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Proportional analysis on *Invitro* antioxidant and antidiabetic properties of three ginger varieties

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Abstract

Ginger (*Zingiber officinale* Roscoe) and Mango-ginger (*Curcuma amada*) are reported asdistinguished and extensively used spices that contain several remarkable bioactive constituents that are responsible forupholdingits health benefits.In this study, the *invitro* antioxidant and antidiabetic effects of the aqueous extracts from the rhizomes of threevarieties (Young, Mature and Mango ginger) were assessed in an effort to liken and validate their medicinal potential. Antioxidant activities were assessed using the DPPH, FRAP and Lipid peroxidation assays. The antidiabetic activity was established using α-glucosidase and α-amylase inhibition assays. This study produced results which corroborate with the findings of a great deal of the previous work in this field. The results of the present study revealed that though all the three extracts presented significant antioxidant activities it was more obvious in mature ginger than the young and mango ginger. The present finding also uncovered that the rhizome extractsefficiently inhibits both α-glucosidase and α-amylase enzymes *invitro* in a dose dependent manner with mature ginger demonstrating greater inhibition potential. This may be attributed to its rich phytochemistry. Thus, concluding that among the selected varieties of ginger, mature ginger has imperative medicinal prospective.

Keywords: Antidiabetic activity, antioxidant activity, ginger, biological effects of ginger

Introduction

Natural bioactive compounds especially from plant sources, including spices have been investigated for their characteristics and health effects. *Zingiber officinale Roscoe* a member of the family Zingiberaceae is well known in Asia and has been cultivated since long as a spice and condiment to add flavor to Indian food[1]. Besides its extensive use as a spice, the rhizome of ginger has also been used in traditional herbal medicine. The health promoting perspective of ginger is often attributed to its rich phytochemistry[2].

The constituents of ginger are numerous and vary depending on the place of origin and form of rhizomes; fresh or dry[3]. It contains appreciable amounts of vitamins and minerals, as well as some enzymes, for example, a potent proteolytic enzyme called zingibain. Researchers have identified more than 60 compounds in fresh ginger grouped into two broader categories; volatiles and non-volatiles[4]. Volatiles compounds including sesquiterpene and monoterpenoid hydrocarbons providing the distinct aroma and taste of ginger and non-volatile compounds include gingerols, shogaols, paradols and zingerone.

The genus Curcuma under the family Zingiberaceae comprises of over 80 species of rhizomatous herb. Mango-ginger (*Curcuma amada*) is a unique spice having morphological resemblance with ginger but imparts raw mango flavour. The rhizomes are used in culinary preparations as a source of raw mango flavour. It is reported to contain ocimene, dihydro-ocimene, α-pinene, α-curcumene, β- curcumene, linalool, cuminyl alcohol, keto-alcohol, camphor, turmerone, linalyl acetate, safrole, curcumin, myristic acid, car-3-ene, myrcene, 1,8-cineol, limonene and perillene . The rhizomes are used by the tribes of Madhya Pradesh for the management of diabetes[5]. The present study was taken up to establish and appraise the antioxidant and antidiabetic

activity of these selected ginger varieties and to ascertain the superlative variety.

Materials and Methods

Samples

Three different varieties of ginger rhizomes young, mature (*Zingiber officinale*) and mango ginger (*Curcuma amada*) were procured from the local market. The rhizomes were authenticated by Professor P. Jayaraman (Certificate No. PARC/3155).

Preparation of plant extract

The ginger rhizomes were shade dried and sliced, made into powder and then it was used for the extraction. The aqueous extracts of the three different rhizomes were prepared separately following the method of Ozougwu and Eyo[6]. The rhizome powder (200g) was soaked in distilled water individually in a glass jar for 2 days at room temperature and filtered. The procedure was repeated 3 to 4 times until the filtrate becomes colourless. The filtrate was distilled and concentrated under reduced pressure in the Buchirotavapour R-200 and freeze dried.

Free radical scavenging assay or DPPH assay

The ability of the samples to scavenge free radical was estimated according to the method of Blois[7]. 1ml of varying concentration (100-500µg) of sample was mixed with 1ml of 0.004% methanol solution of DPPH. The mixture was shaken well and allowed to stand for 30 minutes at room temperature in the dark. The reduction of the DPPH radical was determined by measuring the absorbance at 517nm. The procedure was repeated for the blank and control. The radical scavenging activity was calculated using the equation:

Scavenging effect (%) = (Absorbance blank – Absorbance sample) / Absorbance blank x 100

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP was estimated by using the method of Benzie and Strain[8]. The working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s- triazine (TPTZ) solution and 20 mM $FeCl_3.6H_2O$ in in the ratio of 10:1:1 prior to use and heated to 37°C in a water bath. The blank was determined by adding 200 µl of this working solution to the micro titre plate and read at 593 nm using spectrophotometer. To 100 µl of sample, 3 ml of this working solution and 300 µl of distilled water was added and left for 4 minutes. Ferrous sulphate, in the concentration of 0 to 1000 µM, was used as standard and for calibration. The results are expressed as µmolFe(II)/gm dry weight of the sample.

Lipid peroxidation assay

Lipid peroxidation inhibition assay was performed by the method of Okhawa*et al.*[9]. Lipids were preincubated with samples, lipid peroxidation was induced with 100 µl $FeSO_4$ and 100 µl Potassium dihydrogen phosphate. The volume was made upto 3ml then, 0.5ml of tricholoroacetic acid and 2ml of thiobarbituric acid was added. The reaction mixture was boiled for 30 minutes and centrifuged at 3500rpm for 10 minutes. The absorbance was measured at 534 nm and the results are expressed as nmoles of MDA/gm.

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity was determined using the method of Apostolidis*et al.*[10]. The enzyme solution was prepared by dissolving 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin. It was diluted further to 1:10 ratio with phosphate buffer just before use. Sample solutions of different concentrations: 1 – 5 mg/ml were prepared and 5µl each of the sample solutions or DMSO (sample blank) was then added to 250µl of 20mM para-nitrophenyl-α-D -glucopyranoside and 495µl of 100mM phosphate buffer (pH 7.0). It was pre-incubated at 37°C for 5 minutes and the reaction was initiated by addition of 250µl of the enzyme solution, after which it was incubated at 37°C for exactly 15 minutes. 250µl of phosphate buffer was added instead of enzyme for blank. The reaction was then stopped by the addition of 1000µl of 200mM sodium carbonate solution and the amount of p-nitrophenol released was measured by reading the absorbance of sample against a sample blank at 400nm. The results are expressed as percentage inhibition against different concentration. Acarbose was used as positive control.

% inhibition = (Absorbance of control – Absorbance of test)/ Absorbance control x 100

Alpha amylase inhibitory activity

Alpha amylase inhibitory assay was performed as per the standard method of Hansawasdi*et al*[11]. 2 mg of starch was suspended in each of the tubes containing 0.2 ml of 0.5 M Tris-Hcl buffer (pH 6.9) and 0.2ml of 0.01 M calcium chloride. The tubes containing the substrate solution were boiled for 5 minutes and were then incubated at 37°C for 5 minutes. 0.2 ml of sample dissolved in DMSO was taken in each tube containing different concentrations (1–5mg/ml). Pancreatic amylase was dissolved in Tris-HCl buffer to form a concentration of 2 units/ml and 0.1 ml of this enzyme solution was added to each tube. The reaction was carried out at 37°C for 10 minutes and was stopped by adding 0.5 ml of 50% acetic acid. The reaction mixture was centrifuged at 3000 rpm for 5 minutes at 4°C. The absorbance of the resulting supernatant was read at 540nm. The results are expressed as percentage inhibition against different concentration. Acarbose was used as positive control.

% inhibition = (Absorbance of control – Absorbance of test)/ Absorbance control x 100

Results

The free radical scavenging assay is based on the reduction of 1,1 diphenyl-2-picrylhydrazyl (DPPH). The ability of the samples and the standard ascorbic acid to scavenge the free radical at different concentrations (100µg-500µg) and pair off the odd electron was shown in this assay (table 1). From the results obtained it was observed that the samples showed increase in percentage of inhibition as the concentration increases. However, among the ginger varieties mature ginger exhibited 89.0±1.07%, followed by young ginger with 75.4±0.8% and mango ginger with 62.4±1.4% of free radical scavenging effect at a concentration of 500 µg/ml. This study showed that aqueous mature ginger extracts have good free radical scavenging ability as compared with other ginger varieties.

The ability of the compounds to prevent lipid peroxidation by inhibiting the production of malondialdehyde (MDA) an intermediate formed during oxidation of lipids and the ferric reducing antioxidant power of three varieties of ginger are presented in table 2. The production of MDA was found to be less in aqueous mature ginger extract (155±1.55 nmoles MDA/gm) as compared with other ginger varieties. The ferric reducing ability was also found to be more in mature ginger (767.2±2.45 µmoles Fe(II)/gm dry weight) when compared with other ginger varieties.

Digestive enzymes have been targeted as potential avenues for modulation of blood glucose through inhibition of enzymatic breakdown of polysaccharides and disaccharides to glucose. Acarbose a widely used oral antidiabetic drug acts on this principle and many plant extracts are found to exhibit this effect. Table 3 and 4 represents the inhibitory effects of the samples on alpha amylase and alpha glucosidase and are compared with the standard acarbose. The results demonstrated that all the samples possess better dose dependent alpha glucosidase inhibitory effects than alpha amylase inhibitory effects. The alpha amylase inhibition percentage was found to be higher (61±1.26 %) for acarbose, followed by (30±1.05 %) for mature ginger at a concentration of 100µg. The percentage of inhibition was found to be less in other samples.

The results of alpha glucosidase inhibition revealed that all the samples exhibited dose dependent inhibition of enzyme activity. The higher percentage inhibition was recorded with acarbose (80±0.09 %) followed by aqueous extract of mature ginger (57±0.85 %) at a concentration of 100µg whereas, lesser percentage of inhibition was recorded with other samples at the same concentration.

Discussion

In living organism's free radicals are constantly generated from numerous physiological and biochemical processes[12]. These radicals are responsible for damage to cellular biomolecules leading to many disease conditions[13]. Though all living organisms possess protective antioxidant systems against the free radicals causing oxidative damage, sometimes it is not adequate to prevent all the possible damage. Thus, the use of antioxidants to provide enhanced and greater protection against oxidative damage is increasingly practiced. The radical scavenging activities of ginger varieties were determined using DPPH radical scavenging assay. DPPH is a stable nitrogen based radical that has been extensively used to test the free radical scavenging

abilities of various substances. Usually, a high DPPH scavenging activity confers the high levels of antioxidant activity in the sample[14].

The results of the present study demonstrated that all three varieties of ginger exhibited considerable amount of free radical inhibition effect at higher concentrations. However, highest percentage of inhibition of free radicals was recorded with mature ginger extract. The results obtained are in agreement with Silvia*et al*.,[15]

Lipid peroxidation causes hazardous effects by the way of forming complex mixtures of secondary break down products of lipid peroxides. It is thought to proceed via radical mediated abstraction of hydrogen atom from methylene carbons in polyunsaturated fatty acids[16]. In biological systems, lipid peroxidation if uncontrolled may lead to precipitation of chronic diseases.[17]

In the present study lipid peroxidation was measured as nmol/g of MDA which is an important secondary metabolite formed due to oxidation of lipids. From the results it was observed that the MDA levels were significantly less in mature ginger extract when compared with the other samples used in the study. Decrease in the MDA value indicates low lipid peroxidation and high membrane stability. The results obtained in the present study are in close agreement with a similar antioxidant study done by Chatterjee *et al.*[18]on a formulated polyherbal drug.

FRAP assay is used to determine the antioxidant activity depending on the analyte to reduce Fe^{3+}/Fe^{2+} couple[19]. There were significant differences among the FRAP values for three varieties of ginger extracts, suggesting that they have different antioxidant potentials. Mature ginger extract manifested a significant and highest ferric reducing ability when compared with other varieties of ginger, which by virtue indicates its antioxidant activity. The results of the study are in accordance with Maya and Sarada.[20]The key enzymes controlling carbohydrate metabolism associated with hyperglycemia in diabetes are α-amylase and α-glucosidase[21]. Earlier reports show that the inhibitory activities of natural compounds against these two enzymes are attributed to the phenolic contents like gingerol and shogaol in these extracts[22]. In the present study all the three varieties of ginger extracts demonstrated significant dose dependent inhibitory effects and the highest percentage was recorded with aqueous extract of mature ginger for α-glucosidase followed α- amylase.Ginger samples gave mild α- amylase inhibitory activity compared to α-glucosidase. Prior reports had also indicated that excessive inhibition of α- amylase could result in the abnormal bacterial fermentation of undigested carbohydrates in colon and therefore mild α- amylase inhibition activity is useful[23]. The results of the present study are in accordance with those that are reported earlier.[24,25,26]

Conclusion

Based on the upshots of the present study it has been resolved that maximum antioxidant activity combined with antidiabetic activity is documented with aqueous extract of mature ginger when compared to other varieties thereby justifying its traditional claims and strongly recommending its use in the management of diabetic complications .

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Table: 1 Free radical scavenging activity by DPPH assay

oncentration	% inhibition of samples at different concentration		
	ature ginger	oung ginger	ango ginger
00µg	1.7±1.32	5.2±1.4	0.2±0.6
00µg	0.5±2.01	0.5±2.01	8.3±0.7
00µg	3.5±1.20	0.7±1.1	5.6±1.2
00µg	2.3±2.10	7.8±1.5	3.8±1.8
00µg	9.0±1.07	5.4±0.8	2.4±1.4

The values are expressed as Mean of triplicates ± Standard deviation

Table: 2 Lipid peroxidation and FRAP assay for honey and ginger samples

Particulars	Mature ginger	Young ginger	Mango ginger
Lipid peroxidation assay nmoles MDA/gm	155 ± 1.55	232 ± 2.12	158 ± 1.98
FRAP assay µmoles Fe(II)/gm dry weight	767.2±2 .45	680±4.3	310±2.68

The values are expressed as Mean of triplicates ± Standard deviation

Table: 3 Inhibition of alpha amylase by ginger samples

Concentration	% inhibition of samples at different concentration			
	Standard Acarbose	Mature ginger	Young ginger	Mango ginger
20µg	2±1.53	0	0	0
40µg	30±1.45	17±1.01	7±0.75	06±1.02
60µg	39±2.01	21±1.23	12±1.10	10±2.04
80µg	48±1.65	26±1.25	18±0.89	15±1.26
100µg	61±1.26	30±1.05	24±1.08	21±1.56

The values are expressed as Mean of triplicates ± Standard deviation

Table: 4 Inhibition of alpha glucosidase by ginger samples

Concentration	% inhibition of samples at different concentration			
	Standard Acarbose	Mature ginger	Young ginger	Mango ginger
20µg	19±0.23	09±0.59	05±0.45	0
40µg	38±1.16	30±0.28	15±0.68	08±0.84
60µg	53±2.02	43±0.56	24±0.94	14±0.45
80µg	67±1.08	52±1.01	31±0.76	21±0.87
100µg	80±0.09	57±0.85	41±0.87	30±0.62

The values are expressed as Mean of triplicates ± Standard deviation