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Antioxidant, lipid peroxidation inhibition and free radical scavenging efficacy of a diterpenoid compound sugiol isolated from *Metasequoia glyptostroboides*

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ABSTRACT

Objective: To investigate the antioxidant efficacy of a biologically active diterpenoid compound sugiol isolated from *Metasequoia glyptostroboides* (*M. glyptostroboides*) in various antioxidant models. **Methods:** An abietane type diterpenoid sugiol, isolated from ethyl acetate extract of *M. glyptostroboides* cones, was analyzed for its antioxidant efficacy as reducing power ability and lipid peroxidation inhibition as well as its ability to scavenge free radicals such as 1,1-diphenyl-2-picryl hydrazyl, nitric oxide, superoxide and hydroxyl radicals. **Results:** The sugiol showed significant and concentration-dependent antioxidant and free radical scavenging activities. Consequently, the sugiol exerted lipid peroxidation inhibitory effect by 76.5% as compared to α -tocopherol (80.13%) and butylated hydroxyanisole (76.59%). In addition, the sugiol had significant scavenging activities of 1,1-diphenyl-2-picryl hydrazyl, nitric oxide, superoxide and hydroxyl free radicals in a concentration-dependent manner by 78.83%, 72.42%, 72.99% and 85.04%, when compared to the standard compound ascorbic acid (81.69%, 74.62%, 73.00% and 73.79%) and α -tocopherol/butylated hydroxyanisole (84.09%, 78.61%, 74.45% and 70.02%), respectively. **Conclusions:** These findings justify the biological and traditional uses of *M. glyptostroboides* or its secondary metabolites as confirmed by its promising antioxidant efficacy.

1. Introduction

Current life style causes overproduction of free radicals and reactive oxygen species (ROS). Free radicals are generated through normal reactions within the body during respiration in aerobic organisms which can exert diverse functions like signaling roles and provide defense against infections[1]. The ROS such as nitric oxide radical, superoxide radical, hydroxyl radical, hydrogen peroxide

(H₂O₂) and singlet oxygen (O^{2·}) are the by-products of cellular metabolism with most biological significance[1]. The ROS are potentially damaging transient chemical species which are produced by a number of exogenous sources such as ionizing radiations (ultraviolet rays and γ -rays), tobacco smoke, pesticides, pollutants, and some medications. Such species are considered to be important causative factors in decreasing cell fluidity, permeability, cell dysfunction and mutations[2]. Many degenerative human diseases including cancer, cardio- and cerebro-vascular diseases have been recognized as a possible consequence of free radical damage to lipids, proteins and nucleic acids[2]. Natural antioxidants protect the living system from oxidative stress and associated diseases and therefore play an important role in health care system[3].

Food industry has long been concerned with issues such as rancidity and oxidative spoilage of foodstuffs[4]. The enzymatic oxidation as well as auto-oxidation of lipids during storage and processing is the major reaction

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responsible for the deterioration in food quality affecting color, flavor, texture and nutritive value of foods[2]. Antioxidants are often added to foods to prevent the radical chain reactions of oxidation by inhibiting the initiation and propagation steps leading to the termination of the reaction and a delay in the oxidation process.

Synthetic phenolic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroxyquinone (TBHQ) effectively inhibit lipid oxidation. However, these commonly used synthetic antioxidants are restricted by legislative rules, because they are suspected to have toxic effects and are considered carcinogenic by nature[4]. Therefore, there has been a considerable interest by the food industry and a growing trend in consumer preferences for using natural antioxidants over synthetic compounds in order to eliminate synthetic antioxidants in food applications, giving more impetus to explore natural sources of antioxidants. This has encouraged both food scientists and health professionals to work on antioxidant molecules from natural origin. Nowadays, there has been a convergence of interest among researchers to find out the role of natural antioxidants in the diet, and their impact on human health has come under huge attention[5].

Metasequoia glyptostroboides Miki ex Hu (*M. glyptostroboides*) is a deciduous coniferous tree of the redwood family, Cupressaceae. This species of the genus *Metasequoia* has been propagated and distributed in many parts of Eastern Asia and North America as well as in Europe. Previously, we reported various biological properties of various essential oils derived from *M. glyptostroboides* such as antibacterial[6,7], antioxidant/antibacterial[8], antidermatophytic[9] and antifungal[10] activities. In addition, the antibacterial activities of terpenoid compounds from *M. glyptostroboides* have also been reported against foodborne pathogenic bacterial[11].

The biological efficacy of *M. glyptostroboides* has been reported previously both *in vitro* and *in vivo*, however, no research has been reported on the antioxidant and free radical scavenging efficacy of sugiol from *M. glyptostroboides* including its lipid peroxidation inhibitory effect. Hence, in addition to our continuous efforts to investigate biologically active secondary metabolites from *M. glyptostroboides*, in this study, we assayed the antioxidant and free radical scavenging efficacy of sugiol, an abietane type diterpenoid compound in various antioxidant models.

2. Materials and methods

2.1. Chemicals and instrument

The chemicals and reagents used in this study such as 1,1-diphenyl-2-picryl hydrazyl (DPPH), sodium nitroprusside (SNP), Griess reagent, trichloroacetic acid (TCA), bovine

brain extract, nitro blue tetrazolium (NBT), ferric chloride, potassium ferricyanide and gallic acid as well as standard antioxidant compound ascorbic acid, BHA and α -tocopherol were purchased from Sigma-Aldrich (St. Louis, USA) and were of analytical grade. Spectrophotometric measurements were done using a 96-well micro-plate enzyme linked immunosorbent assay (ELISA) reader (Infinite M200, Teacan, Mannedorf, Switzerland).

2.2. Plant material

The male cones of *M. glyptostroboides* were collected locally from Pohang city, Republic of Korea, in November and December 2008 and identified by the morphological features and the database present in the library at the Department of Biotechnology, Daegu University, Korea. The cones were dried under shade at room temperature without exposure of sunlight. A voucher specimen (DUB-0038) was deposited in the herbarium of College of Engineering, Department of Biotechnology, Daegu University, Korea.

2.3. Extraction and isolation of sugiol

Dried cones of *M. glyptostroboides* (2 kg) were milled into powder and then extracted with ethyl acetate at room temperature for 12 days. The extract was evaporated under reduced pressure using a rotary evaporator (EYELA N1000, Japan). The dried ethyl acetate extract (7 g) was subjected to column chromatography over silica gel (230–400 meshes, Merck, Darmstadt, Germany) and was eluted with hexane-ethyl acetate-methanol solvent system to give 20 fractions. Of the fractions obtained, fraction-14 was further purified by preparative thin layer chromatography over silica gel GF254 using hexane-ethyl acetate (2:1, v:v) as a mobile phase to give one compound (122 mg) which was characterized as sugiol on the basis of spectral data analysis[12].

2.4. Determination of DPPH radical scavenging assay

The antioxidant activity of sugiol, based on the scavenging of stable DPPH free radical, was determined by the method described previously with a minor modification[13]. Different concentrations of sugiol and reference compounds such as ascorbic acid and α -tocopherol (25–150 μ g/mL) were added to 0.004% methanolic solution of DPPH (1:1, v:v) in a 96-well microplate. The mixture was incubated at 37 °C in dark for 30 min with shaking at 150 r/min. Absorbance was recorded at 517 nm using the 96-well ELISA reader against a blank sample. All the tests were run in triplicate. Ascorbic acid and α -tocopherol were used as reference compounds. The percent inhibition activity was calculated using the formula: Percent inhibition (%) = $(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100$. (1) where, A_{control} is the absorbance of the control reaction at 517 nm and A_{test} represents the absorbance of a test reaction at 517 nm.

2.5. Determination of nitric oxide (NO) radical scavenging assay

In aqueous solution at physiological pH, SNP automatically generates NO, which intermingles with oxygen to generate nitrite ions that can be anticipated by the Griess reagent (10 g/L sulphanilamide, 20 mL/L phosphoric acid and 1 g/L naphthyl ethylene diamine dihydrochloride)[14]. Scavengers of free radicals result in the reduced production of NO. In this assay, the solution of SNP (10 mmol/L) in phosphate buffer saline (PBS, pH 7.4) was mixed with different concentrations of sugiol, ascorbic acid and α -tocopherol (20–100 μ g/mL). The mixture was incubated at 37 °C for 60 min in light. The half quantity of aliquots was taken and mixed with equal quantity of the Griess reagent, and the mixture was incubated at 25 °C for 30 min in dark. The absorbance of pink chromophore generated during diazotization of nitric ions with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm against a blank[14]. All the tests were performed in triplicate. Ascorbic acid and α -tocopherol were used as standard reference compounds. The percent inhibition activity was calculated using the formula:

$$\text{Percent inhibition (\%)} = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100. \quad (2)$$

where, A_{control} is the absorbance of the control reaction at 546 nm and A_{test} represents the absorbance of a test reaction at 546 nm.

2.6. Determination of superoxide radical scavenging assay

Superoxide radical scavenging activity of sugiol was measured by the reduction of NBT according to the previously reported method with minor modifications[15]. In this assay, the non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce NBT to a purple color formazan. The reaction mixture (150 μ L) contained phosphate buffer (0.2 mol/L, pH 7.4), NADH (73 μ mol/L), NBT (50 μ mol/L), PMS (15 μ mol/L) and various concentrations (50–250 μ g/mL) of the sugiol solution. After incubation for 60 min at room temperature, the absorbance of the reaction mixture was measured at 560 nm against an appropriate blank to determine the quantity of formazan generated. All tests were performed three times. Ascorbic acid and α -tocopherol were used as positive controls. The percent inhibition activity was calculated using the formula:

$$\text{Percent inhibition (\%)} = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100. \quad (3)$$

where, A_{control} is the absorbance of the control reaction at 560 nm and A_{test} represents the absorbance of a test reaction at 560 nm.

2.7. Determination of hydroxyl radical scavenging assay

A previously described method was adopted for determining the hydroxyl radical scavenging activity of sugiol with minor modifications[16]. The assay is based on

quantification of the degradation product of 2-deoxy-2-ribose sugar by condensation with 2-thiobarbituric acid (TBA). Hydroxyl radicals were generated by the Fenton reaction using Fe^{3+} -ascorbate-EDTA- H_2O_2 system. The reaction mixture in a final volume of 240 μ L contained 2-deoxy-2-ribose (3 mmol/L), KH_2PO_4 -KOH buffer (20 mmol/L, pH 7.4), FeCl_3 (0.1 mmol/L), ethylenediamine tetra-acetic acid (EDTA) (0.1 mmol/L), H_2O_2 (2 mmol/L), ascorbic acid (0.1 mmol/L) and various concentrations (100–500 μ g/mL) of sugiol or standard compounds. After incubation for 45 min at 37 °C, 40 μ L of 2.8% (v:v) TCA, and 40 μ L of TBA [0.5% (v:v) in 0.025 mol/L NaOH solution containing 0.2 g/L BHA] were added in the reaction mixture, and the mixture was incubated at 95 °C for 15 min to develop the pink color. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All tests in this assay were performed three times. Ascorbic acid and BHA were used as positive controls. The percent inhibition activity was calculated using the formula:

$$\text{Percent inhibition (\%)} = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100. \quad (4)$$

where, A_{control} is the absorbance of the control reaction at 532 nm and A_{test} represents the absorbance of a test reaction at 532 nm.

2.8. Lipid peroxidation assay

The Fe^{3+} /ascorbic acid dependent non-enzymatic lipid peroxidation in bovine brain extract was performed according to the previous method with minor modifications[17]. The reaction mixture, in the absence and presence of sugiol or reference compounds (50–250 μ g/mL), containing 50 μ L of 5 mg/mL bovine brain phospholipids, 1 mmol/L FeCl_3 and 1 mmol/L ascorbic acid in 20 mmol/L phosphate buffer with a final volume of 330 μ L, was incubated at 37 °C for 1 h. The hydroxyl radicals generated in the reaction initiated the lipid peroxidation, resulting in malondialdehyde (MDA) production that was measured by TBA reaction using an ELISA reader. All tests in this assay were performed three times. BHA and α -tocopherol were used as positive controls. The percent inhibition activity was calculated using the formula:

$$\text{Percent inhibition (\%)} = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100. \quad (5)$$

where, A_{control} is the absorbance of the control reaction at 532 nm and A_{test} represents the absorbance of a test reaction at 532 nm.

2.9. Reducing power assay

The ferric ion (Fe^{3+}) reducing power of the sugiol was determined by the method described previously with minor modifications[18]. Aliquots (50 μ L) of different concentrations of sugiol (5–25 μ g/mL) were mixed with 50 μ L phosphate buffer (0.2 mol/L, pH 6.6) and 50 μ L potassium ferricyanide (10 g/L), followed by incubation at 50 °C for 20 min in dark. After incubation, 50 μ L of TCA (10%, v:v) was added to terminate the reaction and the mixture was subjected to centrifugation at 3 000 r/min for 10 min. For final reaction

mixture, the supernatant (50 μ L) was mixed with 50 μ L distilled water and 10 μ L FeCl₃ solution (1 g/L). The reaction mixture was incubated for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution. A higher absorbance of the reaction mixture indicated greater reducing power ability. All tests were run in triplicate. Ascorbic acid and α -tocopherol were used as positive controls.

2.10. Statistical analysis

All data are expressed as the mean \pm SD by measuring three independent replicates. Analysis of variance using one-way ANOVA followed by Duncan's test was performed to test the significance of differences between means obtained among the treatments at the 5% level of significance using the SAS software (Version SAS 9.1; SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Identification of sugiol

The ethyl acetate extract of *M. glyptostroboides* cones after column chromatography over silica gel yielded a pure compound, which was obtained as yellow glass crystal with a specific melting point (282–284 $^{\circ}$ C). The ¹H NMR data (pyridine-*d*₅, 250 MHz) showing two singlet protons at δ 8.39 and 7.16 (each 1H, s), an aliphatic methine signal at δ 3.59 (1H, m, signals partially overlapped), and five terminal methyl groups at δ 1.35 (3H, d, *J* = 6.8 Hz), 1.33 (3H, d, *J* = 6.8 Hz), 1.11 (3H, s, Me-20), 0.83 (3H, s, Me-19), 0.79 (3H, s, Me-18), as well as the ¹³C NMR data displaying 20 carbon signals including a carbonyl group at δ 197.6 strongly suggested that this compound should be an abietane diterpenoid. The structure of this compound was determined to be sugiol by 1D and 2D NMR analysis and also confirmed by comparing the physical and spectroscopic data with those in the literature^[12,19].

3.2. DPPH radical scavenging activity

Figure 1 shows the percentage of DPPH radical scavenging capacity of sugiol in comparison with ascorbic acid and α -tocopherol as reference compounds. A concentration-dependent response relationship was found in the DPPH radical scavenging capacity and the activity was increased with the increase of sample concentration. The sugiol showed maximum 78.83% inhibition of DPPH radicals, while ascorbic acid and α -tocopherol showed about 81.69% and 84.09% inhibitory effect, respectively within the concentration range. Significant (*P*<0.05) scavenging of DPPH radicals was evident at all the tested concentrations of sugiol and reference compounds (25–150 μ g/mL).

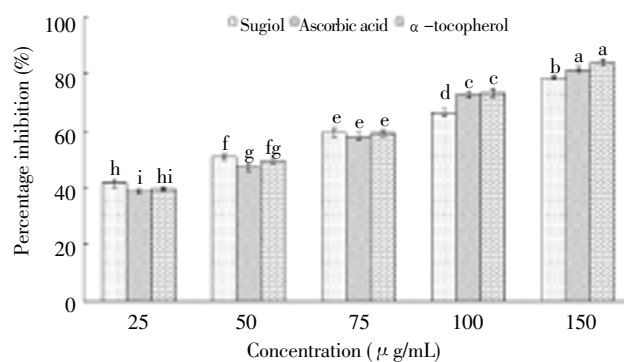


Figure 1. DPPH radical scavenging activity of a diterpenoid sugiol from *M. glyptostroboides* and standard antioxidants, ascorbic acid and α -tocopherol.

Different superscripts in each column indicate the significant differences between the means (*P*<0.05).

3.3. NO radical scavenging activity

As shown in Figure 2, both sugiol and positive controls showed significant NO radical scavenging activity (*P*<0.05) in a concentration-dependent manner. In this assay, sugiol caused a concentration-dependent inhibition of NO, and the highest inhibitory effect (72.42%) was observed at the concentration of 100 μ g/mL. On the other hand, ascorbic acid and α -tocopherol as positive controls (100 μ g/mL) had about 74.62% and 78.61% of inhibitory effect on scavenging of NO radical, respectively.

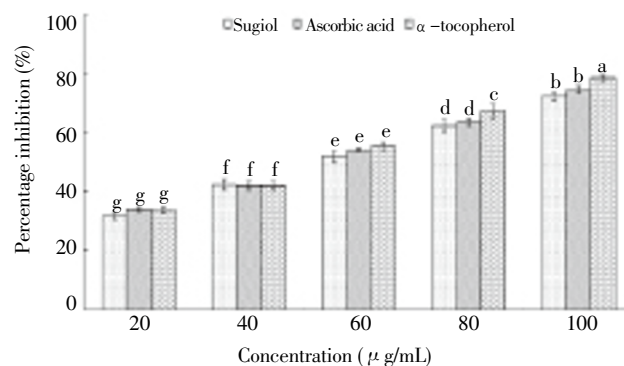


Figure 2. Nitric oxide radical scavenging activity of a diterpenoid sugiol from *M. glyptostroboides* and standard antioxidants, ascorbic acid and α -tocopherol.

Different superscripts in each column indicate the significant differences between the means (*P*<0.05).

3.4. Superoxide anion radical scavenging activity

The effect of the sugiol on superoxide radical was determined by the PMS-NADH superoxide generating system and the results are shown in Figure 3. All the tested samples significantly scavenged the superoxide radicals in a concentration-dependent manner (*P*<0.05). It has been reported that antioxidant properties of some phenolic compounds are effective mainly via scavenging of superoxide anion radicals^[4]. In this assay, addition of sugiol

and standard compounds (ascorbic acid and α -tocopherol) at the concentration of 250 μ g/mL showed 72.99%, 73.00% and 74.45% superoxide radical scavenging effect, respectively.

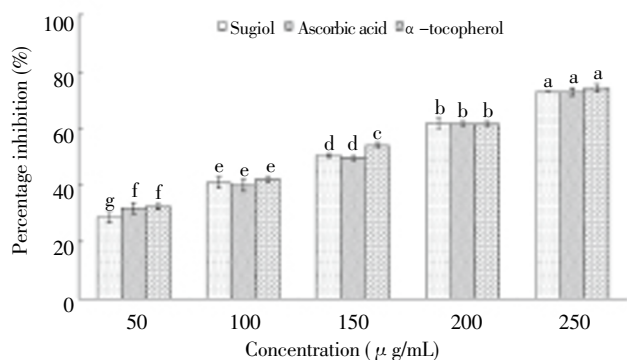


Figure 3. Superoxide radical scavenging activity of a diterpenoid sugiol from *M. glyptostroboides* and standard antioxidants, ascorbic acid and α -tocopherol.

Different superscripts in each column indicate the significant differences between the means ($P < 0.05$).

3.5. Hydroxyl radical scavenging activity

The scavenging effect of sugiol against hydroxyl radicals was investigated using the Fenton reaction. As presented in Figure 4, the percentage inhibition of sugiol, ascorbic acid and BHA on hydroxyl radical scavenging was found to be 85.04%, 73.79%, 70.02%, respectively. The results showed significant antioxidant activity in a concentration-dependent manner ($P < 0.05$). The ability of the sugiol to quench hydroxyl radicals seems to be directly related to the prevention of propagation of lipid peroxidation; because sugiol seems to be a good scavenger of active oxygen species, it will thus reduce the rate of the chain reaction.

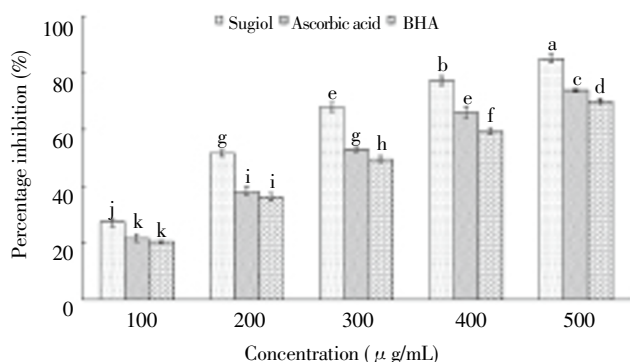


Figure 4. Hydroxyl radical scavenging activity of a diterpenoid sugiol from *M. glyptostroboides* and standard antioxidants, ascorbic acid and BHA.

Different superscripts in each column indicate the significant differences between the means ($P < 0.05$).

3.6. Lipid peroxidation inhibitory activity

In this assay, the peroxidation of bovine brain phospholipid extract was induced by ferric chloride and ascorbic acid as reducing agents. Hydroxyl radicals are generated by mixing

Fe^{3+} and ascorbate which attack the biological material. This leads to the formation of MDA and other aldehydes, which form a pink chromogen with TBA, absorbing at 532 nm. As shown in Figure 5, the sugiol demonstrated considerable amount of lipid peroxidation inhibitory effect by 76.50%, while α -tocopherol and BHA significantly inhibited lipid peroxidation by 80.13% and 76.59%, respectively. The results were concentration-dependent and considered statistically significant ($P < 0.05$).

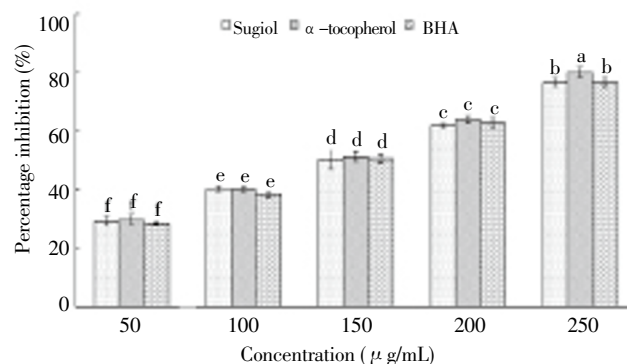


Figure 5. Lipid peroxidation inhibitory effect of a diterpenoid sugiol from *M. glyptostroboides* and standard antioxidants, α -tocopherol and BHA.

Different superscripts in each column indicate the significant differences between the means ($P < 0.05$).

3.7. Reducing power activity

As illustrated in Figure 6, the conversion of Fe^{3+} to Fe^{2+} in the presence of sugiol and reference compounds could be measured as their reductive ability. At the concentration of 25 μ g/mL, the absorbance values of sugiol, ascorbic acid and α -tocopherol were measured to be 1.09, 1.21, and 1.14, respectively. The results showed a concentration-dependent significant increase ($P < 0.05$) in reductive ability of the test samples. These results demonstrated that sugiol had marked ferric ions (Fe^{3+}) reducing ability and had electron donor properties for neutralizing free radicals by forming stable products.

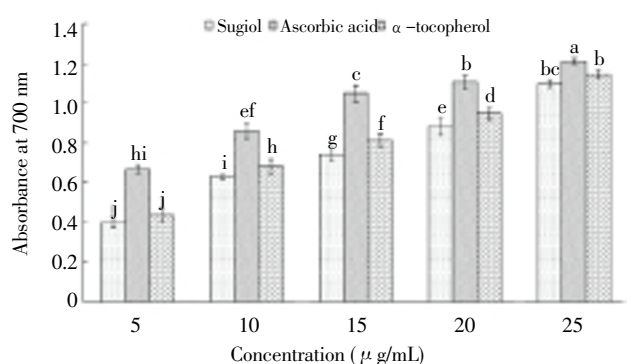


Figure 6. Reducing power activity of a diterpenoid sugiol from *M. glyptostroboides* and standard antioxidants, ascorbic acid and α -tocopherol.

Different superscripts in each column indicate the significant differences between the means ($P < 0.05$).

4. Discussion

The scavenging activity on DPPH radicals assay is generally used as a basic screening method for testing the anti-radical activity of a large variety of compounds^[20]. This assay is based on the measurement of the ability of antioxidants to scavenge the stable radical DPPH, widely used in evaluating the antioxidant activities in a relatively short time as compared to the other methods. In this assay, the color of DPPH radical changes from violet to yellow upon reduction which is demonstrated by the decrease in absorbance at 517 nm. The free DPPH radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. For being an easy and accurate method, it has been recommended to measure the antioxidant activity of samples of different origin^[21]. Free radical scavenging is one of the recognized mechanisms by which antioxidants inhibit lipid peroxidation^[22]. DPPH radical scavenging activity of diterpenoid compounds has been confirmed previously^[23,24].

NO is a free radical with a single unpaired electron. NO is formed from L-arginine by NO synthase^[25]. It can also be formed from the reaction of peroxy radical and NO, polluted air, and smoking^[26]. NO itself is not a very reactive free radical, however, overproduction of NO is involved in ischemia reperfusion, neurodegenerative and chronic inflammatory diseases like rheumatoid arthritis. Nitric dioxide adds to double bonds and extracts labile hydrogen atoms initiating lipid peroxidation and production of free radicals. Marcocci *et al.* reported that NO scavengers compete with oxygen, resulting in a lower production of NO^[27]. The metabolite ONOO⁻ (peroxynitrite) is extremely reactive, directly inducing toxic reactions, including SH-group oxidation, protein tyrosine nitration, lipid peroxidation, and DNA modifications^[28]. NO radical scavenging activity of terpenoid compounds has been confirmed previously^[23,24].

Superoxide anion, which is a reduced form of molecular oxygen, is an initial free radical formed from mitochondrial electron transport systems. Superoxide anions serve as precursors to active free radicals that have the potential to react with biological macromolecules and thereby induce tissue damage^[29]. Superoxide has also been observed to directly initiate lipid peroxidation and plays an important role in the formation of other ROS like hydroxyl radicals, which induce oxidative damage in lipids, proteins, and DNA. Previously diterpenoid compounds have been found to possess significant superoxide radical scavenging activity^[23,24].

Hydroxyl radicals are extremely reactive free radicals formed in biological systems and have been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. These radicals can be formed from a superoxide anion and hydrogen peroxide in the presence of copper or iron ions^[30]. Hydroxyl radicals are very strongly reactive oxygen species, and there is no specific enzyme to defend against them in human^[31]. Therefore, it is important to discover natural compounds with good scavenging capacity against these reactive oxygen species. It is well established that hydroxyl

radical scavenging capacity of samples is directly related to its antioxidant activity^[32]. Hagerman *et al.* explained that high molecular weight phenolic compounds comprising hydroxyl group and aromatic ring serve as potent free radical scavengers^[33]. Hydroxyl radical scavenging efficacy of diterpenoid compounds has been confirmed previously^[23,24].

Several factors to food commodities lead to the quality deterioration. Among these, an undesirable factor, i.e., lipid auto-oxidation, is one of the most concerned. The need of protecting food against oxidative degradation has prompted the wide usage of food additives from natural origin. Lipid peroxidation leads to rapid development of rancid and stale flavors, and it is considered as a primary mechanism of quality deterioration in lipid foods^[34]. Synthetic antioxidants, e.g., BHA, are added in food during processing to suppress lipid peroxidation and consequently to improve food quality and stability. The phenolic compounds and other chemical components may suppress lipid peroxidation through different chemical mechanisms, including free radical quenching, electron transfer, radical addition or radical recombination^[35]. In addition, lipid peroxidation of cell membrane is associated with various pathological events such as atherosclerosis, inflammation and liver injury^[36]. Previous reports have confirmed the lipid peroxidation inhibitory effect of various terpenoid compounds^[23,24].

The reducing property of test compounds indicates they can be used as electron donors which reduce the oxidized intermediates of lipid peroxidation processes, therefore, they can act as primary and secondary antioxidants. In the present study, assay of reducing power was based on the reduction of Fe³⁺/ferricyanide complex to Fe²⁺ in the presence of reductants (antioxidants) in the tested samples. The Fe²⁺ was then monitored by measuring the formation of Prussian blue at 700 nm^[18]. The increased reducing ability observed may be due to the formation of reductants which could react with free radicals to stabilize and terminate radical chain reactions during fermentation, converting them to more stable products^[37]. Some of the terpenoid compounds have been found to be potent reductants^[23,24].

On the basis of the results obtained in the present study, it can be concluded that sugiol exhibited potent antioxidant and free radical scavenging activities. Moreover, the hydrogen donating ability of sugiol has been proven through the assessment of reducing power ability and radical scavenging activities. Sugiol also exerted significant lipid peroxidation inhibitory activity of considerable interest. These results confirm the efficacy of sugiol as a significant source of natural antioxidant to stabilize the foods from oxidative deterioration and to prevent the progress of various oxidative stress-induced diseases. However, the *in vivo* safety needs to be thoroughly investigated in experimental rodent models prior to its practical application.

Conflict of interest statement

We declare that we have no conflict of interest.

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