International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 7, 2018

Original Article

EVALUATION OF FREE RADICAL SCREENING AND ANTIOXIDANT POTENTIAL OF *MORINGA CONCANENSIS* NIMMO-A MEDICINAL PLANT USED IN INDIAN TRADITIONAL MEDICATION SYSTEM

BRINDHA BANU BALAKRISHNAN[1,2]✉, KALAIVANI KRISHNASAMY[1]

[1]Department of Biochemistry, Kongunadu Arts and Science College, Affiliated to Bharathiar University, Coimbatore 641029, Tamil Nadu, India, [2]Department of Biochemistry and Bio-informatics, Dr. MGR-Janaki College of Arts and Science for Women Affiliated to University of Madras, Chennai 600028, Tamilnadu, India
Email: brindha.banu82@gmail.com

Received: 02 Apr 2018 Revised and Accepted: 15 Jun 2018

ABSTRACT

Objective: We aimed to investigate the free radical scavenging, antioxidant and hepatoprotective potential of *M. concanensis* Nimmo leaves.

Methods: Free radical scavenging activity was evaluated by employing various accepted *in vitro* systems, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), hydroxyl (OH) and nitric oxide (NO) radical. Antioxidant potential of *M. concanensis* Nimmo extract was assessed against H_2O_2 in goat liver by determination of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glucose-6-phosphatase dehydrogenase (G-6-PDH), total reduced glutathione (GSH), vitamin C, vitamin E activity, and lipid peroxidation (LPO).

Results: Results showed that the amount of plant extract of *M. concanensis* Nimmo required to scavenge 50% of the DPPH radicals was 401.80 µg/ml, ABTS radical was 353.14 µg/ml, OH radical was 433.71 µg/ml and NO radical was 371.24 µg/ml. Also, the pre-treatment of ethanolic extract of *M. concanensis* Nimmo leaves in goat liver showed a significant protection against H_2O_2 induced oxidative stress by retaining of antioxidants of SOD (4.76±0.25), CAT (26.81±0.25), GPx (6.41±0.34), GST (2.31±0.10), G-6-PDH (0.84±0.02), GSH (37.30±1.12), vitamin C (3.01±0.07), vitamin E (14.43±0.45) within normal range.

Conclusion: In conclusion, promising free radical scavenging, the antioxidant activity of *M. concanensis* Nimmo leaves can be able to treat various diseases caused by free radicals.

Keywords: *Moringa concanensis,* Liver, Antioxidants, Free-radicals, Oxidative stress, Alkaloids

DOI: http://dx.doi.org/10.22159/ijpps.2018v10i7.26403

INTRODUCTION

The liver plays an essential role in the metabolism of xenobiotics, catabolism, biochemical process, physicochemical functions of the body like oxidation, reduction, hydroxylation, hydrolysis, etc. Some hepatotoxic agents cause the liver damage is of severe consequences. In recent years, tremendous of scientific advancement in the field of Hepatology [1]. Oxidative stress is causative agents for various ailments include deoxyribonucleic acid (DNA) damage, cancer, and degeneration of cellular membrane leading to diabetes, liver diseases [2]. The production of oxidative stress can be monitored by an antioxidants system which is robust agents scavenge the free radicals and promotes their decomposition and suppresses their mechanism [3]. The model was very carefully designed to simulate *in vivo* intraperitonial exposure of oxidant. This study was prepared based on the recommendation by the fund for the replacement of animals in medical experimentation (FRAME) to diminish the use of live animals in research and to develop a model system that would simulate *in vivo* conditions [4].

M. concanensis Nimmo is one of the Indian medicinal plants belongs to the family of Moringaceae, used to treat various human diseases [5-8], *M. concanensis* Nimmo plant have confirmed anti-inflammatory [7, 9], anticancer [10], analgesic [9], anti-pyretic [7], anti-implantation [11], anti-convulsant [12], anti-microbial anti-fungal [13] and anti-hyperglycemic activity [14]. To the best of our knowledge, *Moringa concanensis* Nimmo leaves extract have not been investigated for phytochemical profiles, free radical scavenging, and *in vivo* simulated *in vitro* antioxidant properties. Therefore, in this study, we collected leaves to study on phytoconstituents, free radicals and antioxidant activity of the plant with the idea to identify new lead compounds for numerous diseases caused by free radicals.

MATERIALS AND METHODS

Collection of plant sample

Fresh leaves of *M. concanensis* Nimmo were collected from Perambalur, Tamil Nadu, India. The specimen sample was authenticated by the Botanical Survey of India (BSI), Tamilnadu Agricultural University, Coimbatore, Tamil Nadu, India. The voucher specimen (No. BSI/SRC/5/23/2016/Tech-151) was filed in the herbarium cabinet.

Chemicals, reagents and drug

Ascorbic acid, vitamin E, rutin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and H_2O_2 were acquired from Sigma and Aldrich (St. Louis, MO). All other chemicals and solvents were bought from Merck Chemicals Mumbai, India.

Preparation of plant extract

The leaves, rinsed with normal saline, dried in a shaded area to remove water, and then turned into powder using a blender. Of the powdered leaves, 10g was extracted with 100 ml of ethanol using a soxhlet apparatus and filtered by using Whatman No. 1 filter paper. The filtrate was then dried under reduced pressure and controlled temperature and concentrated. The concentrated extracts were kept in a small tight container at-20 ° C until further analysis.

Antioxidant assay: *in vitro*

DPPH radical scavenging activity

The DPPH radical assay was established according to the method of Makris *et al.* [15]. Various concentrations (100-500 µg/ml) of a leaf extract (4 ml) were added to 1 ml of DPPH methanol solution. The

mixture was shaken well and left for 30 min at room temperature, and the absorbance was measured at 517 nm. Ascorbic acid was used as a control. DPPH radical scavenging activity was calculated as follows;

$$\% = A_0 - A_1 \times 100$$

Where A_0 is absorbance of control reaction, A_1 is absorbance of the reaction system of the sample.

ABTS free radical scavenging assay

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging activity was conducted by the following method of Giao *et al.* [16]. The reaction was initiated by the addition of 1.0 ml of diluted ABTSto 10 µl of different concentrations (50-250 µg/ml) of a leaf extract along with 10 µl of ethanol as a control. Ascorbic acid was used as a standard. After 6 min, the optical density was read at 734 nm, and the percentage inhibitions were calculated. The inhibition was calculated according to the equation.

$$\% = A_0 - A_1 \times 100$$

Where, A_0 is absorbance of control reaction, A_1 is absorbance of reaction system of the sample.

Hydroxyl radical scavenging assay

Hydroxyl (OH) radical scavenging activity of the plant extract was calculated by the method of Rajeshwar *et al.* [17] with slight modifications. A reaction mixture of 3.0 ml volume contained, 1.0 ml of 1.5 mmol $FeSO_4$, 0.7 ml of 6 mmol hydrogen peroxide, 0.3 ml of 20 mmol sodium salicylate and 1.0 ml of different concentrations (100-500 mg/ml) of leaf extract was mixed well and incubated for 1 h at 37 °C. The absorbance of the hydroxylated salicylate complex was measured at 562 nm. Vitamin E was used as positive control. The percentage of scavenging potential was calculated as,

$$\text{Scavenging activity} = [1 - \frac{(A_0 - A_1)}{A_2}] \times 100$$

Where A_0 is absorbance of the control, A_1 is absorbance in the presence of the extract; A_2 is absorbance without sodium salicylate.

Nitric oxide scavenging assay

Nitric oxide (NO) radical scavenging potential was measured by the following method of Madan *et al.* [18]. The reaction mixture (6 ml) containing sodium nitroprusside (4 ml), phosphate buffer saline (PBS, 1 ml) and different concentrations (100-500 µg/ml) of a leaf extract (1 ml) in DMSO were incubated at 25 °C for 15 min. At the end of incubation, 0.5 ml of the reaction mixture containing nitrite was removed, 1.0 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotization and then 1.0 ml of naphthyl ethylenediamine dihydrochloride was added, mixed well and kept for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was read at 540 nm. Rutin was used as a standard.

The inhibition was calculated according to the equation,

$$I = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 is absorbance of control reaction, A_1 is absorbance of the test compound.

Antioxidant assay: *in vivo* simulated *in vitro* model

Sample collection and preparation

Briefly, the fresh liver was collected from the local slaughterhouse immediately after the sacrifice of the animal. The tissue was quickly forced into cold, sterile Hanks balanced salt solution (HBSS) with buffer and maintained at 4 °C. Very thin (≈ 1 mm) slices of the tissues were cut by using the sterile scalpel, and 250 mg of tissue was taken with 1.0 ml of sterile HBBS, in wide flat-bottomed flasks. The experiment was performed on goat liver comprising 5 groups. The groups were normal control (group I), H_2O_2 (2 ml/kg tissue) treated (group II), H_2O_2 and plant extract (20 mg/ml) treated (group III), H_2O_2 and Rutin (70 µg/kg tissue) treated (standard drug) (group IV), plant extract treatment (20 mg/ml) (group V). The binding compounds of H_2O_2 and extract were added and incubated at 37 ⍰ for 1h with a mild shaking. Appropriate control groups were also done.

Superoxide dismutase (SOD) activity assay

Superoxide dismutase activity was calculated by the following method of Das *et al.* [19]. Briefly, 1.4 ml aliquots of the reaction mixture (1.11 ml of 50 mmol phosphate buffer-pH 7.4, 0.075 ml of 20 mmol L-Methionine, 0.04 ml of 1% (v/v) Triton X-100, 0.075 ml of 10 mmol HCl and 0.1 ml of 50 mmol EDTA) was added with 100 µl of the sample, and were incubated at 37 °C for 5 min. Followed by 80 µl of riboflavin was added and the tubes were exposed for 10 min to 200 W Philips fluorescent lamps. The control tube contained equal amounts of buffer instead of samples. The sample and its respective control were run together. At the end of the exposure time, 1 ml of Griess reagent was added to each tube, and the absorbance was measured at 543 nm. The unit of enzyme activity was calculated by the amount of SOD ability of inhibiting 50% of nitrite formation under experiment circumstance.

Catalase (CAT) activity assay

Catalase activity was evaluated using the method described by Sinha [20]. Briefly, the reaction mixture of 0.9 ml of phosphate buffer (pH 7.0) and 0.1 ml tissue homogenate was added with 0.4 ml H_2O_2. The reaction was terminated after 15, 30, 45 and 60 seconds of incubation by adding 2 ml dichromate-acetic acid mixture. The tubes were kept in a boiling water bath for 10 min, cooled and the color developed. The absorbance was read at 590 nm. The enzyme activity is expressed as µmoles of H_2O_2 utilized/min/mg protein in tissues.

Glutathione peroxidase (GPx) activity

Glutathione peroxide activity was measured using the method described by Rotruck *et al.* [21]. In brief, the reaction mixture (0.2 ml of Tris buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of sample) was mixed with 0.2 ml glutathione followed by 0.1 ml H2O2. The mixture was thoroughly mixed and incubated at 37 °C for 10 min. At the end of incubation, the reaction was arrested by the addition of 0.5 ml of 10% TCA and centrifuged. The collected supernatant was assayed for glutathione by Ellman's method. Two ml of the supernatant, 3.0 ml Na2HPO4 solution and 1.0 ml of DTNB reagent were added and mixed well. The color developed was measured at 412 nm. The enzyme activity was expressed in term of µg of glutathione consumed/min/mg protein.

Glutathione S-Transferase (GST) activity

Glutathione S-transferase activity was given by the following method of Habig *et al.* [22]. The plant extract (0.1 ml) was added to the reaction mixture containing 1.0 ml of buffer, 1.7 ml of water and 0.1 ml of CDNB and incubated at 37 °C for 5 min. At the end of incubation, 0.1 ml of reduced glutathione was added. The increased optical density of the enzyme was measured at 340 nm. The enzyme activity was calculated in terms of µmoles of CDNB conjugate formed/min/mg protein.

Glucose-6-phosphate dehydrogenase (G-6-PDH) activity

G6PDH activity was tested by the method of Balinsky and Bernstein [23]. Briefly, 0.4 ml of Tris-HCl buffer, 0.2 ml of NADP, 0.2 ml of magnesium chloride, 1.0 ml water and 0.2 ml of sample were added in, and the reaction was started by the addition of 0.2 ml of glucose-6-phosphate, and the increased optical density was measured at 340 nm. The activity was reflected in terms of units/mg protein.

Total reduced glutathione (GSH) activity

GSH activity was measured using the described method of Moron *et al.* [24] with some slight modification. One ml of tissue homogenate was precipitated with using 4.0 ml of metaphosphoric acid, and then the precipitate was removed by centrifugation. Two ml of the supernatant was added to 2 ml of Na_2HPO_4 and 1.0 ml of DTNB reagent. The absorbance was measured within 2 min at 412 nm. The unit of reduced glutathione was expressed as µg/g tissue.

Estimation of vitamin C

Estimation of vitamin C was calculated as described by the method of Omaye *et al.* [25]. Briefly, 1 ml of tissue homogenate was precipitated by adding 5 % ice-cold TCA and centrifuged for 20 min at 3500 rpm. One ml of the supernatant was mixed with 0.2 ml of DTCS reagent and

incubated for 3 h at 37 °C. After that 1.5 ml of ice-cold 65 % sulphuric acid was added, mixed well and the solutions were kept at room temperature for an additional 30 min. The absorbance was measured at 520 nm. The results were expressed as µg/g tissue.

Estimation of vitamin E

Estimation of vitamin E was assayed as described the method of Rosenberg [26] with a few modifications. The homogenate (1.5 ml) was in addition to 1.5 ml of the ethanol and 1.5 ml of water. This was followed by the addition of 1.5 ml of xylene was added to the reaction mixture and centrifuged. Transferred 1.0 ml of xylene layer into another stoppered tube, taking care not to include any ethanol or protein, added 1.0 ml on 2, 2′-dipyridyl reagent to each tube, stoppered and mixed. Pipetted out 1.5 ml of the mixtures into spectrophotometer cuvettes and read the absorbance at 460 nm. The amount of vitamin E was calculated using the formula.

$$\frac{\Delta A520nm - \Delta A460nm \times Conc.[S] \times Total\ Volume}{\Delta A520nm \times Volume\ for\ Experiment \times Weight\ of\ Sample}$$

Estimation of lipid peroxidation (LPO)

The lipid peroxidation assay was measured by the following method of Uchiyama and Mahara [27]. One ml of the homogenate containing various concentrations was added to 2 ml of TCA-TBA-HCl reagent and mixed thoroughly. Then the mixture was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1,000g for 10 min. The absorbance was determined at 535 nm against a blank. The results were expressed as nmoles of MDA formed/min/mg protein.

Statistical analysis

Three replicates data were analyzed by using one-way analysis of variance (ANOVA), and the significance of the difference between the means was analyzed by Duncan's multiple range test ($P<0.05$). Values are expressed as the means±standard deviation of the mean.

RESULTS AND DISCUSSION

Free radical scavenging activity: *in vitro*

DPPH Radical scavenging activity

Globally with a great number of people seeking remedies and management of ailments free from side effects caused by synthetic chemicals. Greater medicinal values of secondary metabolites of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids and steroids from plants have been extensively evaluated their health benefits, because of its richness in antioxidants [28, 29]. Medicinal plants are natural resources. They are delivering new drugs and many of the modern medicine indirectly, and these medicinal plants have organized by refined traditional medicine practices that have been used for thousands of years by people in the world. Since the last few decades, researchers have more attention on medicinal plants, screening for their biological and pharmaceutical properties of the medicinal plants [30].

Free radicals are spontaneously produced as byproducts under certain environmental conditions in the biological systems during the normal metabolic process which may cause extensive damage to tissues and biomolecules by lipid peroxidation, breakdown of DNA strands, denaturation of proteins and disrupting cellular functions leading to various diseases including cancer, diabetes mellitus, chronic inflammation, and neurodegenerative disorders [31]. Although plenty of synthetic drugs are available to protect oxidative damage to the cells, the major drawback is their side effects, so it has restricted to use. To overcome this problem, consumption of natural antioxidants from food supplementation and traditional medicine is an alternative choice. Therefore, in this study, we determined the antioxidant property of *M. concanensis* Nimmo leaves against DPPH, ABTS, $OH^{\bullet}$ and $NO^{\cdot}$free radicals.

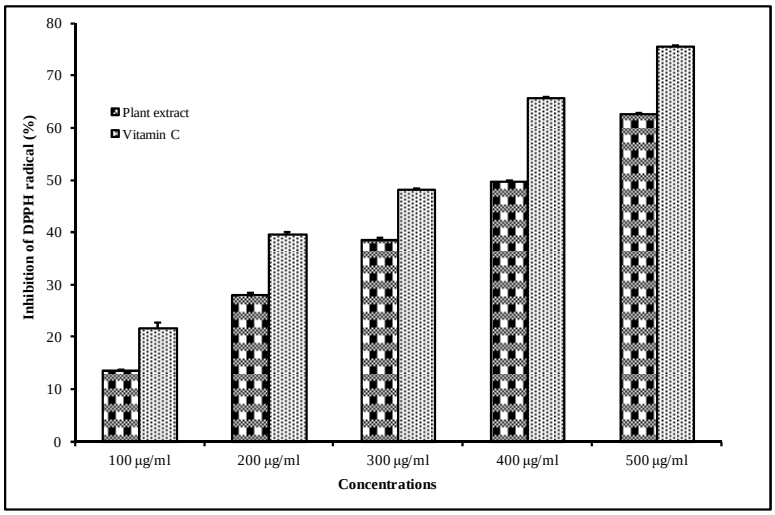

Fig. 1: Determination of DPPH radical scavenging activity (%) of ethanolic extract of *M. concanensis* nimmo leaves and vitamin C. All experiments were done in triplicate, results are represented as mean±Std ($P<0.05$)

The DPPH radical scavenging activity results of various concentrations of ethanol extract of *M. concanensis* Nimmo leaves and known antioxidant vitamin C is represented in fig. 1. Our current study results showed a greater DPPH radical scavenging property increased with increasing concentration of ethanolic extract with the concentration of 100-500 µg/ml. Also, we noted that the concentration which can scavenge 50% of the radical (IC_{50}) values of *M. concanensis* Nimmo extracts, and was 401.80 µg/ml and that of known antioxidant was 312.77 µg/ml.

The DPPH radical scavenging property results showed that the reaction of *M. concanensis* Nimmo leaves extract with purple colored DPPH radical converted to α-diphenyl-β-picryl hydrazine with increased with increasing concentrations. This radical scavenging activity occurs depends upon their antioxidant capacities could be due to the high phenolic and flavonoids contents present in the ethanolic extract of *M. concanensis* Nimmo leaves, especially phenols that can donate a hydrogen proton [32]. Phenols have been stated to own DPPH radical scavenging activity either one by donating hydrogen to the nitrogen-centred free radicals of DPPH altering it to a stable diamagnetic molecule identified as diphenyl-picryl hydrazine or by single electron transfer to ABTS radical [33]. The dose-dependent manner DPPH radical scavenging activity of *M. concanensis* Nimmo leaves extract compared with standard vitamin C. Our study results agreement with Usha and Pushpalatha [34], and Geetha *et al.* [35] who reported that the antioxidant potential of *Grewia heterotricha mast* and *Piper umbellatum* to the greater concentration of phenolic compounds respectively.

ABTS scavenging activity

Fig. 2 depicts the antioxidant activity of ethanolic extract of *M. concanensis* Nimmo leaves against ABTS radical scavenging. The ABTS radical scavenging activity of the extract was varied significantly (P<0.05) and ranged from 23.05% at 100 µg/ml of 60.26% 500 µg/ml concentrations. However, the IC_{50} value of *M. concanensis* Nimmo leaves was found to be 353.14 µg/ml, and known antioxidant compound of vitamin C was 280.25 µg/ml. The highest percentage of scavenging property was noted in our study, and our results suggested the high amount of antioxidant potential of *M. concanensis* Nimmo leaves.

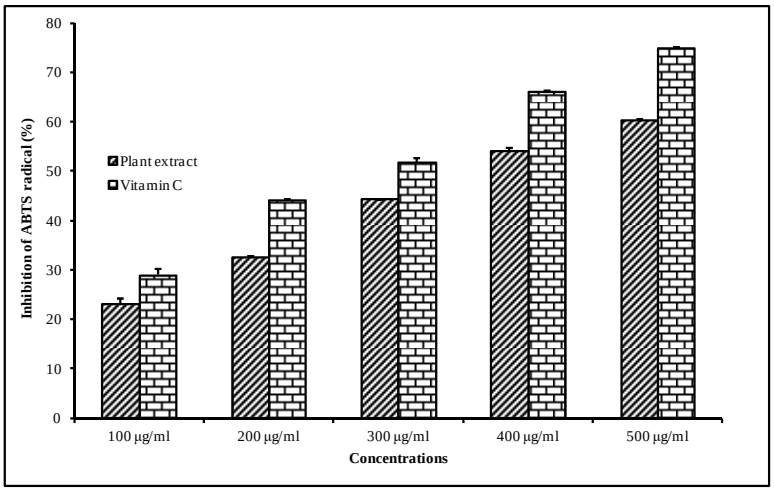

Fig. 2: Determination of ABTS radical scavenging activity (%) of ethanolic extract of *M. concanensis* Nimmo leaves and vitamin C. All experiments were done in triplicate, results are represented as mean±standard deviation (P<0.05)

ABTS is a blue chromophore produced assay. This ABTS radical scavenging activity is measured to prove the ability of the antioxidant compounds of the *M. concanensis* Nimmo leaves extract by hydrogen donating to the ABTS centered free radicals using a blue chromophore. Among our current study results, the *M. concanensis* Nimmo leaves extract showed the antioxidant activity increased with increasing concentration (P>0.05). This greater antioxidant property of the extract may be due to the higher amount of lipophilic and hydrophilic compounds in the leaves. The present study results were agreed with Hagerman *et al.*, [36] and Goncalves *et al.*, [37] who reported in the previous study that the high molecular weight phenolics have more efficient to scavenging free radicals.

Hydroxyl radical scavenging activity

The efficiency of an ethanolic extract of *M. concanensis* Nimmo leaves to inhibit hydroxyl-radical-mediated damage was determined at a concentration of 100-500 µg/ml. It was observed that the *M. concanensis* Nimmo showed the minimum activity of 12.30 % at 100 µg/ml and maximum activity of 58.22 % at 500 µg/ml concentration, indicating that the hydroxyl radical scavenging activity of *M. concanensis* Nimmo occurred in a dose-dependent manner and IC_{50} values of extract was 433.71 µg/ml and known antioxidant (vit E) was 349.01 µg/ml (fig. 3). This result indicates the scavenging capacity of *M. concanensis* Nimmo against hydroxyl radicals.

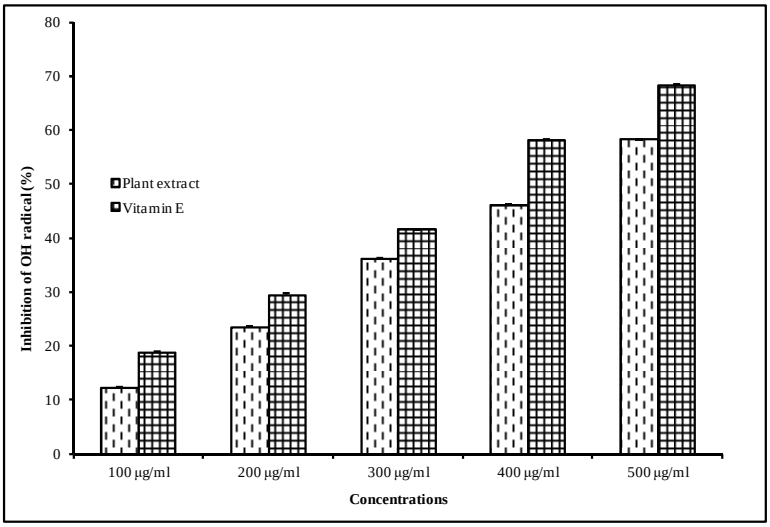

Fig. 3: Determination of Hydroxyl radical scavenging activity (%) of ethanolic extract of *M. concanensis* Nimmo leaves and vitamin E. All experiments were done in triplicate. Results are represented as mean±standard deviation (P<0.05)

The Fenton reaction produces hydroxyl radicals ($OH^{\bullet}$) which degrade DNA deoxyribose, by using reduced transition metals and H_2O_2, which is known to be the most reactive species of all the reduced forms of dioxygen and may cause to DNA fragmentation and DNA strand

breakage [38]. The scavenging of OH• radical is an important for the measurement of antioxidant activity because of very high reactivity of the OH• radical, allowing it to counter with a wide range of biomolecules found in living cells, such as sugars, amino acids, lipids, and nucleotides. Thus removing OH• is very important for the protection of living systems. The OH• radical scavenging potential of *M. concanensis* Nimmo leaves extract is shown in fig. 4. The leaf extracts showing OH• radical scavenging activity was in a dose-dependent manner. This OH• radical scavenging activity may be due to the presence of the hydrogen donating capacity of phenolic compounds in the extract. The results of the current study were agreed with Sutar and Kalaichelvan [39] who reported in the previous study that leaf extract of *Holoptelea integrifolia* showed hydroxyl radical scavenging property with dose dependant manner.

Nitric oxide scavenging activity

In this study, ethanolic extract of *M. concanensis* Nimmo leaf displayed a significant NO scavenging activity at all the selected concentrations 100-500 µg/ml (fig. 4) when compared with that of the known antioxidant Rutin at a concentration of 100-500 µg/ml. It was also noted that the IC_{50} values of *M. concanensis* Nimmo and Rutin were observed to be 371.24 µg/ml and 293.60 µg/ml mg/ml respectively. These results indicate that the NO scavenging activity of *M. concanensis* Nimmo in a dose-dependent manner.

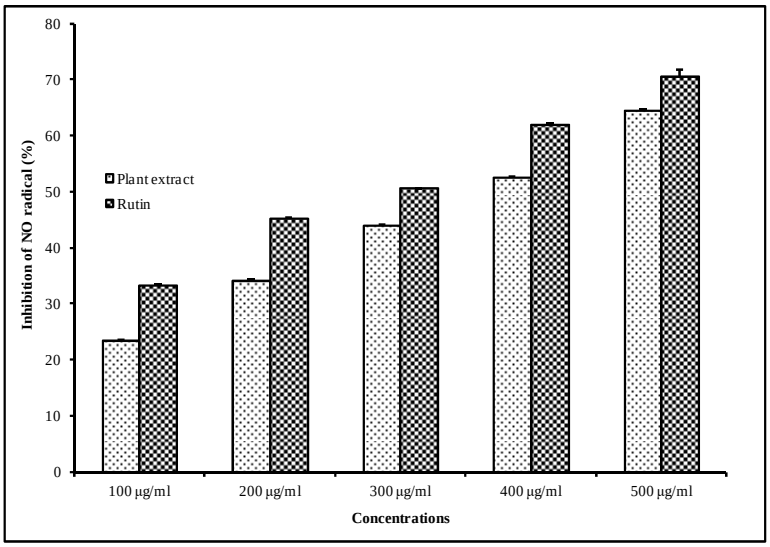

Fig. 4: Determination of nitric oxide radical scavenging activity (%) of ethanolic extract of *M. concanensis* nimmo leaves and rutin. All experiments were made in triplicate, results are represented as mean±standard deviation (P<0.05)

NO is an effective pleiotropic intermediary in physiological development and which reacts with the superoxide anion to form a potentially cytotoxic molecule of peroxynitrite ($ONOO^-$). However, overproduction of NO·radical caused various diseases such as diabetes, carcinomas, and arthritis [40]. Our study results showed that the ethanolic extract of *M. concanensis* Nimmo inhibited NO·radical formation by scavenging NO^-. It was might be due to the phenolic and flavonoid compounds in the extracts, which participate with oxygen to react with NO and thus inhibited the formation of nitrile [41].

Our research data are supported by Jethinlalkhosh JP [42] findings who reported in the previous study, the hydroethanolic extracts of *Pothos scandens* L. showed moderate NO radical scavenging activity. The antioxidant activity of ethanolic extract of *M. concanensis* Nimmo leaves of this plant may aid to prevent the chain reactions prompt by overproduction of NO and may play an important function in preventing inflammatory signalling processes.

Antioxidant activity *in vivo* simulated *in vitro* model

The antioxidant defence machinery includes enzymatic and non-enzymatic antioxidants play an important role in satisfying the physiological level of O_2 and H_2O_2 and destroying the peroxides caused by inadvertent contact with toxic drugs. Some natural medications for antioxidant outlines may help to protect health when took continuously as modules of nutritious food, spices or remedies. Between the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) stand the first line of defence besides oxidative injury of the tissues. SOD is the main factor of the defence mechanism in the antioxidant system involved against oxidative stress through catalyzing the dismutation of superoxide radicals (O_2^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) [43]. Most of the cells contain heme-containing enzyme catalase, which catalyses the breakdown of H_2O_2 to water and oxygen. Cytosolic catalase is considered as essential in the inactivation of various environmental mutagens [44]. In the present study, the H_2O_2 treated group showed the significantly increased the level of enzymatic and non-enzymatic antioxidants. The pretreatment of ethanolic extract of *M. concanensis* Nimmo leaves to the liver homogenate by *in vitro* stimulated *in vivo* model, resulted in significant elevation of enzymatic antioxidant enzymes of SOD and CAT activity in the liver is indicative of the decreased oxidative stress. In this study, the significant increases in the activity of the leaf extract indicate that they can efficiently remove the oxidative stress.

Antioxidant activity of *M. concanensis* Nimmo leaves extract on enzymatic and non-enzymatic antioxidant parameters in H_2O_2 administrated liver tissue are shown in table 1. In this study, H_2O_2 administered group displayed a significantly decreased (P<0.05) on the tissue enzymatic (SOD-3.37±0.26; CAT-15.88±0.59; GPx-3.35±0.21; GST-1.46±0.09 and G6PDH-0.52±0.01) and non-enzymatic antioxidants (GSH-25.49±1.06; vit C-2.29±0.03 and vit E-12.14±0.49) level as compared with control group. EEMCL+H_2O_2 pre-treatment group showed the significant increase in the enzymatic (SOD-4.00±0.16; CAT-23.89±0.36; GPx-5.44±0.14; GST-2.31±0.10 and G6PDH-0.86±0.00) and non-enzymatic antioxidant (GSH-33.36±0.69; vit C-3.07±0.01 and vit E-14.85±1.15) levels when compared with H_2O_2 administrated group (P>0.05). But this increased level was not as great as compared with those treated with Rutin (SOD-5.40±0.18; CAT-27.30±0.77; GPx-6.37±0.07; GST-2.42±0.01 and G6PDH-0.95±0.01; GSH-35.68±0.40; vit C-3.25±0.04 and vit E-16.51±0.15). H_2O_2 intoxicated group showed the significantly elevated level of LPO as indicated by elevated levels of MDA. EEMCL alone pre-treatment group significantly decreased the MDA levels, which were similar to those treated with standard drug group.

Table 1: Efficacy of an ethanolic extract of *M. concanensis* nimmo leaves on enzymic and non-enzymatic antioxidants against H_2O_2 induced oxidative stress in goat liver of control and experimental groups

Group	Group I	Group II	Group III	Group IV	Group V
	Control	H_2O_2 ml/kg	EEMCL 20 mg/kg+H_2O_2	Rutin 70 mg/kg+H_2O_2	EEMCL 20 mg/kg
Protein	10.95±0.12	4.35±0.12[a]	4.86±0.09[b]	6.80±0.11[b]	10.35±0.10[c]
LPO	29.30±0.18	89.63±3.72[a]	43.16±1.92[b]	35.28±2.06[b]	27.79±0.58[c]
SOD	6.41±0.54	3.37±0.26[a]	4.76±0.25[b]	7.29±0.11[b]	5.24±0.15[c]
CAT	30.94±1.09	21.91±0.62[a]	26.81±0.25[b]	29.01±0.57[b]	30.32±0.06[c]
GSH	40.32±0.69	27.34±1.06[a]	37.30±1.12[b]	37.77±0.40[b]	39.39±1.06[c]
GPx	7.35±0.31	4.82±0.32[a]	6.41±0.34[b]	7.01±0.33[b]	7.25±0.23[c]
G6PDH	1.01±0.01	0.57±0.02[a]	0.84±0.02[b]	0.92±0.01[b]	0.98±0.01[c]
Vit C	3.15±0.03	2.46±0.09[a]	3.01±0.07[b]	3.04±0.04[b]	3.09±0.01[c]
Vit E	17.37±0.37	13.23±0.56[a]	14.43±0.45[b]	16.53±0.08[b]	17.01±0.24[c]

Values are expressed as mean±SD (n=3)

Comparison:-a: Group II compared with Group I; b: Group III compared with Group II; c: Group V compared with Group I

Units: Protein-g/dl; LPO-MDA (n mol/mg tissue); SOD-50 % inhibition of nitrite formation/min/mg protein; CAT-µmoles of H_2O_2 decomposed/min/mg protein; GSH-µg/g tissue; GPx-µg of glutathione utilized/min/mg protein; G6PD-0.01 OD change/min/mg protein; Vitamin-C µg/g tissue; Vitamin-E µg/g tissue.

Glutathione-s-transferase (GSH) is a tripeptide, non-enzymatic antioxidant existent in the hepatocytes, which is a primary component of the all antioxidant defence systems that keeps the membrane protein thiols of hepatocytes from the toxic effects of reactive oxygen metabolites like H_2O_2 and superoxide radicals [45]. The decline of GSH and GPx level in the H_2O_2 treated group might be due to its utilization by the excessively generated free radicals in the liver resulting in tissue injury. However, the subsequent recovery in liver tissue treated with ethanolic extract of *M. concanensis* Nimmo leaves might be due to de-novo GSH synthesis or GSH restoration (GSSG to GSH). GSH, apart from being a potent antioxidant by itself also acts as a substrate for antioxidant enzymes like GPx and GST [46]. The lowered level of the GPx may be due to correlated to reduced availability of its substrate. Oxidative stress is regulated by also GPx which in turn requires glutathione as a cofactor. It catalyzes the oxidation of GSH to GSSG at the expenditure of H_2O_2. It is noted in both cytosol (70%) and mitochondria (30%) of various tissues [47]. The level of this enzyme was decreased in the toxic group compared with control. From table 2, the levels of enzymatic and non-enzymatic antioxidants were improved in the treatment group compared with the standard antioxidant rutin. Glucose 6-phosphate dehydrogenase (G6PD) is also one of the key enzymes for the group of NADPH, which is consumed for the regeneration of various antioxidant molecules.

Vitamin-C is an essential nutrient, widely distributed in all tissues of the body and is necessary for a wide range of vital metabolic process in animals and plants. In living organism vitamin-C is an antioxidant, it defends the body against oxidative stress and also it is a cofactor for several dynamic enzymatic reactions. The study results showed the lowered level of vitamin C in the H_2O_2 treated group. This diminished level of vitamin C may be due to the increased level of lipid peroxidation. Retrieval towards standardization of these enzyme produced by *M. concanensis* Nimmo leaves treatment was nearly similar to that retained by rutin. Ethanol extract of *M. concanensis* Nimmo leaves showed the suppressed levels of lipid peroxidation in goat liver homogenate, and this result agreed with Vidya *et al.* [48].

CONCLUSION

The ethanolic extract of *M. concanensis* Nimmo revealed the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, amino acids and proteins in the leaves. The present investigation indicates that *M. concanensis* Nimmo leaves extract, rich in total phenolics and flavonoids. We observed that *M. concanensis* Nimmo leaves accounted for the potent antioxidant properties noticed against DPPH, ABTS, $OH^{\bullet}$, and $NO^{\cdot}$radicals. Our current study indicates that the ethanolic extract of *M. concanensis* Nimmo leaves to improve the enzymic and non-enzymic antioxidant status against *in vivo* simulated oxidative stress induced by H_2O_2 in an *in vitro* model. This study strongly permits closer attention to this traditional medicinal plant for the development of new molecules to treat various complications initiated by free radicals. However, further studies are needed to isolation, characterization of the active antioxidant phytoconstituent in *M. concanensis* Nimmo leaves, and to investigate the antioxidant defence and hepatoprotective mechanisms in *in vivo*.

ACKNOWLEDGEMENT

We are very much thankful to Secretary and Joint Secretary of Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India for providing facilities and their immense support for the success of this research.

AUTHORS CONTRIBUTIONS

Brindha Banu Balakrishnan and Kalaivani Krishnasamy conceived and designed the current study. Brindha Banu Balakrishnan performed the experiment and data analysis. The manuscript of the study was done by Brindha Banu Balakrishnan. The revision of the manuscript was done by Brindha Banu Balakrishnan.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest

REFERENCES

1. Nazeema TH, Brindha V. Antihepatotoxic and antioxidant defense potential of *Mimosa pudica*. Int J Drug Dis 2009;1:1-4.
2. Samuel EO, Edifofon A, Omolola TF, Kevwe BE, Oluyemisi FK. *Annona senegalensis* persoon (Annonaceae): a review of its ethnomedicinal uses, biological activities and phytocompounds. J Pharm Phytochem 2016;5:211-9.
3. Gomathi D, Ravikumar G, Kalaiselvi M, Vidya B, Uma C. *In vitro* free radical scavenging activity of ethanolic extract of the whole plant of *Evoluulus alsinoides* L. Chinese J Int Med 2015;21:453-8.
4. http://www.frame-uk.demon.co.uk/atlahome.htm. [Last accessed on 01 Mar 2018]
5. Anbazhagan S, Dhandapani R, Anandhakumar P, Balu S. Traditional medicinal knowledge on *Moringa concanensis* nimmo of perambalur district, Tamilnadu. Ancient Sci Life 2007;26:42-5.
6. Ravichandran V, Arunachalam G, Subramanian N, Suresh B. Pharmacognostical and phytochemical investigations of *Moringa concanensis* (Moringaceae) an ethnomedicine of Nilgiris. J Pharm Phytother 2009;1:76-81.
7. Jayabharathi M, Chitra M. Evaluation of anti-inflammatory, analgesic and antipyretic activity of *Moringa concanensis* nimmo. J Chem Pharm Res 2011;3:802-6.
8. Santhi K, Sengottuvel R. Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. Int J Curr Microbiol Appl Sci 2016;5:633-40.
9. Rao CV, Hussain T, Verma AR, Kumar N, Vijaykumar M, Reddy GD. Evaluation of the analgesic and anti-inflammatory activity of *Moringa concanensis* tender fruits. Asian J Trad Med 2008;3:95-2.
10. Balamurugan V, Balakrishnan V, Robinson JP, Ramakrishnan M. Anticancer and apoptosis-inducing effects of *Moringa concanensis* using hepG2 cell lines. Bang J Pharmacol 2014;9:604-9.
11. Shukla S, Mathur R, Prakash AO. Anti-implantation efficacy of *Moringa concanensis* nimmo in rats. Int J Crude Drug Res 2008;26:29-32.

12. Joy AE, Kunhikatta SB, Manikkoth S. Anti-convulsant activity of ethanolic extract of *Moringa concanensis* leaves in swiss albino mice. Arch Med Health Sci 2015;1:6-9.
13. Balamurugan V, Balakrishnan V. Evaluation of phytochemical, pharmacognostical and antimicrobial activity from the bark of *Moringa concanensis* nimmo. Int J Curr Micobiol Appl Sci 2013;2:117-5.
14. Brindha Banu B, Kalaivani K. *Moringa concanensis* nimmo ameliorates hyperglycemia in 3T3-L1 adipocytes by upregulating PPAR-γ, C/EBP-α via Akt signalling pathway and STZ-induced diabetic rats. Biomed Pharmacother 2018;108:719-28.
15. Makris DP, Boskou G, Andrikopoulos NK. Recovery of antioxidant phenolics from white vinification solid by-products employing water-ethanol mixtures. Bioresour Technol 2007;98:2963-7.
16. Giao MS, Gonzalez SML, Rivero PMD, Pereira CI, Pintado ME, Malcata FX. Infusions of portuguese medicinal plants: dependence of final antioxidant capacity and phenolic content on extraction features. J Sci Food Agric 2007;87:2638-47.
17. Rajeshwar Y, Senthilkumar GP, Malay AG, Mazumder UK. Studies on *in vitro* antioxidant activities of methanol extract of *Mucuna pruriens* (Fabaceae) seeds. Eur Bull Drug Res 2005;13:131-8.
18. Madan MP, Raghavan G, Ajay KSR, Prabhu P. Free radical scavenging potential of *Saussurea costus*. Acta Pharm 2005;55:297-4.
19. Das S, Vasishat S, Snehlata R, Das N, Srivastava LM. Correlation between total antioxidant status and lipid peroxidation in hypercholesterolemia. Curr Sci 2000;78:486-7.
20. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-4.
21. Rotruck JT, Pope AL, Ganther HE. Selenium, biochemical role as a component of glutathione peroxidase purification and assay. Science 1973;179:588-90.
22. Habig W, Pabst MJ, Jacob WB. Glutathione-S-transferase the first enzyme step in mercapturic acid formation. J Biol Chem 1974;249:7130-9.
23. Balinsky D, Bernstein RE. The purification and properties of glucose-6-phosphate dehydrogenase from human erythrocytes. Biochim Biophys Acta 1963;67:313-5.
24. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582:67-78.
25. Omaye ST, Turnbull TP, Sauberchich HC. Selected methods for determination of ascorbic acid in cells, tissues and fluids. Method Enzymol 1979;62:3-11.
26. Rosenberg HR. Chemistry and physiology of the vitamins. *Interscience Publishers Inc*., New York; 1992. p. 452-3.
27. Uchiyama M, Mihara M. Determination of MDA precursor in tissues by the thiobarbituric acid test. Anal Biochem 1978;86:271-8.
28. Swargiary A, Roy B. Phytochemical screening and antioxidant property of shoot extracts of *Alpinia nigra* (Gaertn.) burtt (Family-Zingiberaceae). Med Plants Int J Phytomed India 2015;7:48-54.
29. Kaur R, Arora S. Alkaloids-important therapeutic secondary metabolites of plant origin. J Crit Rev 2015;2:1-8.
30. Meher R, Rao DM, Reddy KA. Phytochemical screening, antioxidant activity and antimicrobial activity of *Spathodea campunalata* stem extracts. Int J Phytomed 2015;7:209-18.
31. Chanda S, Dave R, Kaneria M. *In vitro* antioxidant property of some Indian medicinal plants. Res J Med Plant 2011;5:169-79.
32. Lugasi A, Hovari J, Sagi KV, Bíro L. The role of antioxidant phytonutrients in the prevention of diseases. Acta Biol Szeged 2003;47:119-25.
33. Kumarappan CT, Thilagam E, Mandal SC. Antioxidant activity of polyphenolic extracts of *Ichnocarpus frutescens*. Saudi J Biol Sci 2012;19:349-55.
34. Usha B, Pushpalatha. *In vitro* antioxidant activity and phytochemical screening of leaf extracts of *Grewia heterotricha mast.* Int J Curr Pharm Res 2016;8:68-72.
35. Geetha S, Irulandi K, Mehalingam P. Evaluation of antioxidant and free radical activities of different solvent extracts of leaves of *Piper umbellatum*. Asian J Pham Clin Res 2017;10:274-6.
36. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, *et al*. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 1998;46:1887-92.
37. Goncalves C, Dinis T, Batista MT. Antioxidant properties of proanthocyanidins of *Uncaria tomentosa* bark decoction: a mechanism for anti-inflammatory activity. Phytochemistry 2005;66:89-98.
38. Halliwell B, Gutteridge J. Formation of a thiobarbituric-acid reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS Lett 1981;128:347-52.
39. Sutar RC, Kalaichelvan VK. Evaluation of antioxidant activity of leaf extracts of *Holoptelea integrifolia* (ROXB) planch. Int J Appl Pharm 2014;16:6-8.
40. Shami PJ, Moore JO, Gockerman JP, Hathorn JW, Misukonis MA, Weinberg JB. Nitric oxide modulation of the growth and differentiation of freshly isolated acute non-lymphocytic leukemia cells. Leuk Res 1995;19:527-33.
41. Sainani GS, Manika JS, Sainani RG. Oxidative stress. A key factor in the pathogenesis of chronic diseases. Med Uptake 1997;7:1-5.
42. Sagbo IJ. Phytochemical analysis and antibacterial properties of aqueous and ethanolic extracts of *Brachylaena elliptica* and *Brachylaena ilicifolia* (MSc Dissertation). South Africa: University of Fort Hare; 2015.
43. Jethinlalkhosh JP, Antony A, Praveena P, Victor Arokia Doss D. Phytochemical, antioxidant and free radical scavenging activities of a hydro ethanolic extract of aerial parts of *Pothos scandens* L. Asian J Pharm Clin Res 2016;9:236-9.
44. McCord JM. The evolution of free radicals and oxidative stress. Am J Med 2000;108:652-9.
45. Masella R, Di Benedetto R, Vari R, Filesi C, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem 2005;16:577-86.
46. Yousef MI, Saad AA, El-Shennawy LK. Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food Chem Toxicol 2009;47:1176-83.
47. Lindholm C, Acheva A, Salomaa S. Clastogenic plasma factors: a short overview. Rad Environ Biophys 2010;49:133-8.
48. Vidya R, Kalaivani K, Venkatesh R. Antioxidant activity of goat liver slices treated with *cucumis melo* (L). Fruit extract. Int J Pharm Sci Res 2014;5:4846-50.