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Research Article

In-vitro Free Radical Scavenging Activity and Radioprotective Property of *Zingiber kangleipakense* (Kishor & Škorničk)

Moirangthem Medhapati Devi*, Paonam Priyobrata Singh, Khoirom Ratipiyari Devi, Dhanaraj Singh Thokchom, Gurumayum Jitendra Sharma

Centre of Advanced Study in Life Sciences, Manipur University, Imphal 795003, India

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ABSTRACT

The purpose of the present study is to investigate the total phenolic, flavonoid and ascorbic acid contents as well as the free radical scavenging potential of *Zingiber kangleipakense* (Kishor & Škorničk) in different solvent systems. Antioxidant properties were determined using thiyl free radical scavenging assay, ferric ion reducing power (FRP) assay, DPPH radical scavenging and metal chelating assays. We also analyzed *in vitro* radioprotective properties of 60% ethanolic extracts of the plant using pBR322 DNA. The plant extracts possess significant contents of total phenolics, flavonoids and ascorbic acid. The observed data indicate that the plant extracts showed higher antioxidant activities with increasing concentrations. 60% ethanol/methanol extract showed higher scavenging activity than aqueous extract. The plant extract dissolved in 60% ethanol protected the DNA damage induced by gamma radiation suggesting that it has potential source of natural antioxidants capable of protection against free radical mediated damages.

Keywords: Reactive oxygen species, antioxidant activity, DPPH, *Zingiber kangleipakense*

INTRODUCTION

Reactive oxygen species (ROS) are free radicals having unpaired electrons that are generated during normal metabolism and exposure to physical and chemical agents, including ionizing radiation and photosensitization. Production of these radicals beyond the antioxidant capacity of the biological system gives rise to oxidative stress[1,2]. Various chronic diseases like cancer, coronary heart diseases, diabetes, atherosclerosis, premature ageing, Alzheimer's disease, inflammatory and neurodegenerative disorders and even senescence processes are associated with over production of free radicals, which results in oxidative damage to the bio-molecules (lipids, proteins, DNA) within the cellular milieu[3-5]. There are many antioxidant defense systems, consisting of enzymatic superoxide dismutase, catalase, glutathione peroxidase and non-enzymatic (ascorbic acid, glutathione and α-tocopherol) compounds that can maintain the balance between free radical generation and further protection from them either by removing or repairing of damage molecules[6]. However, under conditions of severe oxidative stress, these anti-oxidant systems fail to afford complete protection from ROS[7]. Hence, exogenous antioxidants are constantly required to maintain a balance between antioxidants and free radical species inside the cells. An antioxidant may be defined as 'any substance that when present at low concentrations, compared with those of the oxidizable substrate, significantly delays or inhibits oxidation of that substrate'[8]. In recent years, plant-based antioxidants become popular and preferred to synthetic ones because of their multiple mechanisms of action and nontoxic nature. Even though synthetic antioxidants have been widely used, restriction is being imposed to use these compounds due to their carcinogenic and toxicological effects[9-11]. Vegetables, fruits, medicinal herbs and spices, etc. are rich source of free radical scavenging molecules especially polyphenolic compounds, particularly flavonoids and phenolic acids, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites having potential antioxidant activities[12,13]. Phenolic and flavonoid compounds are the most important plant secondary metabolites and widely spread throughout the plant kingdom[14]. The antioxidative properties of phenolic compounds arise because of their redox properties which play important roles as hydrogen donors, stabilizing or neutralizing unpaired electrons or their ability to chelate metal ions and caused termination of Fenton's reaction[15]. Besides these, some reducing compounds such as glutathione and ascorbic acid are potential candidates for enzymatic browning[16]. Various scientific and research investigations suggest that high intake of natural antioxidants has been associated with reduced risks of oxidative stress related diseases[17-20]. There are also reports of antioxidants that could help in delaying or inhibiting the oxidation of lipids or other molecules by hindering the initiation or propagation of oxidative chain reactions[21,22]. An attempt has been made to evaluate the antioxidant and free radical scavenging activities as well as radioprotective properties of *Z. kangleipakense* (Kishor & Škorničk) belonging to Zingiberaceae family. Interestingly, the *Zingiber* species has been reported recently from Manipur, India which falls within the Indo-

*Author for Correspondence

Burmese mega-biodiversity hotspot[23]. Generally, family Zingiberaceae comprises of valuable herbs with important roles in the prevention and treatment of diseases[24] as per traditional Chinese and Indian medicines. The objectives of present study are to evaluate free radical scavenging and radioprotective abilities of rhizome extracts of *Z. kangleipakense*.

MATERIALS AND METHODS

Chemicals and reagents

Agarose, ascorbic acid, curcumin, DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, glutathione, plasmid pBR322, quercetin, TCA (trichloro acetic acid) and trizma base were purchased from Sigma Aldrich, St. Louis, USA. Other chemicals and reagents were of analytical grade obtained from Merck, India.

Collection of plants and preparation of extracts

Healthy plants of *Zingiber kangleipakense* were collected from their natural habitats within various parts of Manipur and were planted in the experimental garden. Freshly collected rhizomes were washed thoroughly in tap water and peeled off the outer covering with a sterile surgical blade. One-gram dry weight equivalent of fresh rhizomes relative to moisture content were ground using mortar and pestle, and the pastes were treated with 10 mL of 60% methanol/ethanol and distilled water and homogenized separately for each extract. The homogenates were collected and centrifuged at 3000g for 10 minutes to get a clear supernatant. Finally, the clear supernatant was decanted and filtered through Whatman No. 1 filter paper and stored at 4°C for experimental use.

Phytochemical analysis

Total phenolic content

Amount of total phenolics in the plant extracts were determined using Folin-Ciocalteu phenol reagent by the method of Singleton and Rossi[25] with slight modification. Equal quantities of 0.4 mL of 60% ethanol and aqueous extract of rhizomes (10 x dilution) were mixed with 0.3 mL of Folin-Ciocalteu reagent (10 x dilution). Then, 0.25 mL of sodium carbonate (7.5% w/v) was added after 3 min and total volume was made up to 4 mL with distilled water. The reaction solution was kept in dark for 30 min. Absorbance was measured at 765 nm against a blank. The flavonoid experiment was carried out in triplicates and the amount of total phenolic content was calculated as gallic acid equivalent (GAE) in mg/100g. The concentration range of gallic acid used for standard curve was 2-10 µg/mL. Equation of the gallic acid curve was found to be $y = 0.088x + 0.024$, $R^2 = 0.996$.

Total flavonoid content

Total flavonoid content of the plant extracts were determined by the colorimetric method as described by Jia *et al*.[26] with minor modification. Equal quantities of 0.5 mL of rhizome extracts prepared in 60% ethanol and aqueous were mixed with 2 mL of 30% ethanol and 0.15 mL of 5% $NaNO_2$. After 6 min, 0.15 mL of 10% Al $(NO_3)_3$ was added. Subsequently, 2 mL of 4% NaOH and 0.2 mL of 30% ethanol were added to the reaction mixture after 6 min and mixed thoroughly. Absorbance was recorded at 510 nm following an incubation of 12 min against a blank. All experiments were carried out in triplicates and expressed as quercetin equivalent (QE) in mg/100g of the extracts. The equation of calibration curve was $y = 0.008x - 0.0094$, $R^2 = 0.998$.

Ascorbic acid content

Ascorbic acid content of the plant sample was estimated as described by the method of Klein and Perry[27] with slight modification. Firstly, 100 mg rhizome extracts made in 60% ethanol/ aqueous were treated with 2 mL of 1% metaphosphoric acid and filtered. Then, the filtrates were mixed with 9 mL of 2,6-dichloroindophenol (0.15 mg/mL), and absorbance was measured at 515 nm. Ascorbic acid content of the plant sample was calculated on the basis of the calibration curve and was expressed in mg/100g. The concentration range of ascorbic acid used for standard curve was 0.3 to 3.5 µg/mL, and equation for the calibration curve was found to be $y = - 0.2564x + 0.9883$, $R^2 = 0.9863$.

Free radical scavenging analysis

Thiyl free radical reactivity assay

Thiyl free radical reactivity assay was carried out as described elsewhere[28,29]. Glutathione was used for the *in vitro* generation of thiyl free radicals following gamma radiation and curcumin was used as reference for assessing the reactivity with free radicals[30,31]. The reaction solution contained 20 µM curcumin in methanol and 15 mM glutathione in distilled water. In this experiment, different volumes (0.4 mL and 0.8 mL) of the 60% methanol extracts with a concentration of 8mg/mL and 16mg/mL were added to the reaction mixture and final volumes were adjusted to 5 mL with methanol. The control solution contains 20 µM curcumin and 15 mM glutathione without sample. Then the solutions were irradiated with 0, 50, 100, 150 and 200 Gy respectively at dose-rate of 0.84 Gy/sec using a ^{60}Co gamma irradiator GC-5000 (BRIT, BARC, Mumbai). The thiyl free radicals generated by glutathione subjecting gamma radiation could deplete the curcumin molecules in the control. Curcumin protection by the sample was determined by observing the absorbance at 425 nm using Beckman DU 640 UV/VIS Spectrophotometer against methanol as blank. Curcumin depletion in the reaction mixture with or without sample and their protective indices (PI) were calculated using the following formulae:

Curcumin depletion (µM) = initial curcumin content – final curcumin content

Protective index (%) = [curcumin depletion (without sample – with sample) / initial curcumin content] ×100.

Ferric ion reducing power assay

Ferric ion reducing power of rhizome extracts was determined according to the method of Oyaizu.[32] Equal volumes each of 0.75 mL of various concentration of the extracts were mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexacyanoferrate (1%, w/v), followed by incubation at 50°C for 20 mins in a water bath. Then, the reaction was made to stop by adding 0.75 mL of trichloroacetic acid (TCA) solution (10%) followed by centrifugation at 3000g for 10 min. Then each of 1.5 mL of the supernatants was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride

($FeCl_3$) solution (0.1%, w/v) and kept for 10 min at room temperature. The absorbance of the resulting solutions was measured at 700 nm as the reducing power. Higher absorbance of the reaction mixtures indicated greater reducing power. The experiments were carried out three times and mean of absorbance was plotted against concentration followed by linear regression analysis.

DPPH free radical scavenging activity

DPPH assay was carried out to assess the free radical scavenging capacity of the plant extracts following the method of Miliauskas *et al.*[33] with slight modification. Each reaction mixture consisted of 100 µM DPPH (2, 2-diphenyl-1-picrylhydrazyl) in absolute methanol with different concentrations of sample. Absorbance was read against a blank at 515 nm after incubation of the reaction mixtures for 30 minutes in dark at room temperature. The percentage of DPPH decoloration was calculated as follows:

% DPPH decoloration = [Abs $_{control}$ - Abs $_{sample}$ / Abs $_{control}$] x 100.

The assay was carried out in triplicates and the results were expressed as mean ± standard error. The degree of decoloration of purple colored DPPH to yellow color signifies the free radical scavenging capacity of the extract. Ascorbic acid was used as standard compound. The IC_{50} value was determined from the DPPH decoloration curve.Metal chelating assay: The ferrous ion chelating ability of plant extracts was monitored by measuring the formation of the ferrous ion-ferrozine complex.[34] The reaction mixture containing 1 mL of various concentrations of the extracts (in triplicates) was mixed with 1 mL of 0.1 mM ferrous sulphate and 1 mL of 0.4 mM ferrozine to initiate the reaction. The reaction mixtures were shaken vigorously and left to stand for 10 mins at room temperature. Absorbance was measured at 562 nm against a blank containing 60% ethanol/ aqueous in place of ferrozine and control consisted of 60% ethanol/ aqueous in place of extract. The ability of sample to chelate ferrous ion was calculated as follows:

Chelating effect (%) = (A $_{control}$- A $_{sample}$)/ A $_{control}$ × 100, Where A $_{control}$ = Absorbance value of control, A $_{sample}$ = Absorbance value of the sample.

Assessment of radiation induced DNA damage and protection

Plasmid pBR322 DNA was used to evaluate protection of gamma radiation-induced DNA damage as described by Sambrook *et al.*[35] The DNA (300 ng) was exposed to 6 and 12 Gy at a dose-rate of 0.06 Gy/sec in the absence and presence of plant extract at different concentration (0.25 to 2 mg/mL) made in 60% ethanol. DNA solution without the sample and radiation was taken as control. After irradiation, the supercoiled (ccc) and open circular (oc) forms of DNA was separated by agarose gel electrophoresis in 1% gel agarose in Tris acetic acid EDTA buffer, pH 8 at constant voltage. The DNA bands stained with ethidium bromide were documented using Vilber Lourmat Gel Documentation Unit. The images of DNA bands were analyzed by PerkinElmer-Gene Tool Software, Version-3.07. The experiments were conducted in three replicates. Radiation induced DNA damage was estimated as an increase in percentage of oc form of DNA.

RESULTS AND DISCUSSION

Total phenol, total flavonoid and ascorbic acid contents: Phenolic compounds are considered secondary metabolites and they play a myriad of important functions, from protection against pathogens, UV radiation and other means of improving health and survivability of plant even though they do not directly involve in crucial functions like growth and reproduction[36]. Phenols are potent antioxidants because of their redox properties which play important roles as hydrogen donors, quenching of singlet and triplet oxygen, neutralizing free radicals and metal ion chelators[15,37]. Flavonoids are naturally occurring secondary metabolites in plants and are thought to have positive effects on human health. Phenolic acids, precursor of flavonoids are reported to form complex with metal ions.[38] Ascorbic acid, an important candidate for enzyme browning and effective scavenger of free radicals has also been determined. The contents of phenol, flavonoid and ascorbic acid for the plant extract are shown in Table 1. The rhizome extract made in 60% ethanol has shown higher value than that of aqueous extract. The standard curves of gallic acid and quercetin for the determination of TPC and TFC are shown in Figures 1&2. Total phenol content (TPC) of plant extract is expressed as gallic acid equivalent (GAE) in 100 g of sample. TPC of 60% ethanol and aqueous extracts are found to be 241.13 ± 0.35 mg GAE/100 g and 97.45 ± 0.17 mg GAE/100 g respectively. Total flavonoid content (TFC) of plant extract is expressed as quercetin equivalent (QE) in 100 g of sample. TFC of extracts made in 60% ethanol and aqueous are found to be 369.32 ± 0.31 mg QE/100 g and 120.0 ± 0.19 mg QE/100 g respectively. In addition, ascorbic acid contents in 60% ethanol and aqueous extracts determined from the standard curve are found to be 0.28 ± 0.02 and 1.95 ± 0.3 mg/100 g respectively.

Thiyl free radicals scavenging assay

The thiyl free radicals generated by the aqueous solution of glutathione during gamma radiolysis can oxidize the chrome yellow compound curcumin and the depletion of color resulting from oxidation can be easily detected[28]. Depletion of curcumin in the reaction solutions with or without the plant extracts are shown in Fig. 3. Supplementation of extract in the reaction mixture substantially decrease the depletion of curcumin but the depleted value increase with increasing radiation dose due to the generation of a larger number of thiyl free radicals which is in correspond to the earlier report[30]. The extracts 8 mg/mL and 16 mg/mL inhibit curcumin depletion to the extent of 6.38 µM and 4.2 µM respectively at 100 Gy. Protective indices (PI) are calculated from the observed values of curcumin depletion with or without samples. At 50 Gy, PI value is found to be low. It increases until the dose of 150 Gy and drops with further increase in radiation dose to 200 Gy (Fig. 4). As such, the initial increased in PI values may be due to the relatively small population of thiyl free radicals, where antioxidant molecules are able to compete with curcumin. However, after reaching a

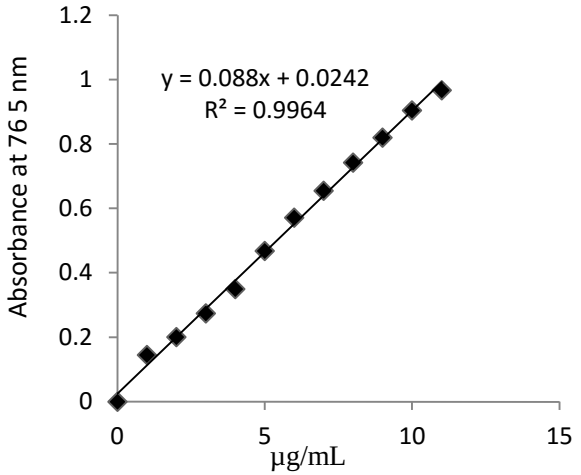

Fig. 1: Standard curve for total phenolic content using gallic acid

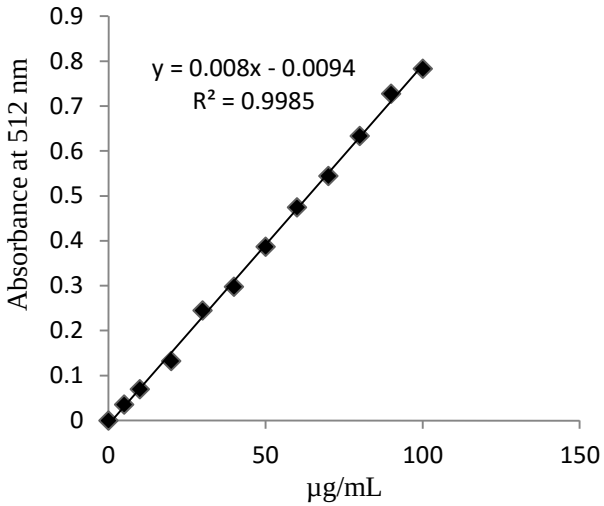

Fig. 2: Standard curve for total flavonoid content using quercetin

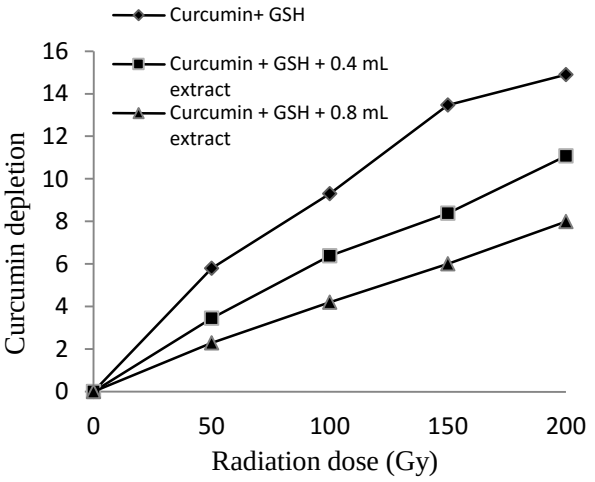

Fig. 3: Curcumin protection against thiyl free radical attack by 60% methanol rhizome extracts of *Z. kangleipakense*

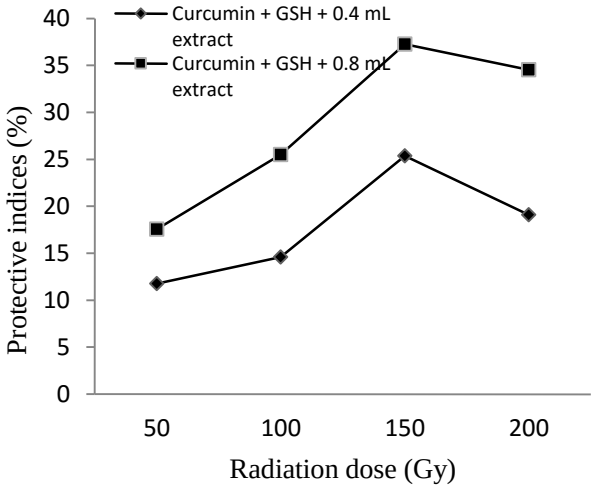

Fig. 4: Protective indices (%) of rhizome extracts of *Z. kangleipakense*

Table 1 : Antioxidant components of rhizome extracts of *Z. kangleipakense*

Plant extract	Total phenolic content (mg /100 g) GAE	Total flavonoid content (mg /100 g) QE	Ascorbic acid content (mg/100 g)
60% ethanol	241.13 ± 0.35	369.32 ± 0.31	0.28 ± 0.02
Aqueous	97.45 ± 0.17	120.0 ± 0.19	1.95 ± 0.03

Data represented as mean ± SE (n =3)

threshold dose, the PI declines due to increased generation of thiyl free radicals. Inhibition of curcumin depletion and protection against thiyl free radicals by the addition of plant extracts suggest that it has potential bioactive molecule having promising antioxidant properties.

Ferric ion reducing power assay

The reducing power has been used as one of the antioxidant capability indicators of plants[39]. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of the compound. The mechanism of this assay is related to the electron donating ability of the plant extracts. The presence of reductants in the tested samples resulted in the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form (Fe^{2+}). The amount of Fe^{2+} complex can therefore be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The reducing power of standard compound and extracts of plant sample increased with increasing concentration. Dose dependent curves for the reducing power of the standard compound and plant extracts are shown in Figures 5 & 6. The observed results may be due to concerted effects such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen substraction, reductive capacity and radical scavenging[40,41]. Such a relation between ferrous reducing activity and total phenolic contents has been reported in the literature.[42] Thus, the reducing capacity of a compound may serve as a

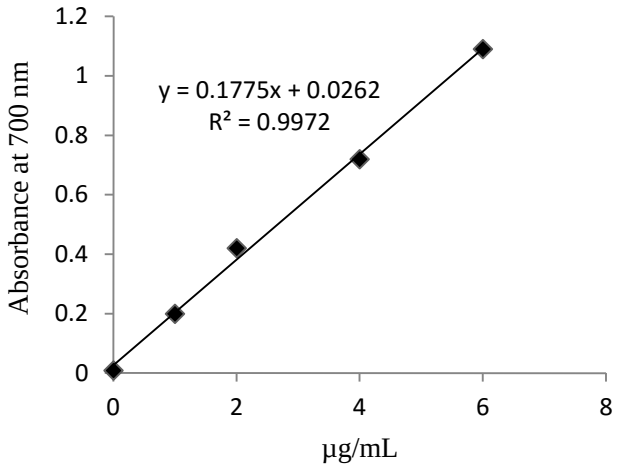

Fig. 5: Ferric ion reducing power assay of pyragallol as standard compound

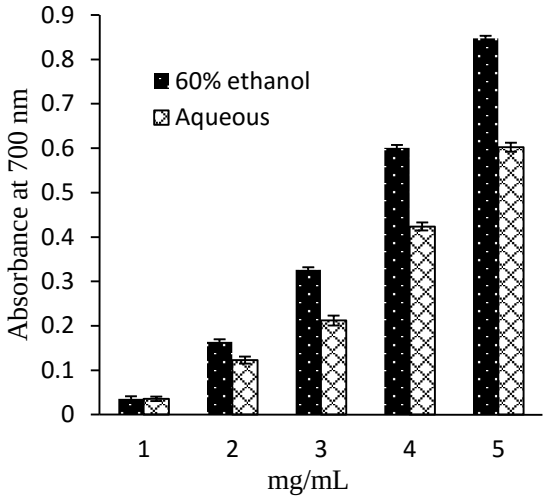

Fig. 6: Ferric ion reducing power assay of 60% ethanol and aqueous extract of *Z. kangleipakense.*

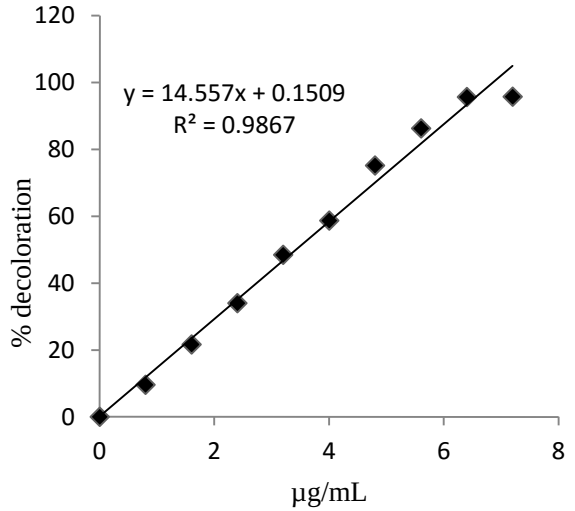

Fig. 7: Percentage decoloration of DPPH against ascorbic acid as standard compound

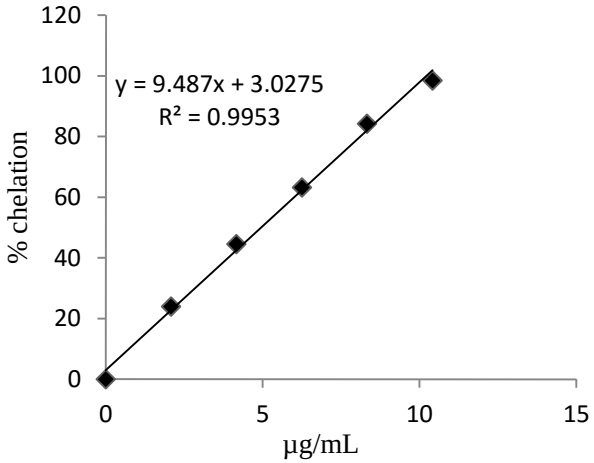

Fig. 8: Ferrous ion chelating activity for Na_2EDTA as standard.

significant indicator of its potential antioxidant activity. Increasing absorbance at 700 nm indicates an increase in reducing ability. The antioxidants present in the fractions of *Z. kangleipakense* cause reduction of Fe^3+/ ferricyanide complex to the ferrous form, thereby proving presence of good reducing power.

DPPH free radical scavenging activity

DPPH scavenging activity method has been widely applicable for evaluating antioxidant properties in a number of studies because of its easy, sensitive and short duration required for analysis[43]. The well known free radical, DPPH possess a characteristic absorption at 515 nm. DPPH• solutions upon reaction with a hydrogen donating antioxidant, gets reduced to non-radical form of DPPH-H and color changes from deep-violet to light-yellow[44,45]. The radical scavenging activity in the presence of antioxidant can be monitored through the decrease in absorbance of DPPH solution. In our experiment, addition of plant extracts/ ascorbic acid to the DPPH solution result in rapid decrease in absorbance and resulting values in terms of IC_{50} are shown in Table 2. DPPH radical scavenging activity is found to be higher in case of ethanol extract as compared to aqueous extract but lesser than that of ascorbic acid. IC_{50} of this assay for 60% ethanol and aqueous extracts are found to be 4.15 ± 0.32 mg/mL and

Table 2: DPPH free radical scavenging assay and ferrous ion chelating activity (IC_{50} values) of 60 % ethanol and aqueous extract of *Z. kangleipakense* and standard compound

Activity	Extract/ standard	IC $_{50}$ values
DPPH radical scavenging assay	60% ethanol	4.15 ± 0.32 mg/mL
	Aqueous	13.37 ± 0.51 mg/mL
	Ascorbic acid	3.42 ± 0.40 µg/mL
Ferrous ion chelating assay	60% ethanol	0.36 ± 0.23 mg/mL
	Aqueous	1.77± 0.05 mg/mL
	Na_2 EDTA	4.81± 0.10 µg/mL

Data represented as mean ± SE (n =3)

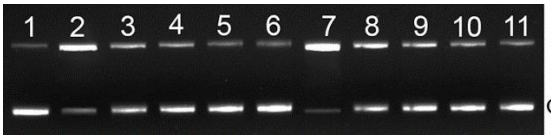

Fig. 9: Agarose gel electrophoresis pattern of pBR322 DNA exposed to different doses of gamma radiation in the absence and presence of *Z. kangleipakense* (60% ethanol extract).
Lane1: control (without extract and radiation);
Lane 2: DNA + 6 Gy;
Lane 3: DNA + 0.25mg/mL extract + 6 Gy;
Lane 4: DNA + 0.5 mg/mL extract + 6 Gy;
Lane 5: DNA + 1mg/ mL extract + 6 Gy;
Lane 6: DNA + 2mg/mL extract + 6 Gy;
Lane 7: DNA + 12 Gy;
Lane 8: DNA + 0.25mg/mL extract + 12 Gy;
Lane 9: DNA + 0.5mg/mL extract + 12 Gy.
Lane 10. DNA + 1mg/mL extract + 12 Gy.
Lane 11. DNA + 2mg/mL extract + 12 Gy.
Abbreviations: 'oc' denotes open circular or linear form of DNA and 'ccc' denotes covalently closed circular form or supercoiled form of DNA.

13.37 ± 0.51 mg/mL respectively and that of ascorbic acid is found to be 3.42 ± 0.40 µg/mL as shown in Fig.7. Reduction of DPPH radical reveals that the plant rhizome possesses radical inhibitors or scavengers with possibility to act as primary antioxidant.

Ferrous ion chelating assay

In living system, transition metal ions such as iron, copper, nickel, arsenic, etc. having one or more unpaired electrons can act as free radicals and powerful catalysts for oxidation reactions even though they play important roles for oxygen transport, respiration and activities of many enzymes. Specially, ferrous ion has been known to initiate/stimulate lipid peroxidation by Fenton reaction and can also accelerate lipid peroxidation by decomposing lipid hydro-peroxides into peroxyl and alkoxyl radicals that can perpetuate the chain reaction[46,47]. However, the chelating agent may have the capacity to inactivate, catalyze and even inhibit radical generation by stabilizing transition metals thereby reducing free radical damages in the living system. Beside this, some phenolic compounds exhibit antioxidant activity through the chelating of metal ions[48].In ferrous ion chelating assay, ferrozine-ferrous complex, a purple color compound is formed with maximum absorbance at 562 nm. There is reduction in the purple coloration with the addition of the extracts and standard compound indicating the interruption of further complex formation. Thus, intensity of color depletion is in equivalence to the metal chelating activity[49]. Chelating activity of Na_2EDTA and *Z. kangleipakense* (60% ethanol/ aqueous extract) are shown in Fig. 8 and Table 2 respectively. Na_2EDTA shows maximum chelating activity with IC_{50} 4.81 ± 0.10µg/mL and that of *Z. kangleipakense* are 0.36 ± 0.23 mg/mL for 60% ethanol and 1.77 ± 0.05 mg/mL for aqueous extract respectively. Ethanol extract shows more chelating activity than the aqueous one. This study shows that the plant extract has significant capacity for iron binding, suggesting the presence of polyphenolic compounds that has potent ferrous ion chelating power.

Table 3: Protection of plasmid PBR 322 by plant extract from radiation-induced damages

Treatment	OC form (%)		Protection (%)	
	6 Gy	12 Gy	6 Gy	12 Gy
DNA	86.72	88.34	-	-
DNA + 0.25 mg/mL	43.46	47.58	56.54	52.42
DNA + 0.5 mg/mL	37.38	42.73	62.62	57.27
DNA + 1 mg/mL	32.27	38.66	67.73	61.34
DNA + 2 mg/mL	28.35	31.42	71.65	68.58

Radioprotective properties

Plasmid pBR322 DNA is predominantly supercoiled (ccc) in native form. Exposure to ionizing radiation produces a variety of lesions in DNA such as single strand breaks, double strand breaks, DNA-DNA cross-links together with damage to nucleotide bases[50]. Evaluating such kind of lesions is considered as an important parameter in the examination of the sequence of events leading to mutagenic, carcinogenic and lethal effects of radiation. In this experiment, DNA when exposed to the gamma radiation at doses of 6 and 12 Gy lead to the conversion of supercoiled (ccc) form to open circular (oc) form or linear form. The disappearance of ccc form of DNA can be taken as an index of DNA damage induced by the radiation exposure. Treatment of plasmid DNA with different concentrations of plant extracts (0.25 to 2 mg/mL) before one hour of irradiation results a dose dependent protection of pBR322 DNA as shown in Fig. 9 and Table 3. At low concentration of 0.25 mg/mL, plant sample is able to provide 56.54% and 52.42 % protection of DNA from exposure of gamma radiation of 6 and 12 Gy respectively. Similarly, plant extract (2mg/mL) provides 71.65% and 68.58 % protection from 6 and 12 Gy respectively. Presence of plant extract reduces the conversion of ccc form (or retain the ccc form) to oc form of plasmid DNA indicating its protecting ability against gamma radiation-induced strand breaks.

CONCLUSION

Based upon the results obtained in the present study, it is concluded that the rhizome extract of *Z. kangleipakense* contains considerable amount of phenolic and flavonoid compounds and ascorbic acid, exhibits high free radical scavenging activities and antioxidant properties. It also chelates iron and has reducing power. Our results suggest that phenolic and flavonoid compounds and ascorbic acid may be the major contributors for the antioxidant activity as the IC_{50} values of radical scavenging activity of various soluble fractions of *Z. kangleipakense* and the contents of phenolics or flavonoids exhibited significant correlation. In addition, it is also shown to protect plasmid DNA effectively against ionizing radiation in an *in vitro* system independent of all cellular defense mechanisms, including

DNA repair. Further investigation on potential antioxidant activity and radioprotection of *Z. kangleipakense* through *in vivo* systems are currently in the process.

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REFERENCES

1. Aruoma OJ. Asssement of potential prooxidant and antioxidant actions. Journal of American Oil Chemist Society 1996; 739(12): 1617-1625.
2. Zima TS, Fialova L, Mestek O, Janebova M, Crkovska J, Malbohan I *et al*. Oxidative stress, metabolism of ethanol and alcohol-related diseases. Journal of Biomedical Science 2001; 8: 59-70.
3. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause, or consequence, Lancet 1994; 344:721-724.
4. Poulson HE, Prieme H, Loft S. Role of oxidative DNA damage in cancer initiation and promotion. European Journal of Cancer Prevention 1998; 7(1): 9-16.
5. L. Menezes-Benavente, F. K. Teixeira, C. L. A. Kamei, and M. Margis-Pinheiro, "Salt stress induces altered expression of genes encoding antioxidant enzymes in seedlings of a Brazilian indica rice (*Oryza sativa* L.)," Plant Science 2004; 166 (2): 323–331.
6. Sun J, Chen C, Li M, Ge Z. Role of antioxidant enzymes on ionizing radiation resistance. Free Radical Biology and Medicine 1998; 42: 586-592.
7. Cesaratto L, Vascotto C, Calligaris S, Tell G. The importance of redox state in liver damage. Annals of Hepatology, 2004; 3:86-92.
8. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 2nd ed. Oxford: Clarendon Press; 1989.
9. Ito N, Fukushima S, Hasegawa A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F334 rats. Journal of National Cancer Institute 1983; 70: 343-346.
10. Shah MA, Bosco SJD, Mir SA. Plant extracts as natural antioxidants in meat and meat products. Meat Science 2014; 98 (1):21–33.
11. Liu J, Jia L, Kan J, Jin C-H. In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). Food and Chemical Toxicology 2013; 51: 310–316.
12. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry 2001; 49: 5165-5170.
13. Cai YZ, Sun M, Corke H. Antioxidant activity of betalains from plants of the Amaranthaceae. Journal of Agricultural and Food Chemistry 2003; 51: 2288-2294.
14. Hwang SJ, Yoon WB, Lee O-H, Cha SJ, Kim JD. Radical-scavenging-linked antioxidant activities of extracts from black chokeberry and blueberry cultivated in Korea. Food Chemistry 2014; 146:71–77.
15. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science 1997; 2: 152-159.
16. Jiang YM, Fu JR. Inhibition of polyphenol oxidase and the browning control of lichi fruit by glutathione and citric acid. Food Chemistry 1998; 62: 49-52.
17. Johnson IT. New approaches to the role of diet in the prevention of cancers of the alimentary tract. Mutation Research 2004; 551: 9-28.
18. Joshipura KJ, Ascherio A, Manson JE, Stampfer MJ, Rimm EB, Speizer FE et al. Fruit and vegetable intake in relation to risk of ischemic stroke. Journal of American Medicine Association 1999; 282: 1233-1239.
19. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of Carcinogenesis by dietary polyphenolic compounds. Annual Review of Nutrition 2001; 21: 381-406.
20. Sun J, Chu YF, Wu XZ, Liu RH. Antioxidant and antiploriferative activities of common fruits. Journal of Agricultural and Food Chemistry 2002; 50: 7449-7454.
21. Gulcin I, Kirecci E, Akkemik E, Topal F, Hisar O. Antioxidant and antimicrobial activities of an aquatic plant: Duckweed (Lemna minor L.). Turkish Journal of Biology 2010; 34(2): 175-188.
22. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables,and grain products. Journal of Agricultural and Food Chemistry 1998; 46: 4113-4117.
23. Kishore R, Skornickova JL. *Zingiber kangleipakense* (Zingiberaceae): a new species from Manipur, India, Garden's Bulletin Singapore 2013; 65(1): 39-46.
24. Wang F, Duan Y. Ginger, garlic and green onion as medicine. Pelanduk Publications 1999; 1-3.
25. Singleton VL, Rossi JA. Colourimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 1965; 16:144-158.
26. Jia ZM, Tang J. The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 1999; 64: 555-599.
27. Klein BP, Perry AK. Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of United States. Journal of Food Science 1982; 47: 941-945.
28. Chirangini P, Sharma GJ, Sinha SK. Sulphur free radical reactivity with curcumin as reference for evaluating antioxidant properties of medicinal Zingiberales. Journal of Environmental Pathology, Toxicology and Oncology 2004; 23(3):227-236.
29. Singh OM, Devi SN, Thokchom DS, Sharma GJ. Novel 3-alkanoyl/ aroyl/ heteroaroyl-2H-chromene-2-thiones: Synthesis and evaluation of their antioxidant activities. European Journal of Medicinal Chemistry 2010; 45:2250-2257.
30. Devi MD, Devi HS, Sharma GJ, James G, Tipping I, Willson RL. Curcumin: visible destruction of the conjugated unsaturated compound as a reference indicator of sulphur free radical reactivity. International Journal of Radiation Biology 1992; 62:105.
31. Aquino D, Bullion C, Chopra M, Devi D, Devi S, Dunster C, James G, Komuro E, Kundu S, Niki E, Raza F, Robertson F, Sharma J Wilson R. Sulfhydral free

radical formation enzymatically, by sonolysis, by radiolysis and thermally:Vitamin A, curcumin, muconic acid and related conjugated olefins as references. Methods in Enzymology 1994; 233: 34-36.
32. Oyaizu M, Jap JN. Studies on the antioxidant and free radical- scavenging effects of sinapic acid: An *in vivo* and *in vitro* model. Pharmacology 2004; 231-255.
33. Miliauskas G, Vnskutonis PR, Van BT. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 2004; 85: 231-237.
34. How YL, Yau YL. Evaluation of antioxidant activitities of the Methanolic Extracts of selected Ferns in Malaysia. International Journal of Environmental Science and Development 2011; 2(6): 442-447.
35. Sambrook K, Fritsch J, Maniatis T. Molecular cloning: A laboratory manual 2nd edn., Vol. 1, Cold Spring Harbour Laboratory Press, New York 1989; 6.3–6.17.
36. Bagniewska-Zadworna A, Zenkteler E, Karolewski P, Zadworny M. Phenolic compound localization in *Polypodium vulgare* L. rhizomes after mannitol-induced dehydration and controlled dessication. Plant Cell Reports 2008; 27:1251-1259.
37. Dzoyem JP, Eloff JN. Anti- inflammatory, anticholinesterase and antioxidant activity of leaf extracts of twelve plants used traditionally to alleviate pain and inflammation in South Africa. Journal of Ethnopharmacology 2015; 160:194–201.
38. Pratt DE, Birac P. Source of antioxidant activity of soybean and soy products, Journal of Food Science 1979; 44:1720-1722.
39. Bhandari MR, Kawabata J. Organic acid, phenolic content and antioxidant activity of wild yam (Dioscorea spp.) tubers of Nepal. Food Chemistry 2004; 88:163-169.
40. Diplock AT. Will the good fairis please proves to us that vitamin E lessens human degenerative of disease? Free Radical Research 1997; 27: 511-532.
41. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V. Comparision of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf Ex DC), Sage (*Savia triloba* L.) and Black tea (*Camellia sinensis*) extracts. Journal of Agriculture and Food Chemistry 2000; 48(10): 5030-5034.
42. X. Goa, L. Brork, V. Trajkovski, M. Uggla. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. Journal of the Science of Food and Agriculture 2000; 80: 2021-2027.
43. Brad-Williams W, Cuvelier ME, Berset C. Use of a radical method to evaluate antioxidant activity, Lebensen-wiss A Technol. Food Science and Technology 1995; 28: 25-30.
44. Koleva II, Beek TAv, Linssen JPH, Groot1 A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis 2002; 13: 8–17.
45. L. Sun, J. Zhang, X. Lu, L. Zhang, and Y. Zhang, Evaluation to the antioxidant activity of total flavonoids extract from persimmon (*Diospyros kaki* L.) leaves. Food and Chemical Toxicology 2011; 49 (10): 2689–2696.
46. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agricultural and Food Chemistry. 1998; 48: 4113-4117.
47. Halliwell B. Antioxidants in human health and disease. Annual Review of Nutrition 1996; 6: 33-50.
48. Zhao H, Fan W, Dong J, Lu J, Chen L, Shan L. Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chemistry 2008; 107: 296-304.
49. Singh R, Singh B, Singh S, Kumar N, Kumar S, Arora S. Investigation of ethyl acetate extract/ fractions of Acacia nilotica willd. Ex Del as potent antioxidant. Records of Natural Products 2009; 3(3): 131-138.
50. Scholes G. Radiation effects on DNA: The Silvanus Thomson memorial lecture. British Journal of Radiology 1982; 56: 221–223.