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Free Radical Scavenging Activity of *Grangea maderaspatana* Poir.

Patel Veena[1], Shukla Sangita[2]*, Patel Sandip[2]

[1] Institute of Science and Technology for Advanced Studies and Research, Vallabh Vidyanagar, Anand-388120, Gujarat, India.
[2] Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Anand-388121, India.

** Author for correspondence: Mrs. Sangita Shukla, Department of Pharmacognosy, Indukaka Ipcowala College of Pharmacy, P.O. Box 53, New Vallabh Vidyanagar, Dist. Anand- 388121, Gujarat, India. E-mail : shshukla@rediffmail.com*

ABSTRACT

The present study was aimed to investigate the antioxidant activities of the methanolic extract of *Grangea maderaspatana* Poir (Compositae). The antioxidant activity of the extract was evaluated using five *in vitro* assays and was compared to standard antioxidant (Ascorbic acid). Further, Total phenolic contents of the extract were determined by using Folin-Ciocalteu method in order to evaluate a relationship between the antioxidant activity and the phytochemical constituents. The total phenolic content was found to be 121.45 ± 2.56 µg Gallic acid equivalent of phenol. The extract and ascorbic acid were found to have different levels of antioxidant activity in the systems tested. Methanolic extract of *Grangea maderaspatana* (GMME) exhibited significant ($p<0.05$) reducing power ability, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, nitric oxide radical scavenging activity, hydrogen peroxide (H_2O_2) scavenging activity and inhibition of β-carotene bleaching. In DPPH radical scavenging activity NO scavenging activity,H_2O_2 scavenging activity, and β-carotene bleaching assay the IC_{50} values obtained for GMME were found to be 46.55 ± 1.67 µg/mL,120.73 ± 0.694 µg/mL, 30.54 ± 1.11 µg/mL and 209.73 ± 4.63 µg/mL respectively and for Ascorbic acid the IC 50 values were found to be 24.96 ± 1.95 µg/mL, 236.37± 1.394 µg/mL, 57.34± 1.29 µg/mL and 339.16 ± 5.30 µg/mL respectively. The antioxidant property depends upon concentration and increased with increasing amount of the extract. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compounds present in the extract. The results obtained in the present study indicate that *Grangea maderaspatana* is a potential source of natural antioxidants.

KEYWORDS: Compositae, *Grangea maderaspatana, In vitro* antioxidant activity

INTRODUCTION

Research on relationships between antioxidants and prevention of non-communicable disease, such as cardiovascular disease, cancer and diabetes has been increasing sharply in recent years. Free radicals have been claimed to play a key role, affecting human health by causing severe diseases, such as cancer and cardiovascular diseases by cell degeneration. These free radicals can be generated during normal body function, and can be acquired from the environment. Interestingly the body possesses defense mechanisms against free radical-induced oxidative stress, which involve preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) etc. Where as non-enzymatic antioxidants are ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids etc (1). All these act by one or more of the mechanisms like reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. It is possible to reduce the risks of chronic diseases and prevent disease progression by either

enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants. This is one of the reasons why discovery and synthesis of novel antioxidants is a major active area. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in processed foods have side effects and are carcinogenic (2). In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value. Epidemiological and *in vitro* studies strongly suggest that food containing phytochemicals with antioxidants have potentially protective effects against many diseases, including cancer, diabetes and cardiovascular diseases (3). Foods such as fruits, vegetables and grains are reported to contain a wide variety of antioxidant components, including phytochemicals. Phytochemicals, such as phenolic compounds, are considered beneficial for human health, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation. These compounds have been reported to be well correlated with antioxidant potential (4).

Grangea is a small genus of herbs distributed in tropical and sub-tropical parts of Africa and Asia. *Grangea maderaspatana* Poir. (Compositae) is found in moist situations throughout India, particularly Bengal. Some important chemical constituents of plant include steroidal constituents, hardwickiic acid, the corresponding 1, 2-dehydro-derivative acetylenic compounds (5), auranamide (6), clerodane diterpenes (7), eudesmanolides (8), penta and hexamethoxy flavones (9). The root is an appetizer, astringent to the bowels, diuretic, anthelmintic, useful in griping, in troubles of the chest and lungs, headache, rheumatism in the knee joint, piles, pain in the muscles, diseases of the spleen and the liver, troubles of the ear, the mouth and the nose and lessens perspiration (10,11). Further, plant has reported to have analgesic, oestrogenic and antiimplantation activities (12,13).

There is paucity in the data of antioxidant activity of the plant and thus the present study was to investigate antioxidant activity of the methanol extract of the *G. maderaspatana* Poir using five *in vitro* models. Total phenolic content of the extract was also determined in order to evaluate a relationship between the antioxidant activity and the phytochemical constituents.

MATERIAL AND METHODS

Plant material and extraction

The aerial parts of the plant were collected between the month of February-April 2008 from Anand and Mahemdabad district of Gujarat State, India. The collected plant was authenticated by Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar. The Voucher specimen of *Grangea maderaspatana* was deposited under ARGH-11 in A.R.College of Pharmacy, Vallabh Vidyanagar. After collection, the plant material was washed thrice with water to remove dust and debris. The washed plant material was dried under shade and powdered (60#). 50 g plant powder was defatted with 400 mL of petroleum ether for 72 h by maceration. Defatted powder was further extracted with 400 mL of methanol by continuous extraction method using soxhlet apparatus. The methanol extract was filtered and concentrated by evaporating the solvent under reduced pressure using rotary vacuum evaporator.

Determination of total polyphenols

Total polyphenols were determined by the Folin–Ciocalteu procedure (14). Aliquots (0.1 mL) of test-solution were transferred into the test tubes and volumes brought up to 0.5 ml by water. After addition of 0.25 mL Folin–Ciocalteu reagent and 1.25 ml 20% aqueous Na_2CO_3 solution, tubes were vortexed and absorbance of blue-colored mixtures recorded after 40 min at 725nm against blank, containing 0.1 mL of extraction solvent. The amount of total polyphenols was calculated from the calibration curve of gallic acid standard solutions, covering the concentration range between 0.1 and 200 µg/mL, and expressed as % (w/w), with respect to dry plant material weight.

$y = 0.0041x - 0.0692$ ($r^2 = 0.9649$)

Reducing power ability

The reducing power of GMME was determined by the method of Oyaizu (15). The capacity of extract to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex (prussian blue in colour) was determined by recording the absorbance at 700 nm after incubation. For this purpose, different concentrations of plant extract (50–1000 µg/mL) in 1 ml of distilled water were mixed with phosphate buffer (2.5mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. Aliquots (2.5 mL) of trichloro acetic acid (TCA, 10%) were added to the mixture. The upper layer of solution (2.5mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm by spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing capability.

DPPH radical scavenging activity

The free radical scavenging activity of GMME was measured *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH)

assay (16). The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. 0.1mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was added to 3.0 mL of extract solution in water at different concentrations (31–1000µg/mL).The mixture was incubated at room temperature for 45 minutes and the absorbance was measured at 517 nm against corresponding blank solution. Ascorbic acid was taken as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading, (which contained DPPH and distilled water without any extract) using the following equation:

DPPH Scavenged (%) = $(A_{cont} – A_{test}) / A_{cont} \times 100$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the extract/ standard.

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/mL) of extracts that inhibits the formation of DPPH radicals by 50%.

Nitric oxide scavenging activity assay

This assay was performed according to the method described by Sreejayan *et al.* (17). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess–Illosvoy reaction. Briefly, 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture, 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. Ascorbic acid was taken as standard. The nitric oxide radical scavenging activity was calculated according to the following equation:

% Inhibition = $[(A_0 – A_t) / A_0 \times 100]$

Where A_0 was the absorbance of the control (without extract) and A_t was the absorbance in the presence of the extract/ Standard.

H_2O_2 scavenging activity

H_2O_2 scavenging ability of extract was determined according to the method of Ruch *et al* (18). A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). 3.4 ml (20–200 µg/mL) extract in phosphate buffer were added to H_2O_2 solution (0.6mL, 40mM). Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of H_2O_2 scavenging of extract and ascorbic acid (standard compound) was calculated as:

% Scavenged $H_2O_2 = [(A_{control} – A_{sample}) / A_{control}] \times 100$

Where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance in the presence of the sample of extract or standard.

β-carotene–linoleate bleaching assay

The antioxidant activity of GMME was assayed based on the β-carotene bleaching method. Ascorbic acid was used as the standard. β-carotene (0.2 mg in 1 mL chloroform), linoleic acid (0.02 mL) and Tween 20 (0.2 mL) were transferred into a round-bottomed flask. The mixture was then added to 0.2 mL of extract or standard or methanol (as control). Chloroform was removed at room temperature under vacuum at reduced pressure using a rotary vacuum evaporator. Following evaporation, 50 mL of distilled water was added to the mixture, and then shaken vigorously to form an emulsion. 2 mL aliquots of the emulsion were pipetted into test tubes and immediately placed in a water bath at 50°C. The absorbance was recorded at 470 nm at 30 min intervals for 2 h. The antioxidant activity of extracts was based upon two different parameters, namely antioxidant activity (AA) and the oxidation rate ratio (ROR).

Antioxidant activity (AA) was expressed as percent of inhibition relative to the control using the following formula (19):

$A_A = [(R_{CONTROL} - R_{SAMPLE\ OR\ STANDARDS})/R_{CONTROL}] \times 100$

Where, Rcontrol and Rsample represent the bleaching rates of β-carotene without and with the addition of antioxidant, respectively.

Degradation rates (RD) were calculated according to first-order kinetics:

$R_D = \ln (A_t/A_x) \times 1/t$

Where, ln is natural log, A_t is the initial absorbance at 470 nm at t = 0 and A_x is the absorbance at 470 nm at t = 30, 60, 90,120 min.

The oxidation rate ratio (ROR) was calculated as

R_{OR} =Rsample/Rcontrol

Where Rsample and Rcontrol are as described earlier.

Statistical analysis

The experimental data were expressed as mean ± standard deviations (n=3). Student's t-test was carried out to determine significant differences ($p < 0.05$) between the means by Sigma stat 2.03.

RESULTS AND DISCUSSION

Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities and also decrease cardiovascular complications (20). The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups (18). Total phenolic assay using Folin-Ciocalteu reagent is a simple, convenient and reproducible method. Total phenol content (TPC) was determined in comparison with standard gallic acid and the results expressed in terms of mg Gallic Acid Equivalent (GAE)/g dry sample. The TPC for GMME (1 mg) was found to be 121.45 ± 2.56 µg Gallic Acid Equivalent, using equation y= 0.0041x-0.0692 (r^2=0.9649).

The antioxidant activity has been reported to be concomitant with the development of reducing power. The reducing power of GMME and ascorbic acid is shown in Table 1. The reducing power increased as the extract concentration increased. At different concentrations (62–1000 µg/mL) GMME (r^2= 0.9633) and ascorbic acid (r^2= 0.975) demonstrated powerful reducing capacity and these differences were statistically significant ($p<0.05$). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The antioxidant activity has been attributed to various mechanisms, among which are the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging (21).

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. Table 2 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of the GMME and standards. The scavenging effect of GMME and ascorbic acid on the DPPH radical decreased in the order of GMME > ascorbic acid and at the concentration of 1000 µg/mL, the resulting inhibition were 94.91%, and 91.347%, respectively. GMME and ascorbic acid significantly quenched DPPH in dose dependent manner: r^2= 0.6185 ($p< 0.05$) for GMME; r^2= 0.6487 ($p< 0.05$) for ascorbic acid. IC_{50} values (concentration of sample required to scavenge 50% free radical or to prevent lipid peroxidation by 50%) were found to be 46.55 ± 1.67 and 24.96 ± 1.95 µg/mL for GMME and ascorbic acid, respectively. Free radical scavenging activity was also increased with an increasing concentration. These data clearly indicate that GMME is a powerful free radical inhibitor or scavenger.

In vitro inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Nitric oxide is a free

Table 1: Reducing power ability of GMME and ascorbic acid

Concentration (µg/ml)	Absorbance at 700 nm	
	GMME	Ascorbic acid
62	0.0523 ± 0.0027	0.116 ± 0.0139*
125	0.111 ± 0.0030*	0.231 ± 0.0023*
250	0.298 ± 0.0047*	0.402 ± 0.0029*
500	0.536 ± 0.0029*	0.477 ± 0.0080*
750	0.651 ± 0.0094*	0.687 ± 0.0111*
1000	0.869 ± 0.0146*	0.909 ± 0.0057*

Values are mean ± S.E.M (n=3)
*P<0.05 Compared to control
GMME- Methanolic extract of *Grangea maderaspatana*

Table 2: Free radical scavenging activity of GMME and Ascorbic acid in DPPH method

Concentration (µg/mL)	% free radical scavenging activity of GMME	Linear equation (r^2)	% free radical scavenging activity of Ascorbic acid	Linear equation (r^2)
10	20.49 ± 0.92*		26.93 ± 1.39*	
15	32.94 ± 1.21*		30.49 ± 1.47*	
31	53.76 ± 1.38*		55.78 ± 1.86*	
62	57.72 ± 2.32*		59.41 ± 0.87*	
125	70.49 ± 1.11*	y = 0.0587x + 47.267 r^2 = 0.6185	72.553 ± 2.73*	y = 0.0564x + 48.592 r^2 = 0.6478
250	79.667 ± 1.64*		81.467 ± 1.53*	
500	84.247 ± 1.91*		87.393 ± 3.01*	
1000	94.91 ± 0.95*		91.347 ± 2.85*	
IC_{50} value	46.55 ± 1.67 µg/mL		24.96 ± 1.95 µg/mL	

Values are mean ± S.E.M (n=3)
*P<0.05 Compared to control
GMME- Methanolic extract of *Grangea maderaspatana*

radical which plays an important role in the pathogenesis of pain, inflammation, etc (22). Table 3 illustrates decrease in the concentration of nitric oxide free radicals due to the scavenging ability of GMME and standard. A 1000 µg/mL of GMME and ascorbic acid (std.) exhibited 77.60% and 77.93% inhibition, respectively. GMME and ascorbic acid significantly scavenged nitric oxide free radicals in dose dependent manner: r^2= 0.9568 ($p< 0.05$) for GMME; r^2= 0.9608 ($p< 0.05$) for ascorbic acid. IC_{50} values were found to be 120.73 ± 0.694 µg/mL and 236.37 ± 1.394 µg/mL for GMME and ascorbic acid (std.) respectively. IC_{50} values indicate that GMME has antioxidant activity at lower dose as that of ascorbic acid. GMME decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. This may be due to the antioxidant properties in the extract which compete with oxygen to react with nitric oxide thereby inhibiting the generation of peroxynitrite.

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but is an intracellular precursor of hydroxyl radicals which is very toxic to the cell (23). Thus, scavenging of H_2O_2 is a measure of the antioxidant activity of the extract. Table 4 illustrates decrease in the concentration of hydrogen peroxide free radicals due to the scavenging ability of methanolic extract and standards. At 200 µg/mL of GMME and ascorbic acid (std.) exhibited 72.983 ± 1.02 and 76.357 ± 1.14 %inhibition, respectively. GMME and ascorbic acid significantly scavenged hydrogen peroxide radicals in dose dependent manner: r^2= 0.8246 ($p< 0.05$) for GMME; r^2= 0.7714 ($p< 0.05$) for ascorbic acid. IC_{50} values were found to be 30.548 ± 1.11 µg/mL and 57.345 ± 1.29 µg/mL for GMME and ascorbic acid respectively. IC_{50} values indicate that GMME has antioxidant activity at lower dose as that of ascorbic acid. GMME scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water.

In the β-carotene bleaching assay, linoleic acid produces hydroperoxides as free radicals during incubation at 50 °C. The presence of antioxidants in the extract will minimize the oxidation of β-carotene by hydroperoxides. Hydroperoxides formed in this system will be neutralized by the antioxidants from the extract (4). In this study, we evaluated the antioxidant activity of GMME by the β-carotene–linoleate bleaching method as β-carotene shows strong biological activity and is a physiologically important compound. Thus, the degradation rate of β-carotene–linoleate depends on the antioxidant activity of the extract. There was a correlation between

Table 3: Free radical scavenging activity of GMME and Ascorbic acid in NO scavenging method

Concentration (µg/mL)	% free radical scavenging activity of GMME	Linear equation (r^2)	% free radical scavenging activity of Ascorbic acid	Linear equation (r^2)
100	46.86 ± 1.016*	y = 0.0328x + 46.049 r^2 = 0.9568	46.23 ± 0.903*	y = 0.0359x + 41.514 r^2 = 0.9608
200	51.82 ± 0.569*		46.93 ± 2.509*	
400	63.67 ± 1.344*		54.72 ± 0.986*	
600	66.48 ± 0.857*		66.79 ± 1.693*	
800	71.58 ± 0.888*		67.60 ± 1.21*	
1000	77.60 ± 0.557*		77.93 ± 0.485*	
IC_{50} value	120.73 ± 0.694 µg/mL		236.37 ± 1.394 µg/mL	

Values are mean ± S.E.M (n=3)

*<0.05 Compared to control

GMME- Methanolic extract of *Grangea maderaspatana*

Table 4: Free radical scavenging activity of GMME and Ascorbic acid in H_2O_2 scavenging method

Concentration (µg/mL)	% free radical scavenging activity of GMME	Linear equation (r^2)	% free radical scavenging activity of Ascorbic acid	Linear equation (r^2)
20	44.483 ± 1.023*	y = 0.1424x + 48.025 r^2 = 0.8246	33.067 ± 0.73*	y = 0.2365x + 36.443 r^2 = 0.7714
40	54.147 ± 1.866*		40.687 ± 1.28*	
60	58.113 ± 0.619*		52.420 ± 1.58*	
80	62.993 ± 0.926*		62.924 ± 1.57*	
100	66.64 ± 0.989*		71.455 ± 0.98*	
200	72.983 ± 1.02*		76.357 ± 1.14*	
IC_{50} value	30.548 ± 1.11 µg/mL		57.345 ± 1.29 µg/mL	

Values are mean ± S.E.M (n=3)

*P<0.05 Compared to control

GMME- Methanolic extract of *Grangea maderaspatana*

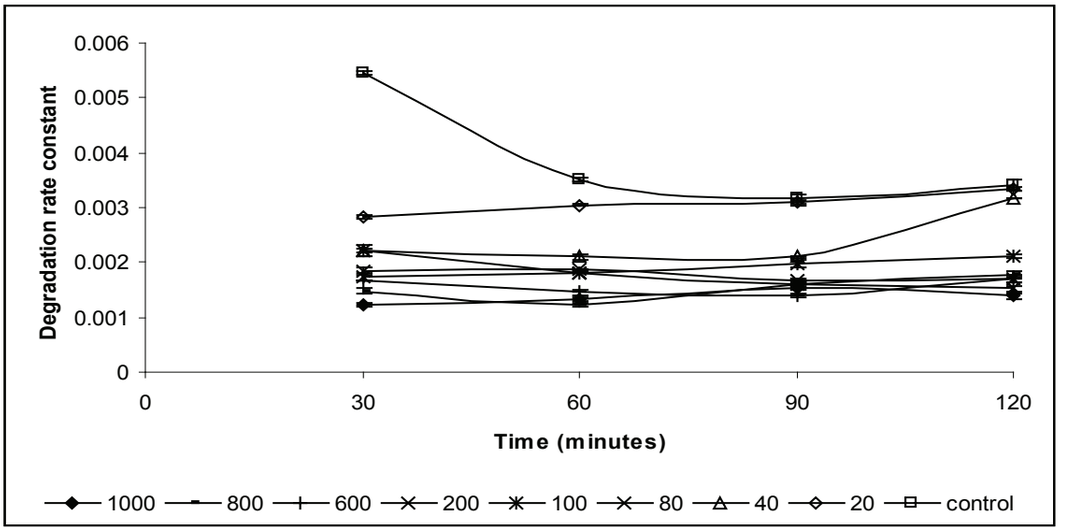

Figure 1: *Degradation rates of different concentration of Methanolic extract of Grangea maderaspatana*
Values are mean ± S.E.M (n=3)

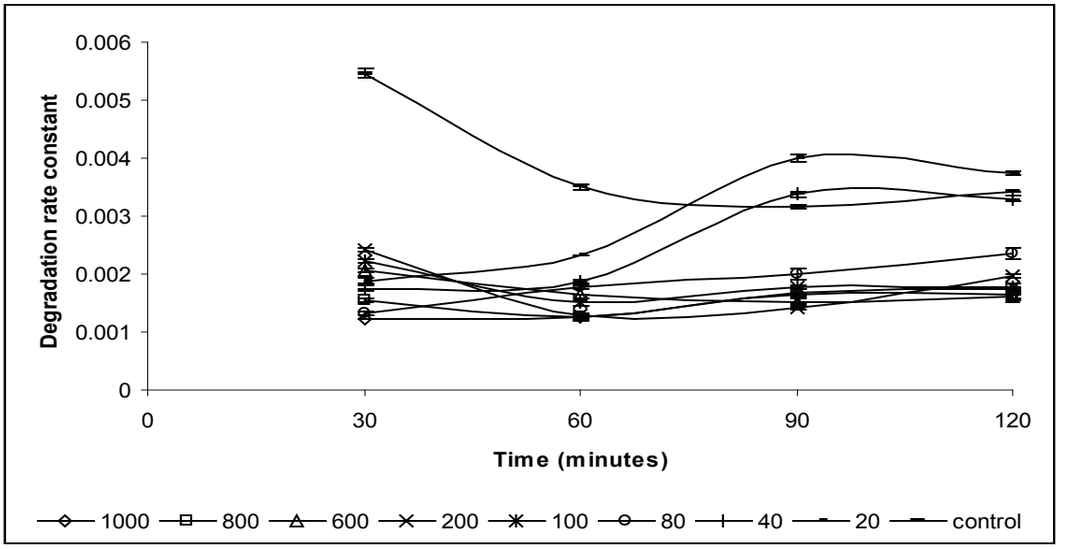

Figure 2: *Degradation rates of different concentration of Ascorbic acid*
Values are mean ± S.E.M (n=3)

Table 5: Antioxidant activities of GMME and Ascorbic acid by β-carotene–linoleate bleaching method

Concentration (µg/mL)	% Antioxidant activity		Oxidation rate Ratio	
	GMME	Ascorbic acid	GMME	Ascorbic acid
1000	68.135 ± 1.696 *	67.422 ± 2.443*	0.353 ± 0.0095*	0.381 ± 0.0065*
800	62.942 ± 1.528*	62.739 ± 1.426*	0.389 ± 0.0055*	0.414 ± 0.0083*
600	60.877 ± 1.214*	59.942 ± 2.37*	0.404 ± 0.0079*	0.448 ± 0.0056*
200	52.82 ± 1.752*	56.225 ± 1.516*	0.461 ± 0.0017*	0.464 ± 0.0099*
100	51.604 ± 3.092*	53.354 ± 1.187*	0.485 ± 0.0052*	0.473 ± 0.0048*
40	37.771 ± 1.052*	36.878 ± 1.864*	0.626 ± 0.0045*	0.687 ± 0.0128*
20	21.443 ± 0.897*	27.013 ± 1.903*	0.82 ± 0.0183*	0.808 ± 0.0219*

Values are mean ± S.E.M (n=3)
*P<0.05 Compared to control
GMME- Methanolic extract of *Grangea maderaspatana*

degradation rate and the bleaching of β -carotene; the extract with the lowest β-carotene degradation rate exhibited the highest antioxidant activity. It was depicted from figure 1 and 2, GMME and ascorbic acid retarded β-carotene degradation rate at significant extent as compared to control. At 1000 µg/mL of GMME and ascorbic acid (std.) exhibited 68.135 ± 1.696 and 67.422 ± 2.443 % inhibitions, respectively. GMME and ascorbic acid significantly reduced β- carotene bleaching in dose dependent manner: r^2= 0.5139 ($p< 0.05$) for GMME; r^2= 0.5001 ($p< 0.05$) for ascorbic acid. IC_{50} values were found to be 209.70 ± 4.63 µg/mL and 339.16 ± 5.30 µg/mL for GMME and ascorbic acid respectively (Table 5). Linoleic acid hydroperoxides attack the β-carotene molecule and, as a result, it undergoes rapid decolorization. The corresponding decrease in absorbance can be monitored spectrophotometrically. The presence of antioxidant extracts can hinder the extent of β- carotene bleaching by acting on the free radicals formed in the system. Oxidation rate ratio bears an inverse relationship with antioxidant activity index (4).

CONCLUSION

Based on the results obtained, it may be concluded that the methanol extract of the *Grangea maderaspatana* Poir showed strong antioxidant activity when compared to standard. Due to the antioxidant effect of the plant, further studies can be carried out to investigate its use in various diseases in which free radicals are involved in their pathogenesis. Further studies will also be needed to evaluate the *in vivo* potential of the extract and its fractions in various animal models and the isolation and identification of the antioxidant principles.

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