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Research Article

In Vitro Antioxidant and Antiglycation Activity of *Zingiber zerumbet* (Wild Zinger) Rhizome Extract

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ABSTRACT

Rhizomes of *Zingiber zerumbet* (Family: Zingiberaceae) is also known as wild zinger or shampoo zinger. Most of the biological activities reported for this plant are attributed to phenolic contents and volatile principles. Hence, a detailed investigation of antioxidant and antiglycation activities of *Zingiber zerumbet* rhizome was carried out. The ethyl acetate extract of *Zingiber zerumbet* rhizome (ZZE) was prepared and tested for phytoconstituents. Antioxidant and antiglycation activity of ZZE was determined by standard methods. Upon phytochemical investigations of ZZE showed the presence of phenols, flavonoids and terpenoids. ZZE had a total phenolic content of 331.93±1.23 mg/ml gallic acid equivalent and total flavonoid content of 198±2.65 mg/ml of quercetin equivalent. ZZE also showed a significant total antioxidant activity (86.04±0.98 mg/ml ascorbic acid equivalent). Further, ZZE scavenged DPPH and ABTS radical with an IC_{50} of 117.65±1.45 and 78.72±1.12 µg/ml respectively. Similarly, hydroxyl, superoxide, hydrogen peroxide and nitric oxide radicals were scavenged with an IC_{50} of 39.90±0.77, 185.49±0.97, 144.71±3.75 and 89.87±1.02 µg/ml respectively. The IC_{50} values in *O*-phenanthroline (iron-chelating capacity), β-carotene bleaching and lipid peroxidation assays were 189.63±2.21, 317.24±0.34 and 94.45±2.76 µg/ml respectively. The observed antioxidant activity can be attributed mainly to the total phenolics and flavonoids present in ZZE. Furthermore, ZZE also demonstrated antiglycation activity in hemoglobin-glucose and BSA-glucose assays, but the activity was not significant when compared to the standards quercetin and aminoguanidine. Results of this study confirm the antioxidant and antiglycation potentials of *Zingiber zerumbet* rhizomes.

Keywords: Antioxidants; flavonoids; phenols; shampoo zinger; wild zinger; *Zingiber zerumbet* rhizome

INTRODUCTION

Antioxidants are defined as the substances that deactivate the free radicals or their action. Each cell in human body is gifted with several endogenous antioxidants to tackle the oxidative damage by free radicals (Devasagayam et al, 2004) Endogenous antioxidants such as superoxide dismutase (SOD), catalase, peroxidases and reductases are few examples of enzymatic antioxidants. Glutathione, thiols, vitamins and some essential micronutrient are the examples of nonenzymatic antioxidants. In diseases, the homeostatic balance between antioxidant defenses and generation of free radicals will be lost leading to 'oxidative stress' and worsen the morbidity as well as mortality (Durackova, 2010). The supplementation of exogenous antioxidants has proven to be beneficial in cancer, diabetes, cardiovascular, liver and kidney diseases where there is a prevalence of oxidative stress. The increased oxidative stress causes the proteins cross-linking with monosaccharides such as glucose, leading to formation of advanced glycation end products (AGEs). In diabetes, the AGEs are linked to the diabetic related metabolic complication such as nephropathy, retinopathy and neuropathy. Targeting AGEs has been explored for last two decades but, till today none of the new molecules has come to therapeutics. Many natural antioxidants have been proven to be blocking AGEs formation both *in vitro* and *in vivo* (Reddy and Beyaz, 2006).

There are few biologically proven synthetic-antioxidants but, they are generally not used in practice as compared to the natural antioxidants from plant sources which have been consumed by human from his inception (Wojcik et al, 2010). Curcumin, resveratrol, many polyphenols and flavonoids isolated from the plants have been proven to be good antioxidants (Seifried et al, 2007). Hence, there is a huge scope for search of natural products as antioxidants.

The plant *Zingiber zerumbet* (family: *Zingiberaceae*), grows wildly in India and other south-east Asian countries. Locally, the plant is known as wild ginger or shampoo ginger. This plant is also cultivated in gardens throughout the tropics. The rhizome of this plant is used in traditional medicine in inflammatory conditions

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for swollen sores, and also for loss of appetite. *Zingiber zerumbet* rhizome extract is reported to contain flavonoids such as kaempferol and its glycosides along with curcumin (Nakatani et al, 1991). The rhizomes are also reported to contain zerumbone, humulene and camphrene as volatile principles (Jang et al, 2004, 2005). The majority of the biological activities of this plant, has been attributed to the presence of volatile principles and polyphenols (Yob et al, 2011). Though the antioxidant activity of *Zingiber zerumbet* rhizome is known, there is so far no report of detailed antioxidant studies (Yob et al, 2011). Hence, the objective of the present study was to evaluate in detail, the *in vitro* antioxidant and antiglycation potential of *Zingiber zerumbet*. Ethyl acetate was used as solvent for extraction, as the interest was on the major phenolic content.

MATERIALS AND METHODS

Plant Material and the Chemicals: The rhizome of the plant *Z. zerumbet* was obtained in the month of January from the Udupi District, Karnataka, India and identified by botanist Dr. K. Gopalakrishna Bhat, Retired Prof and Head, Department of Botany, Poornaprajna College, Udupi. A specimen of the plant was kept in the department for future references. Remaining chemical such as DPPH [2,2-diphenyl-1-picrylhydrazyl], ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)], bovine serum albumin (BSA), *O*-phenanthroline, quercetin, butylated hydroxytoluene (BHT), gallic acid, and aminoguanidine (AG) were purchased from Sigma-Aldrich, USA. Sodium nitroprusside, ferrous sulphate, ammonium molybdate, Folin-Ciocalteu reagent, ferric chloride nitro blue tetrazolium, hemoglobin and thiobarbituric acid, were purchased from HiMedia, Mumbai, India. The remaining chemicals were purchased from standard suppliers with LR and AR grade.

Preparation of *Zingiber zerumbet* Ethyl Acetate Extract (ZZE): The rhizomes of *Z. zerumbet* were chopped and dried in a hot-air oven at 50°C. The dried rhizomes were then powdered in a mixer grinder and the powder was used for extraction. The flavonoid-rich extract was made by extracting the dried rhizome powder with ethyl acetate using soxhlet apparatus. The extract was concentrated using rotavapor (Buchi, Switzerland) under low pressure and evaporated to dryness under vacuum in a desiccator. ZZE thus obtained was then tested for phytochemical constituents by standard procedures.

Determination of Total Phenolic Content and Total Flavonoid Content: Total phenolic content was determined using Folin-Ciocalteu (FC) reagent and was expressed as gallic acid equivalent (GAE) as per the earlier literature (Aiyegoro and Okoh, 2010). Total flavonoid content was determined with aluminium chloride ($AlCl_3$) using quercetin as standard and expressed as quercetin equivalent as per the standard literature (Aiyegoro and Okoh, 2010).

Determination of *In vitro* Antioxidant Activity of ZZE

Total antioxidant capacity of ZZE: The assay was based on the reduction of molybdate-VI (Mo^{VI}) to molybdate-V (Mo^{V}) by the extract and subsequent formation of a green phosphate-Mo^{V} complex in acidic pH. The assay was performed described previously (Prieto et al, 1999). The total antioxidant capacity is expressed as weight equivalents of ascorbic acid.

DPPH radical scavenging assay: DPPH radical scavenging activity was determined as reported earlier with suitable modifications (Narla and Rao, 1995). A solution of DPPH (100 µM) in methanol was added to an equal volume of different concentrations of ZZE in methanol, mixed well and kept in dark for 20 min The absorbance at 517 nm was measured using the spectrophotometer UV-1650, Shimadzu. The percentage scavenging of DPPH radical was calculated from the following equation

% scavenging = [(Absorbance of blank-Absorbance of test) /Absorbance of blank] X 100

The percentage scavenging of DPPH radical was plotted against concentration using Microsoft-Excel computer software, and IC_{50} was calculated from this plot. Quercetin was used as standard.

ABTS radical cation scavenging assay: This assay was carried out as per the literature with necessary modification (Prabhakar et al, 2006). To the reaction mixture containing 0.5 ml of different concentration of extract-ZZE, 1.7 ml of phosphate buffer (20 mM) and 0.3 ml of 100 µM $ABTS^{\bullet-}$ [prepared by mixing 2 mM ($ABTS^{2-}$) with 0.17 mM potassium persulphate in 20 mM phosphate buffer pH 7.4; kept overnight before use] was added. Immediately, the decrease in absorbance was measured at 734 nm. Quercetin was used as standard. The % scavenging and the IC_{50} values were calculated as mentioned in the DPPH assay.

Superoxide radical ($O_2^{\bullet-}$) scavenging assay: This assay was carried out as per the previous report with appropriate modifications (Prabhakar et al, 2006). To 50 µl of different concentrations of extract/standard, 100 µl alkaline DMSO (1.0 ml DMSO containing 5 mM NaOH in 0.1 ml water) and 20 µl of 20 mM NBT (in phosphate buffer, pH 7.4) were added. The absorbance was measured at 560 nm. The experiment was performed in triplicate. The % scavenging and the IC_{50} values were calculated as mentioned in the DPPH assay.

Hydroxyl radical ($^{\bullet}OH$) scavenging assay: This assay was performed as per the method reported earlier with appropriate modification (Kunchandy and Rao, 1990). To the reaction mixture containing ascorbic acid (0.1 mM), deoxy-D-ribose (3 mM), ferric chloride (0.1 mM), EDTA (0.1 mM), hydrogen peroxide (2 mM) in phosphate buffer (20 mM, pH=7.4), extract-ZZE in a volume of 0.3 ml (various concentrations) were added, to obtain a final volume of 3.0 ml. After incubation for 30 min at ambient temperature, 1.0 ml of TCA-TBA

reagent (equal volumes of TCA-2.8% and TBA-0.5% in 4 mM NaOH) was added, followed by boiling the tubes in a water bath for 30 min. The tubes were then cooled and the absorbance was measured at 532 nm. Simultaneously, a blank absorbance was determined without the extract. The percentage scavenging and IC_{50} values were calculated as given in DPPH method and it was compared with the standard quercetin.

Nitric oxide (NO) scavenging assay: This assay was performed with as per literature with necessary modifications (Sreejayan and Rao, 1997). The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, 1.0 ml) and 1.0 ml of extract/standard were incubated at 25ºC for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrate was mixed with 1.0 ml of sulphanilic acid reagent and allowed to stand for 5 min for completion of diazotization. Then 1.0 ml of naphthylethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 min in diffused light at room temperature. The absorbance was measured at 540 nm against a corresponding blank solution without sodium nitroprusside. IC_{50} value obtained is the concentration of the sample required to inhibit 50 % nitric oxide radical as explained in DPPH assay.

Hydrogen peroxide (H_2O_2) radical scavenging: This assay was carried out as per the method reported earlier with suitable modification (Ak and Gülçin, 2008). The solution of hydrogen H_2O_2 (20 mM) was prepared in PBS (pH 7.4). To the test tubes containing 1.0 ml of different concentrations of extract/standard 2.0 ml of H_2O_2 was added. The absorbance of H_2O_2 decreases upon its oxidation. Absorbance of H_2O_2 was determined 10 min later at 230 nm against a blank solution containing the PBS without H_2O_2. The % scavenging and IC_{50} were determined as explained in DPPH assay.

Reduction of ferric ions by *O*-phenanthroline: This assay was carried out as per the literature with necessary modifications (Shirwaikar et al, 2006). The reaction mixture containing 50 µl of *O*-phenanthroline (3 mM), 100 µl of 200 µM ferric chloride (3.24 mg in 100 ml distilled water) and 100 µl of various concentrations of the extract/ standard were incubated at room temperature for 10 min and the absorbance was measured at 510 nm. The experiment was performed in triplicate. The % scavenging and IC_{50} was determined as explained in explained in DPPH assay.

β-Carotene bleaching assay: The assay reagent was prepared by mixing 3 ml β-carotene solution from the stock solution of 5 mg per 50 ml chloroform with 40 mg of linoleic acid and 400 mg of Tween 40. Chloroform was removed by evaporation while incubating at room temperature. Distilled water was added to make 100 ml of aqueous emulsion of linoleic acid and β-carotene and the absorbance at 470 nm was measured immediately against blank (Takada et al, 2006). The extract/standard (50 µl) was treated with 3 ml of the above reagent and the tubes were kept in a water bath at 50°C for 1 h. When linoleic acid undergoes oxidation, it causes bleaching of β-carotene which can be measured at 470 nm. BHT was used as the standard. The antioxidant activity was expressed as percentage inhibition with reference to control after 1 h of incubation using the following formula:

$$AA = \left(1 - \frac{DR_S}{DR_C}\right) \times 100$$

Where, AA is the antioxidant activity; DR_C is degradation rate of the control and DR_S is degradation rate in the presence of sample.

Lipid peroxidation assay: Wistar rat was sacrificed by cervical dislocation and whole brain was dissected out, blotted dry, weighed and a 10% w/v homogenate was prepared using ice cold 0.154 M potassium chloride. The homogenization was performed carefully in a homogenizer tube placed in a beaker containing ice cold water, to form a smooth homogenate without frothing. The homogenate thus obtained was centrifuged at 10000 rpm for 10 min at 4°C. The supernatant was used for lipid peroxidation assay. To 0.5 ml of rat brain homogenate, 1.0 ml of the different concentrations of extract/quercetin (standard) was added. Lipid peroxidation was stimulated by adding 1.0 ml of 100 µM ferrous sulphate. The reaction was stopped after 30 min by the addition of ice cold 2 ml TCA-TBA-HCl reagent. The test tubes were heated in a water bath at 80°C for 15 min followed by centrifugation at 10000 rpm for 10 min. The absorbance of the supernatant was measured at 535 nm (Narla and Rao, 1995). The % inhibition of lipid peroxidation and IC_{50} were determined as explained in DPPH assay.

Determination of *In Vitro* Antiglycation Activity of ZZE

Hemoglobin-glucose assay: The extract was weighed and dissolved in 10% DMSO to obtain a stock solution of 1.0 mM. Various concentrations of ZZE / standard in 4.0 ml final reaction mixtures was added to a mixture containing glucose (2%), haemoglobin (0.6%) and gentamycin (0.2%) in 10 mM phosphate buffer (pH 7.4). The test tubes were then incubated in dark at room temperature for 72 h with intermittent shaking. To the resulting mixtures, 1.0 ml of NBT reagent (0.5 mM NBT in 0.2 M sodium carbonate buffer, pH 10.4) was added and the mixture was incubated at 37°C for 1 h. Absorbance was measured at 530 nm against a reagent blank (Somani et al, 1989). The % inhibition of glycosylation was calculated as follows:

[(Absorbance of Control – Absorbance of Test)/ Absorbance of Control] X 100

The IC_{50} values of test compounds were evaluated from the dose-response curves of each experiment using Microsoft-Excel computer software. Aminoguanidine (AG) was used as standard.

BSA-glucose assay: This assay was carried out after suitably modifying previously reported methods (Wu

and Yen, 2005). Briefly, BSA (50 mg/ml) was non-enzymatically glycated by incubation in 1.5 M phosphate buffer (pH 7.4) at 37°C for 7 days in the presence of 0.8 M glucose and 0.2% gentamycin. The reaction mixture of 4 ml with various concentrations of extract/ standard was incubated for 7 days. At the end of 7th day, fluorescence was measured at excitation and emission maxima of 330 and 410 nm respectively, versus an un-incubated blank containing the protein, glucose, and inhibitors. Quercetin was used as a standard. The % inhibition of glycation and IC_{50} were calculated as mentioned in hemoglobin glucose assay.

RESULTS

Phytochemical Screening: The phytochemical analysis conducted on ZZE revealed the presence of phenols, flavonoids and terpenoids. The total phenol content, total flavonoid content and total antioxidant activity were obtained by interpolation from the standard plot (Figure 1) and the results are given in Table 1. These phytochemicals are known to support the biological activities of ZZE and could thus be responsible for the antioxidant activities.

Antioxidant Activity of ZZE

The IC_{50} values for various antioxidant activities of ZZE are represented in Table 2. ZZE had significant DPPH-scavenging effects with increasing concentration in the range of 12.5–800 µg/ml when compared to ascorbic acid (Figure 2A). Though the IC_{50} values of the extract was much higher than that of ascorbic acid, it is still considerably good owing to the fact that the extract is a crude one.

The extract showed concentration-dependent scavenging of ABTS-radical (Figure 2B). At lower doses, the extract was poor in scavenging, but at higher doses (>200 µg/ml) it scavenged ABTS more effectively than ascorbic acid. The IC_{50} values of extract and ascorbic acid in scavenging ABTS-radical was 78.72 ± 1.12 and 37.00 ± 0.76 µg/ml respectively. This antioxidant assay enables us to assess the electron transfer capability of the extract. ZZE was as potent as to ascorbic acid in this assay.

The superoxide radical scavenging potency of ZZE was comparatively low, but the activity was dose-dependent (Figure 2C). The IC_{50} values of extract and ascorbic acid for scavenging superoxide radical were found to be 185.49 ± 0.97 and 27.25 ± 0.56 µg/ml respectively.

The extract ZZE reduced ferric ions significantly in the *O*-phenanthroline assay (Figure 2D), though the IC_{50} values were higher than ascorbic acid (IC_{50} of extract = 189.63 ± 2.21; ascorbic = 50.31 ± 0.63 µg/ml).

Extract ZZE showed a graded increase in hydroxyl radical scavenging activity with concentration (Figure 2E). The extract ZZE proved to be a superior hydroxyl radical scavenger than quercetin (IC_{50} extract = 39.90 ± 0.77, quercetin = 85.01 ± 0.33 µg/ml).

The extract ZZE showed concentration-dependent NO-

Table 1: Preliminary phytochemical investigation of ZZE

Parameter tested	Activity per gram of crude dry rhizome powder
Total phenolic content	331.93±1.23 mg of gallic acid equivalent
Total flavonoid content	198±2.65 mg of quercetin equivalent
Total antioxidant capacity	86.04±0.98 mg of ascorbic acid equivalent

Table 2: Antioxidant activity of ZZE in various free radical scavenging assays

Assay	IC_{50} (µg/ml)	
	Extract (ZZE)	Standard
DPPH scavenging assay	117.65±1.45	36.26±0.43
ABTS radical cation decolourization assay	78.72±1.12	37.00±0.76
Superoxide radical scavenging assay	185.49±0.97	27.25±0.56
Reduction of ferric ions by *O*-phenanthroline method	189.63±2.21	50.31±0.63
Hydroxyl radical scavenging assay	39.90±0.77	85.01±0.33 [1]
Nitric oxide scavenging assay	89.87±1.02	48.73±0.85
Hydrogen peroxide radical scavenging assay	144.71±3.75	94.47±1.05
β-Carotene bleaching assay	317.24±0.24	23.21±0.54 [2]
Lipid peroxidation assay	94.45±2.76	140.59±1.43 [1]

Data are expressed as mean ± SEM; Ascorbic acid was used as standard except at [1] quercetin and [2] butylated hydroxyl toluene (BHT).

Table 3: Antiglycation activity of ZZE

Assay	IC_{50} (µg/ml)	
	Extract (ZZE)	Standard
Hemoglobin-glucose assay	340.85±10.57	29.75±0.48 (Aminoguanidine)
BSA-glucose assay	1849.12±13.52	350.70±2.23 (quercetin)

Data are expressed as mean ± SEM

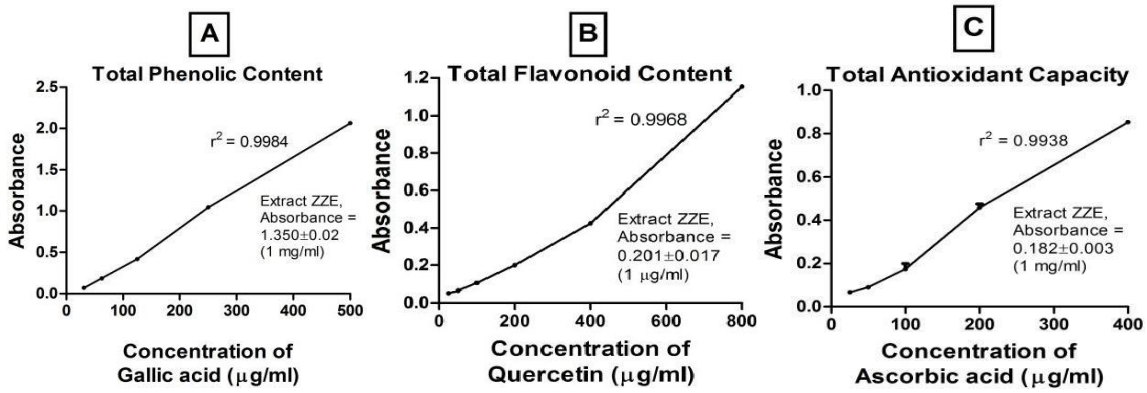

Figure 1: Total phenolic and flavonoid content in ZZE and total antioxidant capacity

[A] Total phenolic content: determined using gallic acid as standard; [B] Total flavonoid content: determined using quercetin as standard; [C] Total antioxidant capacity: determined using ascorbic acid as standard.

scavenging activity (Figure 2F) and it scavenged 50% NO at a concentration of 89.87 ± 1.02 µg/ml which is comparable to ascorbic acid.

The capacity of ZZE to scavenge H_2O_2 was measured by its ability to decompose H_2O_2. H_2O_2 solution (20 mM) was added to different concentrations of ZZE and the reduction in absorbance at 240 nm was measured. There was a graded increase in H_2O_2 scavenging activity (Figure 2G) with IC_{50} 144.71 ± 3.75 µg/ml which was less potent than ascorbic acid (94.47 ± 1.05 µg/ml).

The discoloration of β-carotene by linoleate-free radical was significantly protected by the extract ZZE (Figure 2H). The IC_{50} values of ZZE and BHT were found to be 317.24 ± 0.34 and 23.21 ± 0.54 µg/ml respectively. Therefore, ZZE could effectively scavenge linoleate-free radical although the potency of ZZE was found to be much lower than that of BHT.

Effect of ZZE on the ferrous-stimulated lipid peroxidation was studied and compared to quercetin. The extract ZZE showed dose dependent inhibition of lipid peroxidation (Figure 2I) and was more potent than quercetin (94.45 ± 2.76 and 140.59 ± 1.43 µg/ml for ZZE and quercetin respectively).

Antiglycation activity of ZZE: The antiglycation activity is given in the table 3. The extract-ZZE exhibited antiglycation activity but the results were not significant. The antiglycation activity was graded and dose-dependent at low concentration, but at higher doses it could not prevent glycation in a dose-dependent manner (Figure 3A and 3B). Among the two assays carried out, the antiglycation activity of ZZE was better in hemoglobin-glucose assay compare to BSA-glucose assay (Figure 3A).

DISCUSSION

The benefits of antioxidants have been the subject of thousands of studies in recent years due to their possible role in preventing heart disease, cancer and other illnesses were oxidative stress is the underlying cause (Kohen and Nyska, 2002). Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions. Many synthetic antioxidants such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) are effective *in vitro* and at preclinical level. However, they possess certain limitations with regard to human consumption. Hence, there is increasing interest in the natural antioxidants such as polyphenols, flavonoids and tannins from plants which have been consumed by humans over the years (Dimitrios, 2006).

Zingiber zerumbet is one such medicinal plant consumed by several ethnic groups as medicine for ages. Traditionally the plant has been used for inflammation, fever, toothache, indigestion, constipation, diarrhea, severe sprains, spasms and rheumatism. This plant is reported as antinociceptive, anti-inflammatory, antipyretic, hepatoprotective, antiallergic, immunomodulatory, antiplatelet aggregation, anticancer, antihyperglycemic, antiamoebic, antigiardial, antimicobacterial and antimicrobial (Yob et al, 2011). This study reports the detailed antioxidant activity of this plant for the first time though there are reports on the total phenolic content (Lako et al, 2007)> In the present study it was found that the total phenolic content obtained was much higher (331.93 ± 1.23 mg GAE per gram of crude dry rhizome powder) than what was reported earlier by Lako *et al* (16 mg per 100 g GAE). The difference in the total phenolic content depends mostly on the solvent ethyl acetate used for extraction in this study. Further, the extract has substantial amount of flavonoid content (198 ± 2.65 mg of quercetin equivalent).

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. DPPH has a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers. It is also well accepted that the DPPH free radical scavenging by antioxidants is due to their hydrogen-donating ability (Goupy et al, 2002). After scavenging by antioxidants (through donation of hydrogen), the DPPH molecule will become stable. Hence by this assay, it can be concluded that the extract ZZE has ability to donate pro-

tons probably because of the presence of high phenolic and flavonoid content. Additionally, the extract also has ability to donate electrons and act as an antioxidant, as revealed from ABTS-radical scavenging activity. ABTS-cation has absorption maximum at 734 nm. The scavenging of this cation is a measure of antioxidant property of the extract and it takes place through electron transfer (Thaipong et al, 2006). ZZE was equally potent as ascorbic acid in electron donating abilities.

The superoxide anion radical is a potent reactive oxygen species generated after oxygen is taken into living cells. It is converted to other harmful reactive oxygen

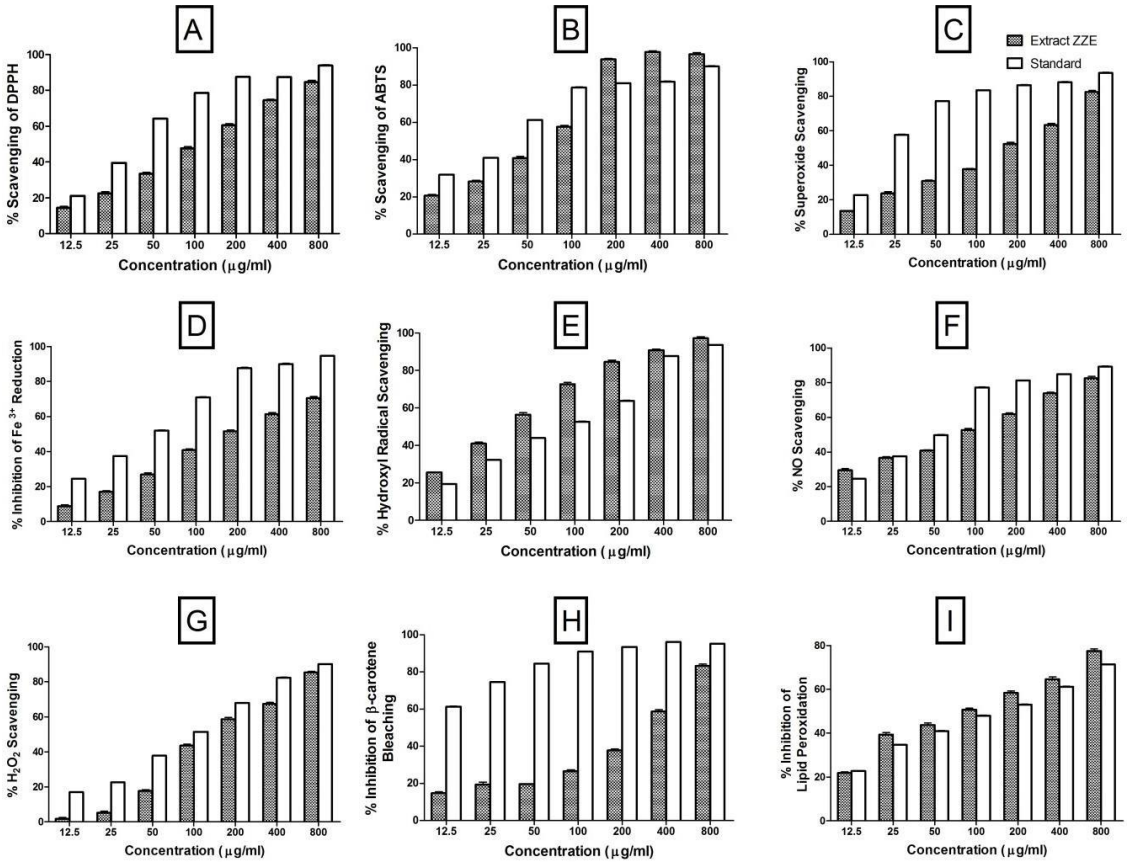

Figure 2: Antioxidant activity of ZZE in various free radical scavenging assays

The percentage radical scavenging activity was plotted against various concentrations of ZZE in [A] DPPH radical scavenging activity; [B] ABTS radical cation decolourization activity; [C] Superoxide radical scavenging activity; [D] Reduction of ferric ions by O-phenanthroline; [E] Hydroxyl radical scavenging activity; [F] Nitric oxide scavenging activity; [G] Hydrogen peroxide radical scavenging activity; [H] Inhibition of β-Carotene bleaching acitivity; [I] Inhibition of lipid peroxidation assay; Data are expressed as mean ± SEM; Ascorbic acid was used as standard except at [1] quercetin and [2] butylated hydroxyl toluene (BHT).

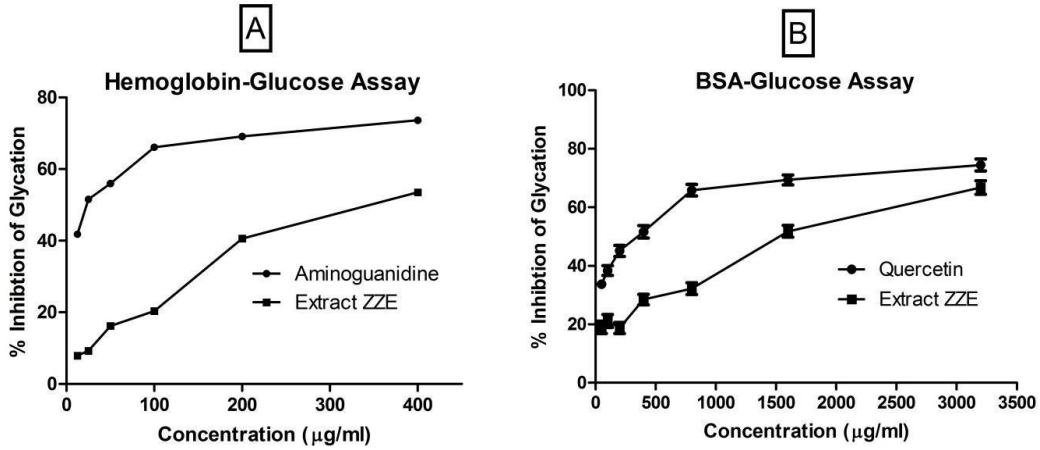

Figure 3: Antiglycation activity of extract ZZE

The percentage antiglycation activity was plotted against concentration of ZZE in [A] Hemoglobin-glucose assay and [B] BSA-glucose assay.

species and free radicals such as hydrogen peroxide and hydroxyl radical (Jones, 2008). Hence superoxide scavenging activity is considered superior to hydroxyl radical and hydrogen peroxide scavenging abilities. The extract demonstrated good superoxide radical scavenging ability.

Ortho substituted phenolic compounds such as *O*-phenanthroline, chelates with iron in its reduced state (Fe^{2+}). Such a chelate has absorption maxima at 510 nm (Berker et al, 2007). The absorbance of the final reaction mixture is directly proportional to the antioxidant activity of the extract. The extract ZZE was found effective in reducing ferric ions although the potency was much lower than that of ascorbic acid.

Hydroxyl radicals are the major reactive oxygen species causing lipid peroxidation, DNA damage and enormous biological impairment (Fang et al, 2002). Hydroxyl radical was produced *in vitro* by incubating ferric-EDTA with ascorbic acid and H_2O_2 at pH 7.4 and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with TBA at low pH. The presence of antioxidant/ extract prevented the reaction by quenching hydroxyl radicals from the sugar. The extract ZZE scavenged hydroxyl radical more potently than standard quercetin. The hydroxyl radical scavenging activity has direct correlation for many biological activities of plant products.

It is well known that nitric oxide has an important role in various inflammatory processes. Sustained levels of production of this radical are directly toxic to tissues and it contributes to vascular collapse associated with septic shock. The chronic expression of nitric oxide is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Pacher et al, 2007). The toxicity of nitric oxide increases greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion ($ONOO^-$). In the *in-vitro* assay, nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extract ZZE inhibited nitrite formation by directly competing with oxygen in the reaction with nitric oxide. Further, the extract demonstrated significant scavenging of H_2O_2 and prevention of β-carotene discolouration by linoleate-free radical *in vitro*, supporting its antioxidant activity (Takada et al, 2006); Wang and Jiao, 2002).

The extract ZZE demonstrated potent inhibition of ferrous-stimulated lipid peroxidation and the potency ¾ fold higher than that of quercetin. Inhibition of lipid peroxidation has more biological relevance than other radical scavenging activity by *in vitro* system (Tribble et al, 1987). This activity could be because of the high polyphenolic components in ZZE. This potent activity could also be the reason for its reported biological activities.

Chronic oxidative stress usually leads to protein-glycation associated with many diseases such as diabetes (Fu et al, 1994). Hence, the antiglycation potential of ZZE was studied. The extract ZZE showed antiglycation activity however, the activity was not significant compared to standard antiglycation molecules such as quercetin and aminoguanidine. ZZE prevented glycation of hemoglobin at better potency in comparison to glycation of BSA. Hence, the extract could play a potential role in preventing diabetes related complications such as nephropathy and retinopathy.

The high phenolic and flavonoid content in ZZE could be the major reason behind the radical scavenging and antiglycation activity. Additionally, the extract contained terpenoids and glycosides. These terpenes could also contribute to the antioxidant activity. Thus, the present detailed antioxidant study amply supports the mechanism of action for the biological activity reported in literature (Yob et al, 2011).

CONCLUSION

Zingiber zerumbet rhizomes are a potential source of antioxidant and antiglycation principles. This antioxidant and antiglycation property of *Zingiber zerumbet* rhizomes could very well be one of the chief mechanisms of action for most of its reported biological activity.

REFERENCES

Aiyegoro, O.A., Okoh, A.I. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC Complem Alter Med. Vol. 10, 2010, pp. 21.

Ak, T., Gülçin, I. Antioxidant and radical scavenging properties of curcumin. Chemico Biol Inter. Vol. 174. 2008, pp. 27-37.

Berker, K.I., Güçlü, K., Tor İ, Apak R. Comparative evaluation of Fe(III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents. Talanta. Vol. 72, 2007, 1157-1165.

Devasagayam, T.P., Tilak, J.C., Boloor, K.K., Sane, K.S., Ghaskadbi, SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India. Vol. 52, 2004, pp. 794-804.

Dimitrios, B. Sources of natural phenolic antioxidants. Trends Food Sci Tech. Vol. 17, 2006, pp. 505-512.

Durackova, Z. Some current insights into oxidative stress. Physiol Res. Vol. 59, 2010, pp. 459-469.

Fang, Y.Z., Yang, S., Wu, G. Free radicals, antioxidants, and nutrition. Nutrition. Vol. 18, 2008, pp. 872-879.

Fu, MiX., Wells-Knecht. K.J., Blackledge J.A., Lyons, T.J., Thorpe SR, Baynes JW. Glycation, Glycoxidation, and Cross-Linking of Collagen by Glucose: Kinetics, Me-

chanisms, and Inhibition of Late Stages of the Maillard Reaction. Diabetes. Vol. 43, 1994, pp. 676-683.

Goupy. P., Dufour. C., Loonis, M., Dangles, O. Quantitative Kinetic Analysis of Hydrogen Transfer Reactions from Dietary Polyphenols to the DPPH Radical. J Agr Food Chem. Vol. 51, 2002, pp. 615-622.

Jang, D.S, Han AR, Park G, Jhon GJ, Seo EK. Flavonoids and aromatic compounds from the rhizomes of Zingiber zerumbet. Arch Pharm Res. Vol. 27, 2004, pp. 386-389.

Jang, D.S., Min, H.Y., Kim, M.S., Han, A.R., Windono, T., Jeohn, G.H. Humulene derivatives from Zingiber zerumbet with the inhibitory effects on lipopolysaccharide-induced nitric oxide production. Chem Pharm Bull. Vol. 53, 2005, pp. 829-831.

Jones, D.P. Radical-free biology of oxidative stress. Am J Physiol Cell Physiol. Vol. 205, 2008; pp. C849-C868.

Kohen, R., Nyska, A. Oxidation of Biological Systems: Oxidative Stress Phenomena, Antioxidants, Redox Reactions, and Methods for Their Quantification. Toxicol Pathol. Vol. 30, 2002, pp. 620-650.

Kunchandy, E., Rao, M.N.A. Oxygen radical scavenging activity of curcumin. Int J Pharm. Vol. 58, 1990, pp. 237-40.

Lako, J., Trenerry, V.C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., Premier, R. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chem. Vol. 101, 2007, pp. 1727-1741.

Nakatani, N., Jitoe, A., Masuda, T., Yonemori, S. Flavonoid Constituents of Zingiber zemmbet Smith (Organic Chemistry). Agr Biol Chem. Vol. 55, 1991, pp. 455-460.

Narla, R.S., Rao, M.N.A. Scavenging of free-radicals and inhibition of lipid peroxidation by 3-phenylsydnone. J Pharm Pharmacol. Vol. 47, 1995, pp. 623-625.

Pacher, P., Beckman, J.S., Liaudet, L. Nitric Oxide and Peroxynitrite in Health and Disease. Physiol Rev. Vol. 87, 2007, pp. 315-424.

Prabhakar, K.R., Veeresh, V.P., Vipan, K., Sudheer, M., Priyadarsini, K.I., Satish, R.B. Bioactivity-guided fractionation of Coronopus didymus: A free radical scavenging perspective. Phytomedicine. Vol. 13, 2006, pp. 591-595.

Prieto. P., Pineda, M., Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. Vol. 269, 1999, pp. 337-341.

Reddy, V.P., Beyaz, A. Inhibitors of the Maillard reaction and AGE breakers as therapeutics for multiple diseases. Drug Discov Today. Vol. 11, 2006, pp. 646-654.

Seifried, H.E., Anderson, D.E., Fisher, E.I., Milner, J.A. A review of the interaction among dietary antioxidants and reactive oxygen species. J Nutr Biochem. Vol. 18, 2007, pp. 567-579.

Shirwaikar, A., Prabhu, K.S., Punitha, I.S. In vitro antioxidant studies of Sphaeranthus indicus (Linn). Indian J Exp Biol. Vol. 44, 2006, pp. 993-996.

Somani, B.L., Sinha, R., Gupta, M.M. Fructosamine assay modified for the estimation of glycated hemoglobin. Clin Chem. Vol. 35, 1989, pp. 497.

Sreejayan, Rao. M.N.A. Nitric Oxide Scavenging by Curcuminoids. J Pharm Pharmacol. Vol. 49, 1997, pp. 105-107.

Takada, H., Kokubo, K., Matsubayashi, K., Oshima, T. Antioxidant activity of supramolecular water-soluble fullerenes evaluated by β-carotene bleaching assay. Biosci Biotech Biochem. Vol. 70, 2006, pp. 3088-3093.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Hawkins, B.D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal. Vol. 19, 2006, pp. 669-675.

Tribble, D.L., Aw, T.Y., Jones, D.P. The pathophysiological significance of lipid peroxidation in oxidative cell injury. Hepatology. Vol. 7, 1987, pp. 377-386.

Wang, S.Y., Jiao, H. Scavenging Capacity of Berry Crops on Superoxide Radicals, Hydrogen Peroxide, Hydroxyl Radicals, and Singlet Oxygen. J Agr Food Chem. Vol. 48, 2000, pp. 5677-5684.

Wojcik, M., Burzynska-Pedziwiatr, I., Wozniak, L.A. A review of natural and synthetic antioxidants important for health and longevity. Curr Med Chem. Vol 17, 2010, pp. 3262-3288.

Wu, C.H., Yen, G.C. Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation endproducts. J Agr Food Chem. Vol. 53, 2005, pp. 3167-3173.

Yob. N.J., Jofrry, S.M., Affandi, M.M., The, L.K., Salleh, M.Z., Zakaria, Z.A. Zingiber zerumbet (L.) Smith: A Review of Its Ethnomedicinal, Chemical, and Pharmacological Uses. eCAM. Vol. 2011, Article No. 543216.