electron donors and respond with free radicals, changing over them into more stable products and along these lines ending radical chain reactions. The methanol extract showed high level of antioxidant activity because of the presence of high amount of phenolics. *M. concanensis* could be developed as an effective antioxidant against numerous kinds of oxidative degenerative diseases. Usage of this plant product will be in a favorable position to mankind and expanded utilization will help in preclusion of chronic lifestyle diseases. From this study, it can be inferred that the plant *M. concanensis* leaves have better antioxidant activity and can be used for further research to define drugs for treating diseases related to oxidative stress.

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