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In vitro antioxidant and inhibitory activities of polyphenolic-rich extracts of *Syzygium cumini* (Linn) Skeels leaf on two important enzymes relevant to type II diabetes mellitus

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Abstract: In this study, the effect of free and bound polyphenolic-rich extract of *Syzygium cumini* (Linn) Skeels leaf on antioxidant as well as α-amylase and α-glucosidase activities were determined using *in vitro* model. Polyphenolic-rich extract of *Syzygium cumini* (Linn) Skeels leaf was prepared accordingly and the capability of the extract to inhibit antioxidants as typified by ferric reducing power (FRAP) and 1,1-diphenyl-2-picryl-hydrazil (DPPH) among other free radicals scavenging abilities were quantified spectrophotometrically, added to this, the activities of α-amylase and α-glucosidase were also assessed. The bound phenolic extract exhibited more *in vitro* antioxidant properties as represented by their high radicals scavenging ability in all the free radicals evaluated. Also, the polyphenolic-rich extracts inhibited α-amylase and α-glucosidase, with bound phenolics showing significant ($p<0.05$) increase in a dose-dependent manner than free phenolics. Therefore, this study suggests the use of *Syzygium* cumini leaf as a nutraceutical in the management/ control of type II diabetes mellitus patients.

Keywords: Antioxidant, α-amylase, α-glucosidase, Polyphenolic-rich, *Syzygium cumini.*

INTRODUCTION

Diabetes mellitus is a metabolic syndrome characterized by either a defect in secretion of insulin or insulin action or both (Naik, 2011). Kwon *et al* (2016) reported that deficiency of insulin can trigger prolonged hyperglycemia with instability in nutrients (e.g. carbohydrate, fat, protein etc.) metabolism. There are two types of diabetes mellitus with type II accounting for more than 90% in both developed and developing countries (Rambabu *et al*., 2014). Sharifi-Rad *et al* (2016) documented that more than 382 million individuals are living with diabetes mellitus globally, with a projection of 471 million people by 2035 if urgent action were not taken.

Diabetes mellitus can be controlled/managed by either diet, exercise, herbal, insulin or oral hypoglycaemic agents (e.g. metformin, glybaclamide etc.) (Olabiyi *et al*., 2016). Type II diabetes mellitus patients are at times characterized by obesity coupled with insulin resistance syndrome. Management of diabetes mellitus is to enhance glycemic control, reduce body weight and ameliorate the risk of cardiovascular heart disease, which accounts for more than 70% of deaths in people living with the disease (Olabiyi *et al*., 2016).

The main function of α-amylase in mammals is to breakdown α-1→4 glycosidic bond in polysaccharides, like in starch. Starch is the main staple food for humans, it start digestion in the mouth using α-amylase for breaking polysaccharides into short-chain saccharides (Naik, 2011). Also, the pancreas has the potential to secrets α-amylase into the small intestine, which continues this digestion process on some escaped polysaccharide from the mouth as described by Perry *et al* (2004). Thereafter, α-glucosidase will go on with the digestion by breaking down the disaccharides (short-chain saccharides) into monosaccharides like glucose, which can be freely absorbed into the blood stream, using epithelial cells of the small intestine (Ajiboye *et al*., 2018a). Therefore, inhibitions of α-amylase and α-glucosidase are very essential in the management of type II diabetes mellitus, the main mechanism of action of commercially available inhibitors like miglitol, acarbose, etc. (Abirami *et al*., 2014).

Moreover, Sharifi-Rad *et al* (2016) documented that continuous hyperglyceamia in diabetes mellitus patients may trigger oxidative stress both at tissues and organs levels, which may be managed by antioxidant nature of polyphenolics compounds. Oboh *et al* (2012) reported

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that phenolics may be either free (aglycone) or bound forms (glycosides). The free phenolics are more easily absorbed while bound phenolics are mainly not digestible by human enzymes, but it has health benefits by releasing some phytochemicals locally, using flora bacteria in both stomach and small intestine (Adefegha and Oboh, 2015).

Sanches *et al* (2016) acknowledged that *Syzygium cumini* (L.) has been used for therapeutic reasons by Indian Ayurvedic medicine practitioners for many years now. In addition, Ayyanar and Subash-Babu (2012) reported that *S. cumini* (known as Jamun) is now cultivated worldwide including Nigeria, especially in the Northern part of the country for different medicinal values. The mediated synthesis of silver nanoparticles (AgNPs) from *Syzygium cumini* (L.) Skeels polyphenolic-rich leaf extracts were reported by (Ojo *et al*., 2018). The antidiabetic activities of the fruits and seeds have been investigated with a scanty report on the leaves. Hence, this research was to examine the *in vitro* antioxidant potential and inhibitory activities of polyphenolic-rich extract of *Syzygium cumini* (Linn) Skeels leaf on two crucial enzymes (α-amylase and α-glucosidase) germane for diabetes mellitus.

MATERIALS AND METHODS

Sample collection: Fresh *Syzygium cumini* leaf (Linn) Skeels was obtained in August, 2016 from the premises of Zamani College, Kaduna, Kaduna State, Nigeria. The leaves were identified and authenticated by a taxonomist at Department of Plant Biology, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria. The leaves were then washed and rinsed with distilled water. Then air dried for 7 days, blended using electrical blender into a powdered form and stored at 10 °C until required for analysis.

Chemicals and reagents

Chemicals and reagents such as 1,10-phenanthroline, deoxyribose, garlic acid, and Folin-Ciocalteu's reagent were procured from Sigma-Aldrich, Inc., (St Louis, MO). TCA (trichloroacetic acid), dinitrophenyl hydrazine, hydrogen peroxide, acetone, ethylacetate, sodium carbonate, aluminum chloride, iron sulphate, potassium ferricyanide, dinitrosalicylic acid, p-nitrophenylglucopyranoside, amylase, glucosidase, sodium carbonate, 1% sulfanilamide, 2% H_3P0_4, N-1-naphthyl ethylenediaminedihydrochloride, Tris-HCl, thiobarbituric acid and others were obtained from BDH Chemicals Ltd., (Poole, England), while distilled water used was glass distilled.

Extraction of phenolics

Extraction of free phenolics

This was done using the method of Chu *et al* (2002). One hundred grams (100 g) of the powdered *S. cumini* leaf sample was extracted with 80% acetone in 1 ratio 5 weight per volume and filtered using Number 4 Whatman filter paper. Thereafter rotary evaporator (vacuum at 45°C) was used to dry the obtained filtrate. Hence, the acquired extract was kept at -4°C for subsequent analyses with the achieved residue used for the extraction of bound phenolics.

Extraction of bound phenolics

Also, the procedure outlined by Chu *et al* (2002) was followed in this extraction. The residue obtained from Section 2.2.1 was flushed with nitrogen and hydrolyzed using 20 mL of 4 M NaOH solution at room temperature (25°C) for one hour with intermittent shaking. The mixture pH was adjusted to two using concentrated HCl. And the bound phenolics were extracted using six times of ethylacetate. This was then evaporated to dryness at 45°C using rotary evaporated.

In vitro antioxidants determination

Ferric reducing antioxidant power (FRAP)

The of Pulido *et al* (2000) was employed in this determination. Two and a half (2.5) mL aliquot of the extract was mixed with 2.5 mL 200mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes and then 2.5 mL of 10% TCA was added to stop the reaction. This was centrifuged at 650 g for 10 minutes. Hence, 5 mL of obtained supernatant was mixed with an equal volume of water plus 1 mL of 0.1% ferric chloride. The gallic acid solution was used as a standard and the absorbance was read at 700 nm. This was calculated and expressed as gallic acid equivalent.

1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging ability

This was determined using a procedure described by Blois (1958). In this assay, the reagent was prepared by mixing the mixture of 1 mL, 0.4 mM methanolic solution containing DPPH radicals. Then 1mL of this solution was pipetted and added to 3 mL of the extract at different concentrations. The mixture was incubated for 30 minutes and absorbance was read at 517 nm; gallic acid was used as a standard. All the tests were performed in triplicate.

Metal ion chelation ability

The method described by Minotti and Aust (1987) with the slightest modification by Puntel *et al* (2005) was used in this assay. Freshly prepared 500 μmol/L of $FeSO_4$ (150 μL) was added to the reaction mixture containing 168 μL of 0.1 mol/L Tris-HCl of pH 7.4, 218 μL saline and polyphenolics extracts of *S. cumini* leaf at different concentrations. This was incubated for 5 minutes and 13 μL of 0.25% phenanthroline (1:10 w/v) was added. The absorbance was subsequently read at 510 nm by means of a spectrophotometer.

Nitric oxide radical scavenging activity

Briefly, sodium nitroprusside (10 mM) in phosphate-buffered saline was mixed with different concentrations of phenolic extracts and incubated at 30°C for 2 hours. The same reaction mixture without the extract but the equivalent amount of ethanol served as the control. After the incubation period, 0.5 mL of Griess reagent [1% sulfanilamide, 2% H_3P0_4 and 0.1% N-(1-naphthyl ethylenediaminedihydrochloride)] was added. The absorbance was immediately read at 550 nm (Garrat, 1964).

Hydrogen peroxide radical scavenging

This was determined in accordance with the method of Ruch *et al* (1989). Phenolics extracts were dissolved in phosphate buffer (0.1nM) of pH 7.4 at various concentrations and mixed with 600 µL of hydrogen peroxide solution. Ascorbic acid was used as the standard. The absorbance of the mixture was read at 230 nm after 10 minutes of incubation at room temperature.

α-Amylase inhibitory activity

This was carried out according to the method of Shai *et al* (2010). A volume of 250 µL of the *S. cumini* leaf extract (at difference concentrations) was incubated at 25°C for 10 minutes with 500 µL of hog pancreatic amylase (2 U/mL) in 100 mmol/l phosphate buffer at pH of 6.8. Then 250 µL of 1% starch dissolved in 100 mmol/l phosphate buffer (pH 6.8) was then added to the reaction mixture and incubated at 25°C for 10 minutes. Also, 1 mL of dinitrosalicylic acid (DNS) (color reagent) was added and boiled for 10 minutes. The absorbance of the resulting mixture was read at 540 nm.

α-Glucosidase inhibitory activity

The effect of the polyphenolic extracts of *Syzigium cumini* leaf on α-glucosidase activity was determined according to the method described by Kim *et al* (2005), using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenylglucopyranoside (pNPG) was prepared in 20 mM phosphate buffer of pH 6.9. Then 100 µL of α-glucosidase was pre-incubated with 50 µL of the different concentrations of the extracts for 10 minutes. Then 50 µL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37°C for 20 minutes and stopped by adding 2 mL of 0.1 M Na_2CO_3. Thereafter, the absorbance was read at 405 nm.

STATISTICAL ANALYSIS

The result of triplicate experiments was pooled and expressed as mean ± standard error of the mean. The mean was analyzed using one-way analysis of variance (ANOVA) and Duncan test was used for the *post hoc* treatment. The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) 16.0 software.

RESULTS

Table 1 shows the ferric reducing antioxidant potential (FRAP) scavenging ability of polyphenolic-rich extracts of *S. cumini* leaf. There was a significant ($p<0.05$) increase in FRAP inhibitory activity as the extracts concentration increases.

As presented in fig. 1, an increase in the concentration of the polyphenolic-rich extracts (free and bound) of *S. cumini* leaf led to a significant ($p<0.05$) increase in the DPPH radical scavenging ability of the extracts.

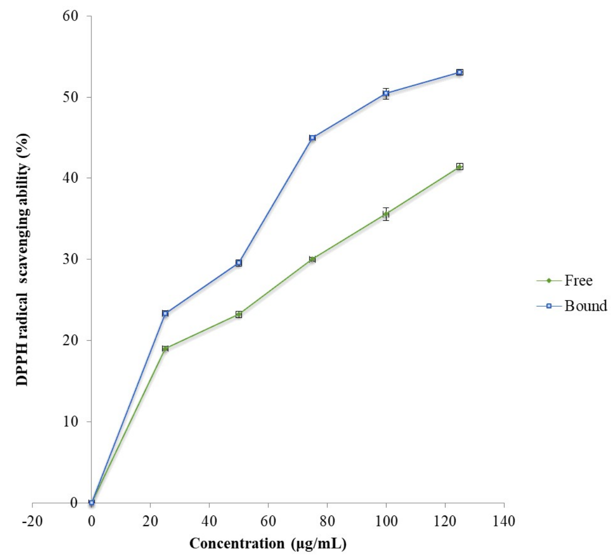

Fig. 1: DPPH radical scavenging ability of polyphenolic-rich extracts of *Syzigium cumini* leaf. Values are represented as mean ± Standard Error of Mean (SEM) of triplicate experiment

The metal ion chelating radical scavenging ability of polyphenolic-rich extracts of *S. cumini* leaf increased with increasing concentration (table 2) i.e. at higher concentrations, the metal ion chelating radical scavenging ability of the extracts were also increase. In addition, bound phenol exhibited higher metal radical scavenging ability when compared with free phenol.

An increase in concentrations of polyphenolic-rich extracts of *S. cumini* leaf led to an increase in nitric oxide scavenging ability of the extracts (table 2), showing that at higher concentrations, nitric oxide scavenging ability of the extracts were higher and at lower concentrations, the nitric oxide scavenging ability of the extracts were low.

Table 2 exhibits the hydrogen peroxide scavenging ability of polyphenolic-rich extracts of *Syzigium cumini* leaf. It shows that an increase in the concentration of polyphenolic-rich extracts of *Syzigium cumini* leaf led to an increase in the concentration of H_2O_2 scavenging ability which was higher at a higher concentration than at lower concentration. Also, bound phenol exhibits higher H_2O_2 scavenging ability compared to free phenol. The polyphenolic-rich extracts of *S. cumini* leaf were proficient in scavenging hydrogen peroxide.

Fig. 2 shows the inhibitory effects of polyphenolic-rich extracts of *S. cumini* leaf against *in vitro* alpha-amylase. It shows that there is a significant ($p<0.05$) increase in inhibitory effects of both free and bound phenol against α-amylase as the concentration of plant extracts increases. However, bound phenol has a higher inhibitory effect of α-amylase compared to free phenol. Also, inhibitory effects of polyphenolic-rich extracts of *S. cumini* leaf against *in vitro* alpha-glucosidase (fig. 3) shows that there is a significant ($p<0.05$) increase in inhibitory effects of both free and bound phenol against α-glucosidase as the concentration of plant extracts increases. There is a higher inhibitory effect of α-glucosidase in bound phenol than in free phenol.

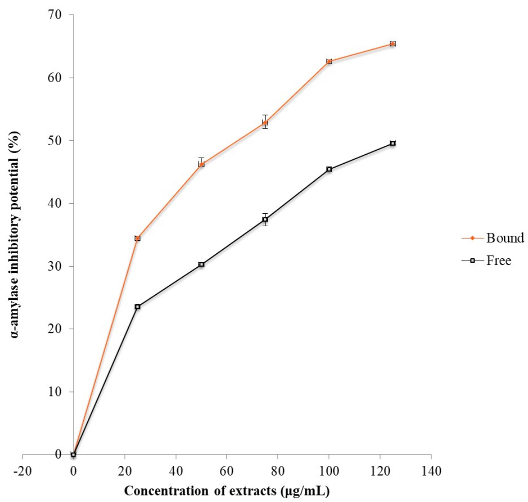

Fig. 2: Inhibition of *in vitro* alpha-amylase activity by polyphenolic-rich extracts of *S. cumini* leaf. Values are represented as mean ± Standard Error of Mean (SEM) of triplicate experiment

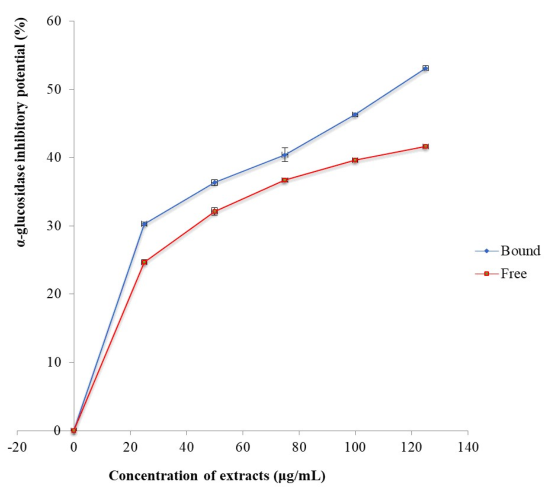

Fig. 3: Inhibition of *in vitro* alpha-glucosidase activity by polyphenolic-rich extracts of *S. cumini* leaf. Values are represented as mean ± Standard Error of Mean (SEM) of triplicate experiment

DISCUSSION

The radical scavenging abilities of the polyphenolic-rich extracts of *S. cumini* leaf could be useful in the management/control of diabetes mellitus because free radicals are convoluted in the pathogenesis of diabetes mellitus by producing reactive oxygen species which leads to the autoimmune destruction of the pancreas, particularly the beta cells (Ademosun and Oboh, 2015). In this study, the antioxidant ability which is a purpose of radical scavenging ability of the extract was evaluated using five (5) different models: Ferric reducing antioxidant potential (FRAP), 1,1-diphenyl-2-picryl-hydrazil (DPPH), nitric oxide (NO), metal ion chelating activity and hydrogen peroxide (H_2O_2). It shows that even though both polyphenolic-extracts had a good ferric reducing antioxidant potential scavenging ability, bound phenol had better FRAP scavenging ability than free phenol. FRAP radical scavenging ability of a plant extract may serve as a significant indicator because of its antioxidant activity (Siddhuraju and Becker, 2007; Ojo *et al*., 2019). It has also been suggested that FRAP is extensively used in evaluating the antioxidant constituent of dietary polyphenols (Luximon-Ramma *et al*., 2003). Therefore, the antioxidant potential of polyphenolic-rich extracts of *S. cumini* leaf was estimated by its ability to reduce Fe (III) complex to Fe (II). This property may provide protection to the affected organs by the ability of the plant extract to clean up harmful metabolites released during pathological states (Ajiboye *et al*., 2016).

The scavenging ability of bound phenol increased compared to that of free phenol showing that bound phenol of the plant extract had a better DPPH scavenging activity. Generally, the extracts were able to scavenge DPPH radical in concentration dependent manner. Scavenging of stable 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical model is broadly used to evaluate the antioxidant activities of the extracts. DPPH is a stable radical which can readily undergo scavenging by antioxidants (Lu and Foo, 2001). It is used to access the ability of an extract as free-radical scavengers or hydrogen donors and to evaluate the anti-oxidative activity of plant extracts. The polyphenolic-rich extracts of *S. cumini* leaf had high levels of DPPH radical scavenging activity showing that the plant extracts have antioxidant potentials. This may be associated with the antioxidant compounds in the extract which may have neutralized the free radical character of DPPH by transferring either electrons or hydrogen atoms to DPPH (Siddhuraju and Becker, 2007; Ojo *et al*., 2014).

Iron is a vital mineral for normal body functioning, but an excess of it may result in cellular damage. If it undergoes Fenton reaction, it may form reactive hydroxyl radicals and thereby contribute to oxidative stress (Siddhuraju and Becker, 2007). An important mechanism of antioxidant

Table 1: FRAP scavenging ability of polyphenolic-rich extracts of *Syzigium cumini* leaf

Extracts	Values (Ascorbic Acid Equivalent) (mg/100g)
Free Phenol	165.95 ± 3.45[a]
Bound Phenol	261.94 ± 5.33[b]

Each value is a mean of triplicate ± SEM .Values with different superscript (a-b) down the column are significant different (p<0.05)

Table 2: Some *in vitro* antioxidant parameters of polyphenolic-rich extracts of *Syzigium cumini* leaf.

Concentration(µg/mL)	0	25	50	75	100	125
Metal ion chelating radical scavenging ability (%)						
Free phenol	0±0.00[a]	26.66±0.18[a]	28.78±1.22[a]	30.74±0.45[a]	33.32±0.16[a]	37.34±0.34[a]
Bound phenol	0±0.00[a]	29.82±0.34[b]	32.65±0.56[b]	33.78±0.21[b]	37.29±0.23[b]	43.21±0.13[b]
Nitric oxide scavenging ability (%)						
Free phenol	0±0.00[a]	10.15±1.12[a]	13.33±0.23[a]	14.53±0.10[a]	16.65±0.12[a]	24.64±0.14[a]
Bound phenol	0±000[a]	12.82±089[b]	17.39±1.08[b]	21.89±0.68[b]	23.34±0.20[b]	30.82±0.40[b]
Hydrogen peroxide scavenging ability (%)						
Free phenol	0±0.00[a]	11.83±0.23[a]	18.18±0.32[a]	22.72±0.21[a]	30.15±0.42[a]	36.95±0.42[a]
Bound phenol	0±0.00[a]	17.66±0.56[b]	21.15±0.89[b]	26.42±0.11[b]	34.86±1.09[b]	47.57±0.35[b]

Values with different superscript (a-b) across the column are significant (p<0.05) different. Each value is a mean of triplicate ± SEM

activity is the ability to deactivate transition metals, which possess the ability to catalyze hydrogen peroxide decomposition and Fenton type reactions. Therefore, it is considered important to screen the iron (II) chelating ability of the extracts. The polyphenolic-rich extracts of *S. cumini* leaf were found to have good chelating ability (table 2).

Bound phenol exhibited higher NO scavenging ability than free phenol. Nitric oxide (NO) is a strong mediator that may alter the structure and function of many cellular components and is involved in the regulation of various physiological processes. It is generated by endothelial cells, macrophages, neurons, etc. via nitric oxide synthases (NOSs), and metabolize arginine to citrulline plus formation of NO through an oxidative reaction. Excess concentration of NO is associated with numerous diseases an example is diabetes mellitus. This may be due to the antioxidant properties of the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite (Parul *et al*., 2013).

Hydrogen peroxide (H_2O_2) generally is not reactive, but it may be toxic because it can give rise to hydroxyl radicals in the cells. Hence, removing H_2O_2 is vital for antioxidant defense in plant cells as it can sometimes be poisonous to the cells. Decomposition of hydrogen peroxide into the water may occur according to the nature of antioxidant compounds present in the extract which are good electron donors. They may accelerate the conversion of H_2O_2 to H_2O (Siddhuraju and Becker, 2007).

Inhibition of key enzymes involved in the metabolism of carbohydrates particularly α-amylase and α-glucosidase is one of the main therapeutic methods for ameliorating postprandial blood glucose levels in an attempt to manage diabetes mellitus (Ajiboye *et al*., 2018b). α-amylase is the key enzyme that hydrolyzes complex dietary carbohydrates into oligosaccharides and disaccharides while α-glucosidase is in charge of breaking down oligosaccharides and/or disaccharides into monosaccharides thereby leading to higher postprandial hyperglycemia. Therefore, α-amylase and α-glucosidase inhibitors are acknowledged as starch blockers since they prevent dietary starch from breaking down to monosaccharides. The type of sugar that is easily absorbed through the mucosal border in the small intestine, thereby, lowering postprandial glucose levels. These may have valuable effects on insulin resistance as well as glycemic index control in individuals living with diabetes mellitus (Barrett and Udani, 2011; Talabi *et al*., 2018). This is in accordance with the present study as indicated in Figures 2 and 3. This means that polyphenolic-rich extracts of *S. cumini* leaf have the ability to regulate postprandial blood glucose levels and consequently replicates the effects of nutraceutical on hyperglycemia. This is in agreement with the report of Ojo *et al.* (2017).

CONCLUSIONS

Polyphenolic-rich extracts of *Syzygium cumini* leaf exhibit inhibitory action on crucial enzymes germane to diabetes mellitus, also the extract show antioxidant potentials. This herb show potential in the management of diabetes mellitus as it exhibits inhibitory activities on main enzymes (α-amylase and α-glucosidase) associated with this disease. Therefore, the possible mechanism through which the extract exerted their antidiabetic properties may be by inhibiting α-amylase, and α-glucosidase as well as preventing oxidative stress.

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