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Evaluation of Antimicrobial and Antioxidant Properties of Myanmar Herbal Plants

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Abstract

In this article potential antimicrobial and antioxidant activities of the selected herbal extracts obtained from twelve plants from their different parts (leaf, stem, bark, seed and fruit) that have been traditionally used as general health supplements in Myanmar. The aim of this current research was to evaluatate the antimicrobial and antioxidant potential of ethnolic crude extracts of all these plants, as well as their total phenol contents. In vitro antimicrobial activity was evaluated against six strains; Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Enterococcus faecalis. by agar well diffusion method. Among all of these plant extracts, the extract of Cassia fistula was strongly inhibited against Enterococcus faecalis. The most antioxidant activities against DPPH were displayed by the extract of Celastras paniculata Wild. and Piper nigrum exhibiting 88.38% and 85.43% inhibition respectively. And the highest value of reducing power was observed by the extract of Celastras paniculata Wild.. and followed by Cassia fistula. Similarity, the highest amount of total phenolic contents was found in Cassia fistula. The brine shrimp lethality assay was also obsoleted to examine the toxic potential of plant extracts. Based on the above results, it can be concluded that the plant extract of Cassia fistula have greatest potential as a source of natural antioxidant and antimicrobial agents than other plant extracts. In the future, plant-derived bioactive compounds will be an essential aspect of the therapeutic agents to accelerate their future discovery in

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biomedical and natural product research.

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1. Introduction

In developing countries, the World Health Organization estimates that about three quarters of the population relies on plant based preparations used in their traditional medicinal system and as the basic needs for human primary health care. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy for a variety of ailments of microbial origin [1], [2].

The composition of biologically active compounds of medicinal plants varies widely depending on the plant species, soil type and on their association with microbes [3]. The potential of higher plants as a source for new drugs is still largely unexplored, and among the estimated 250,000- 500,000 plant species, only a small fraction has been submitted to biological or pharmacological screening. The therapeutic activity of the plants is attributed mainly to their antimicrobial activity. The use of plant-derived antimicrobial agents might be effective in reducing the dependence on antibiotics and minimizing the chance of antibiotic resistance in pathogenic microorganisms [4].

Natural antioxidants may have free-radical scavengers, reducing agents, complexes of pro-oxidant metals, quenchers of singlet oxygen etc. Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal products to replace synthetic antioxidants, which are being restricted due to their adverse reaction such as carcinogenicity. Plants therefore constitute the main source of natural antioxidant molecules which have the capacity to eliminate or neutralize the deleterious ROS [5].

Rapid production of free radicals can lead to oxidative damage to biomolecules and may cause disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative diseases, and premature aging [6]. Natural antioxidants, from medicinal or edible plants, have recently received much attention as promising agents for reducing the risk of oxidative stress-induced neurological diseases [7], [8].

Antioxidants are substances that significantly delay or inhibit oxidation of an oxidizable substrate when present at low concentrations in comparison with those of the substrate [9]. The activities of free radicals have been implicated in aging, destruction of DNA, obstruction of arteries, cancer, strokes, cardiac and central nervous system (CNS) disorders which have led to an increase in the investigation of substances that can protect against these reactive oxygen species and thus may play a role in disease prevention [10]. Scientists have reported that polyphenolic compounds significantly constitute to the active substances in plant extracts having multiple protective effects including antioxidant, anti- inflammatory, antibacterial and antiproliferative activities.

Herbal medicine represents one of the most important fields of traditional medicine all over the world and the uses of herbal remedies for various medical conditions have been popularly growing. There is increasing trend in correlating phytochemical constituents of plants with its pharmacological activities [11]. The World Health Organization has also estimated conservatively that 60 to 90 percent of the populations of the developing countries rely, either totally or partially, on medicinal plants to discover their health care needs.

In this research, selected twelve herbal medicinal plants attract special attention and, are being surveyed for their potential antimicrobial and antioxidant capacities from Myanmar.

2. Materials and Methods

2.1. Plant Materials

The twelve herbal medicinal plants were collected from Kyaukse District in Mandalay Division and identified at the Biotechnological Research Department. The information of the screened plants is given in Table 1. The plant parts were thoroughly washed with tap water, air dried, homogenized to fine powder and stored in air tight bottles.

Table 1. Characteristics of Selected Twelve Herbal Medicinal Plants

No.	Plant species	Family	English Name	Parts Used
1.	Aloe vera Linn.	Liliaceae	Barbodos Aloe	Leaves
2.	Aegle marmelos Corr.	Rutaceae	Bael fruit	Fruits
3.	Benincasa cerifera	Cucurbitaceae	White pumpkin	Fruits
4.	Celastras paniculata Wild.	Celastraceae	Staff tree	Barks
5.	Casia siamea	Caesalpinlaceae	-	Leaves
6.	Cassia fistula	Caesalpinlaceae	India labumum	Barks
7.	Ocimum Sanctum Linn.	Labiatae	Sacred basil	The whole plants
8.	Plantago major Linn.	Planta ginaceae	Cart- track plant	Leaves
9.	Psidium guayava Linn.	Myrtaceae	Guava tree	Leaves and fruits
10.	Piper nigrum	Piperaceae	Black pepper	Seeds
11.	Tinospora cordifolia Miers.	Menispermaceae	-	Stem
12.	Viscum album Linn.	Loranthaceae	Mistletoe	Leaves

2.2. Preliminary Phytochemical Screening

The preliminary phytochemical screening of different plant extracts was done to ascertain the presence of bioactive components. The presence of alkaloids, amino acid, carbohydrates, flavonoids, tannins, saponin glycoside, reducing sugar, phenolic compound, acid, base or neutral, glycosides and cyanogenic glycoside were determined by standard methods.

2.3. Extracts Preparation

The dried powder of each plant (500g) was extracted in 70% ethanol for about eight hours by Reflux (hot extraction method) and then filtrated with filter papers. After filtration, the filtrates were examined by using

distillation method. Each extract was dried at desiccator under vacuum and stored in refrigerator for the screening of antioxidant and antimicrobial properties.

2.4. Brine Shrimp Lethality Bioassay

The Brine Shrimp Lethality Assay has been developed for toxicity testing of various concentrations of pure compounds and crude plant extracts according to the method of Teng Wah Sam et.al [12]. A stock solution of extract was made for serial dilutions to prepare different concentrations (range of 10-1000 µg/ml). This is useful in the means of toxicity testing, because it is an important point to determine the concentration range in which there is a linear correlation between the concentration and the lethality of the brine shrimps [13]. Each vial contains the tested crude plant extract, artificial sea water and ten brine shrimp nauplii and transferred into each sample vial. The toxicity of tested plant samples was determined by comparing their LC_{50} values with highly toxic substances suitable to be used potassium dichromate as positive control for this test. The vials were restored in the dark room at 25±1˚C. After 24 hours of exposure to the tested sample, the lethal concentration for 50% mortality (LC_{50}) was determined by counting the survived nauplii. Usually, no deaths were observed to occur in the referenced control after 24 hours.

2.5. Antimicrobial Assay

The extracts were individually tested against the following pathogenic microorganisms: Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Enterococcus faecalis. The antimicrobial activity of the extracts was tested using the agar well diffusion method. Microorganisms were grown about 6 hours at 30˚C in Mueller- Hinton Broth. Each plant extract was dissolved in 70% ethanol and sterilized by filtration. The inoculated microorganism was spread on the surface of Mueller-Hinton agar plates. Wells with 6-mm diameters were cut off and filled with 12.5mg/25µl of each extract. Ampicillin (10µg/25µl) and 70% ethanol were used as negative controls. The plates were incubated at an appropriate growth temperature (37˚C) for 24 hours. The assessment of antimicrobial activity was based on the measurement of inhibition zones on the agar surface around the well. Each antimicrobial assay was performed in triplicate.

2.6. Antioxidant Assay

2.6.1. DPPH (1,1-diphenyl-2-picryl-hydrazyl) Radical Scavenging Activity

The antioxidant activity of the plant extracts was evaluated by DPPH radical scavenging mechanism as described earlier with some modifications [14]. DPPH is a free radical compound that has widely been used to test the free radical scavenging abilities of various types of samples.

From the stock solution, different concentrations of extract (2.5 - 500 µg/ml) were prepared. 1ml of each concentration was mixed with 1ml of methanolic DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance of the mixture was then measured at 517 nm control and Vitamin C (L-ascorbic acid) was used as a positive. Scavenging ability of the sample to DPPH radical was determined

according to the following formula.
Inhibition (%) = [(Absorbance of control - Absorbance of test)/Absorbance of control] × 100
DPPH radical scavenging activity was measured in triplicate.

2.6.2. Ferric Reducing Power Assay

The reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm. Ferric reducing antioxidant power (FRAP) was determined following the method [15]. Firstly, the various concentrations of the extracts was mixed with 0.2 mol/L phosphate buffer (pH 6.6) and potassium ferricyanide (1g/100ml water) were mixed and incubated at 50 ˚C for 20 min. After this, 10g/100ml water of 10% trichloroacetic acid was added and the tubes were centrifuged at 3,000 rpm for 10 min. The upper layer of the solution was mixed with 5.0 ml of distilled water and 1 ml of 0.1% ferric chloride and then the absorbance of the reaction mixtures was measured at 700 nm. The final results were expressed as mg ascorbic acid equivalent/g of dry weight.

2.6.3. Total Phenolic Content Determination

The level of total phenols in the crude extracts was determined by using Folin–Ciocalteu reagent and standard with gallic acid. 0.5ml of methanolic extract solution and 2.5ml of 10% of Folin–Ciocalteu reagent were added and the contents mixed thoroughly [16]. After a few min, 2.5 ml of 75% $NaHCO_3$ was added, and then the mixture was allowed to stand at 45 C for 45 min in a thermostat. The absorbance was measured at 765 nm using a spectrophotometer (Thermo Fisher Scientific, model 4001/4). The concentration of the total phenolics was calculated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve. The determination of total phenolic contents in the crude extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

3. Results and Discussion

3.1. Preliminary Phytochemical Screening of Plant Samples

From phytochemical screening of medicinal plant samples, we observed that alkaloids and saponin glycosides gave positive results for all plant samples. And the test of cyanogenic glycosides indicated the negative results for all plant samples whereas it's potential safety for further experiments. The detailed results of preliminary phytochemical screening of selected plant samples were given in Table 2.

Table 2. Preliminary Phytochemical Screening of Selected Medicinal Plants

Selected Medicinal Plants	Alkaloid	Amino acid	Carbohydrate	Flavonoid	Tannin	Saponin glycosides	Reducing sugar	Phenolic compound	Acid/ Base	Glycosides	Cyanogenic glycosides
Aloe vera Linn.	+	-	-	-	-	+	-	-	Neutral	+	-
Aegle marmelos Corr.	+	+	+	+	+	+	+	+	Neutral	+	-
Benincasa cerifera	+	+	-	+	+	+	+	+	Neutral	+	-
Celastras paniculata Wild.	+	+	-	+	+	+	+	+	Base	+	-
Casia siamea	+	+	-	-	+	+	-	+	Neutral	+	-
Cassia fistula	+	+	-	+	+	+	+	+	Base	+	-
Ocimum Sanctum Linn.	+	+	+	-	+	+	+	+	Base	-	-
Plantago major Linn.	+	+	-	+	+	+	+	+	Neutral	-	-
Psidium guayava Linn.	+	+	+	+	+	+	+	+	Neutral	+	-
Piper nigrum	+	+	+	+	+	+	+	+	Base	+	-
Tinospora cordifolia Miers.	+	+	-	+	+	+	+	+	Base	+	-
Viscum album Linn.	+	+	+	+	+	+	+	+	Base	+	-

+ = Present - = Absent

3.2. Brine Shrimp Lethality Bioassay

The Brine Shrimp Lethality Bioassay is a very useful for preliminary assessment of the toxic potential of plant extracts. The median lethal concentration (LC_{50}) value of plant extracts was shown in Table 3. The preliminary toxicity data obtained by conducting the Brine Shrimp Lethality Assay gives LC_{50} values of all plant extracts which were non-toxic.

Table 3. The median lethal concentration (LC_{50}) value of selected plant extracts

No.	Plant species	LC_{50}(µg/ml) ± SD
1.	Aloe vera Linn.	553.99 ± 1.3
2.	Aegle marmelos Corr.	131.92 ± 1.29
3.	Benincasa cerifera	3122.98 ± 1.12
4.	Celastras paniculata Wild.	999.08 ± 1.16
5.	Casia siamea	3047.61 ± 1.15
6.	Cassia fistula	2056 ± 1.1
7.	Ocimum Sanctum Linn.	1526.19 ± 1.3
8.	Plantago major Linn.	4422.57 ± 1.17
9.	Psidium guayava Linn.	851.14 ± 1.26
10.	Piper nigrum	90.4 ± 2.12
11.	Tinospora cordifolia Miers.	1878.93 ± 1.26
12.	Viscum album Linn.	3841.93 ± 1.13
13.	Potassium dichromate($K_2Cr_2O_7$)	14 ± 2.13

3.3. Antimicrobial Assay

The inhibitory effect of selected plant extracts were tested against six pathogenic microorganisms by agar well diffusion method. The result in different extents of inhibition was given in Table 4. The Staphylloccous aureus strain was inhibited against the extracts of Viscum album Linn., Psidium guayava Linn., Benincasa cerifera and Aloe vera Linn. The extracts of Casia siamea, Cassia fistula and Psidium guayava Linn. exhibited potential antibacterial activity against Enterococcus faecalis whereas the other crude extracts did not exhibit any inhibitory activity against the tested strains.

Table 4. Antimicribial activity of selected plant extracts

Plant species	Zone of Inhibition (mm) in diameter					
	Escherichia coli	Bacillus cereus	Pseudomonas aeruginosa	Staphylococcus aureus	Candida albicans	Entero coccus faecalis
Aloe vera Linn.	0	0	0	11.75	0	0
Aegle marmelos Corr.	0	0	0	0	0	0
Benincasa cerifera	0	0	0	11	0	0
Celastras paniculata Wild.	0	0	0	0	0	0
Casia siamea	0	0	0	0	0	12
Cassia fistula	0	0	0	0	0	16
Ocimum Sanctum Linn.	0	0	0	0	0	0
Plantago major Linn.	0	0	0	0	0	0
Psidium guayava Linn. (Leaves)	0	0	0	11.5	0	11.75
Psidium guayava Linn. (Fruits)	0	0	0	12	0	11.5
Piper nigrum	0	0	0	0	0	0
Tinospora cordifolia Miers.	0	0	0	0	0	0
Viscum album Linn.	0	0	0	15.5	0	0
Ampicillin (Control)	20 ± 0.23					

3.4. Antioxidant Assay

3.4.1. DPPH (1,1-diphenyl-2-picryl-hydrazyl) Radical Scavenging Activity

The antioxidant activity of medicinal plants is mainly contributed by the active compounds. Various type plant extracts have different free radical antioxidant activity which depends upon their different constituents. The antioxidant activities of leaves, bark, seeds, fruits and/or root of the collected medicinal plants was given in Fig. 1. The highest antioxidant activity of ethanolic extracts was displayed by Celastras paniculata Wild. and Piper nigrum causing 88.38% and 85.43% DPPH inhibition at the concentration of 500µg/ml. In comparison, the standard ascorbic acid showed 83.45% DPPH inhibition in the assay. The IC_{50} value of some plant extracts in comparison of standard ascorbic acid was showed in Fig. 2.

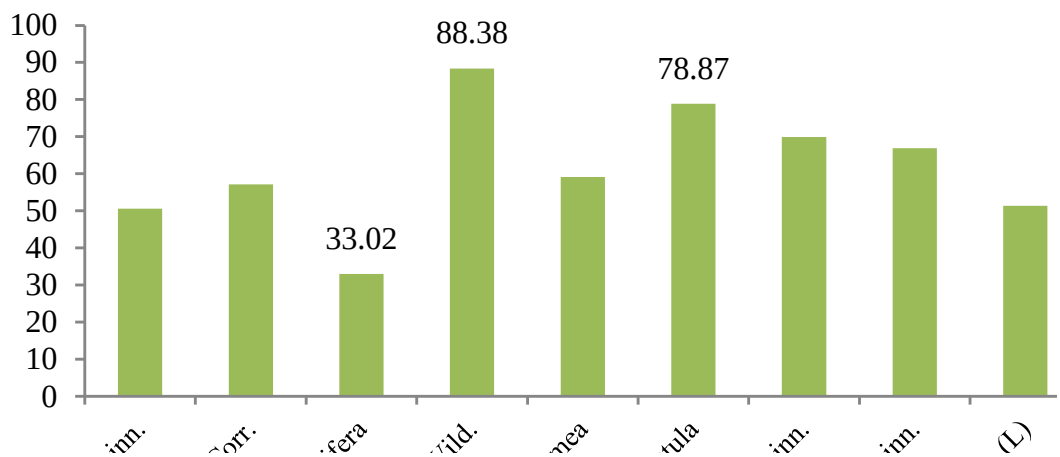

Fig. 1. DPPH radical scavenging activity of selected plant extracts

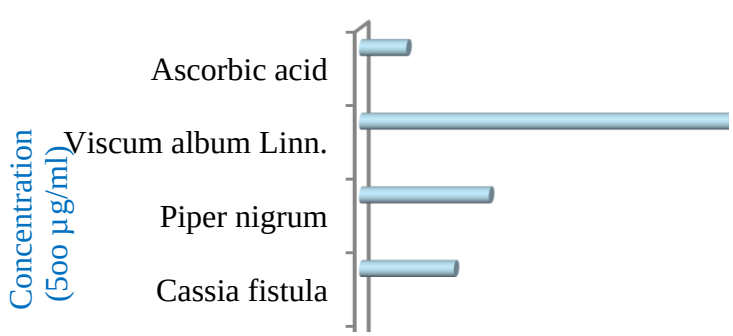

Fig. 2. IC_{50} value of some plant extracts in comparison of standard ascorbic acid

3.4.2. Ferric Reducing Power Assay

The ferric reducing capacity of extracts was investigated by using the potassium ferricyanide (Fe^{3+}) to convert potassium ferrocyanide (Fe^{2+}). The reducing power of the sample extracts and standard concentrations was resulted in Figure 3. In this experiment, the yellow color changed to pale green and blue color depending on the concentration of antioxidants in the samples. The highest value of reducing power was observed by the extract of Celastras paniculata Wild.. The other ethanolic extracts exhibited a higher activity potential than that of positive control (ascorbic acid).

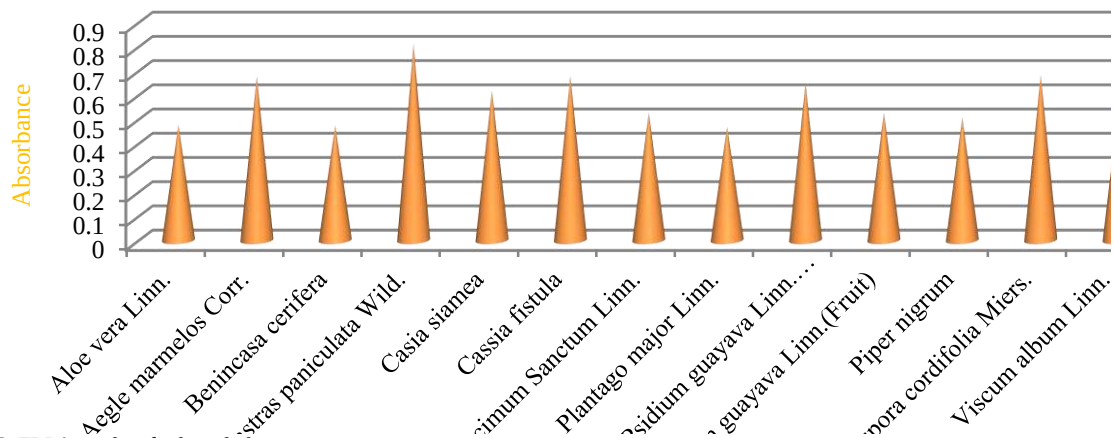

Fig. 3. FRPA results of selected plant extract

3.4.3. Total Phenolic Content Determination

The selected plant extracts were characterized by the presence of considerable amount of phenolic content by Folin–Ciocalteu method. The highest amount of total phenolic was found in Cassia fistula, followed by Benincasa cerifera. The result of our screening on the crude extract was showed in Fig. 4.

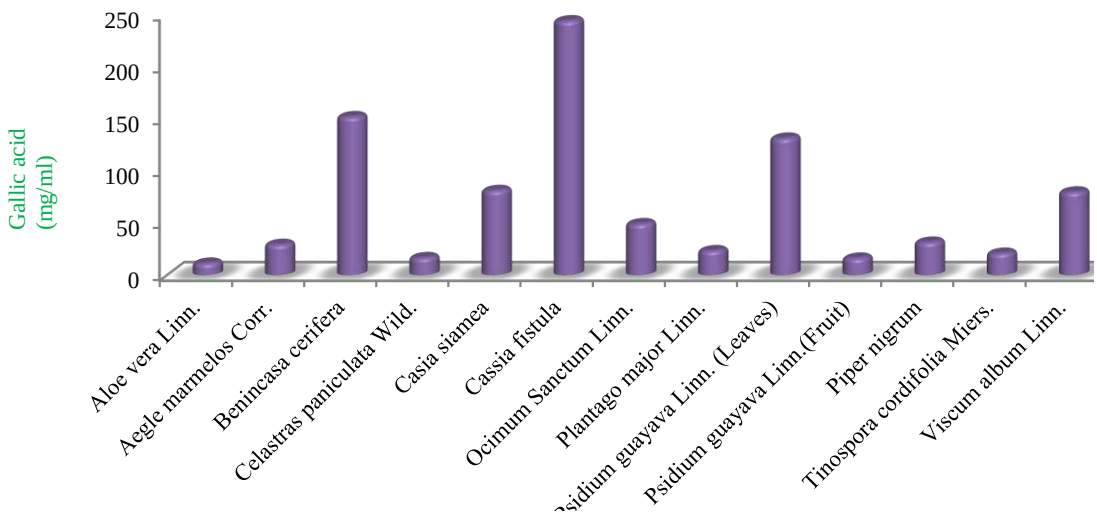

Fig. 4. Total phenolic content of selected plant extracts

Conclusion

The twelve selected herbal plants from Myanmar were evaluated for their potential antimicrobial and antioxidant activities whereas has been towards the alternative health care treatment and in discovery of modern drugs. The crude powder of twelve plant parts were subjected to preliminary phytochemical analysis and the results of other phytoconstituents were present in trace amount or absent. It is possible that these secondary metabolites might be responsible for the bioactivity of the plant extracts. The brine shrimp lethality assay was also obsoleted to examine the toxic potential of plant extracts. In vitro antimicrobial activity, the highest antibacterial activity was shown by the extract of Cassia fistula against Enterococcus faecalis among all of these plant extracts screened. Various assays are used to test for antioxidant activity, the extract of Celastras paniculata Wild. > Piper nigrum > Cassia fistula were exhibited in the highest inhibition DPPH out of 12 extracts. The antioxidant activity of phenolics is largely due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and as well as potential metal chelators. We also evaluated the reducing power of the crude extracts and significant changes were observed in the concentration of the extract of Celastras paniculata Wild.. and followed by Cassia fistula. Moreover, comparatively higher amount of total phenolic contents was showed in Cassia fistula as compared to the other extracts. In this study, the crude extract of Cassia fistula indicated that higher antioxidant activity and higher phenol contents could be significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses and its related disorders.

Statistical analysis

Presented data are mean ± standard deviation of three independent replicates.

Conflict of Interest

None declared.

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