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EVALUATION OF POTENT ANTIOXIDANT ACTIVITY OF GINGER EXTRACT USING IN VITRO STUDY

Himayoon Rashid[1] and G. Umamaheswari*

[1]Research Scholar, Research and PG Department of Biotechnology, Marudupandiyar College, Vallam- Post, Thanjavur, Tamilnadu, India-613403.

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***Corresponding Author**
Dr. G. Umamaheswari
Research Scholar, Research and PG Department of Biotechnology, Marudupandiyar College, Vallam- Post, Thanjavur, Tamilnadu, India-613403.

1. ABSTRACT

Ginger (*Zingiber officinale*) has been used since time immemorial for treating different ailments and as dietary condiment. This rhizome has shown potent anticancer and anti-inflammatory activity. In the current study we evaluated the potent antioxidant activity of methanolic extract of ginger using distinct techniques like DPPH [1,1-diphenyl-2-picryl hydrazyl] assay and nitric oxide scavenging assay. This dietary condiment showed substantial antioxidant activity as determined by the above mentioned assays. While an IC50 of 27.85 was seen for ginger in DPPH assay, IC50 of 59.79µg/ml was obtained for ginger extract in nitric oxide scavenging assay. These findings established that ginger has strong antioxidant activity and may be used as a remedy to treat disorders associated with oxidative stress including cancer and neurodegeneration.

2. KEYWORDS - Phytochemicals, Ginger, DPPH, Thin layer chromatography, NOS) activity.

3. INTRODUCTION

Ginger (*Zingiber officinale*), falls among the frequently consumed dietary condiments. This rhizomatous perennial plant used worldwide as a spice in foods and beverages, is commonly known for its medicinal properties, primarily as a remedy for digestive disorders, including dyspepsia, colic, nausea, vomiting, gastritis and diarrhea.[1] Ginger is known to contain several bioactive phenolic compounds, including non-volatile pungent compounds such as gingerols, paradols, shogaols and gingerones.[2,3] The most abundant phytochemicals,

gingerols, vary in chain length and comprise odiferous components of the fresh root, with 6-gingerol being the most critical.[4] The dehydrated form of gingerols, shogaols, mainly occurs in the dried roots, with 6-shogaol being the most abundant one. The constituent phenolics of ginger have been shown to display anticancer, anti-inflammatory and anti-angiogenesis.[5-7] Ginger extract has been reported to reduce cholesterol level, inhibits LDL oxidation and attenuate development of atherosclerotic lesions.[8] Current therapeutic strategies based on synthetic drugs are expensive and result in genetic and metabolic complications emphasizing the escalating need of developing safe and effective mode of treatment for circumventing disease states.[2] It is well established that oxidative stress triggers several disorders including cancer, neurodegeneration and type 2 diabetes.[9-13] Taking these grim facts into consideration the current study focused on the antioxidant activity of ginger extract and its in vitro validation using extensive techniques like DPPH [1,1-diphenyl-2-picryl hydrazyl] and nitric oxide scavenging assay.

4. MATERIALS AND METHODS

4.1 Plant material

The rhizomes of ginger were purchased from Thanjavur district of Tamil Nadu. These rhizomes were washed shade dried and grinded to a fine powder.

4.2 Preparation of extract

Methanolic extract of powdered ginger was prepared using Soxhlet extractor.[14] 20 g of ginger was taken and 300 mL of methanol was added in the defined extractor. The extract was ultimately evaporated to dryness and the solid extract was stored for further use.[14]

4.3 Thin layer chromatography

Crude methanolic extract was diluted to 10 mg/mL using respective solvents. For TLC analysis, 2 µl of sample was applied as spot. Chloroform: Methanol (950:50) was used as developing solvent system. After development, the plates were air dried, numbers of spots were noted and Rf values were calculated. Spots were visualized using NaOH as detecting agent. For liquid-liquid extraction, 500 mg of crude methanolic extract was solubilized in 10 mL methanol. Then the different solvent starting from hexane, chloroform, Ethyl acetate and Methanol were used for the further extraction using separating funnel. Extracts were diluted to 10mg /ml using respective solvents. 2 ul of sample was used for TLC analysis and solvent system used was chloroform: methanol (950:50).[15] TLC characteristics of ginger chloroform, ginger ethylacetate, and ginger hexane were obtained.

4.4. Purification using column chromatography

The methanolic was reconstituted in methanol and subjected to column chromatography (300mm x 10 mm) on silica gel (60–120 mesh, Merck). The extract was eluted with n-hexane (100%), ethyl acetate–n-hexane (20:80, 50:50 and 90:10) solvent systems. Fractions (5ml each) were collected and pooled based on color to obtain 7 fractions- Fraction (1), Fraction (2) and Fraction (3) did not showed any spots in TLC. Fractions (4), (5), (6) and (7) showed the following spots in TLC. Fourth fraction showed the presence of gingerol.[15, 16]

4.5 Determination of antioxidant activity of methanolic extract using DPPH assay

DPPH [1,1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple colour. Antioxidants reduce DPPH to 1,1-diphenyl-2-picryl hydrazine, colourless compound which is measured at an absorbance of 510 nm. DPPH assay was performed as per standard protocol.[17, 18] Different concentrations tested for reference standard were 0.5, 1.0, 1.5, 2.0, 2.5 µg/mL. The reaction mixture was mixed and incubated at 25°C for 15 minutes. Absorbance was measured at 510 nm using semi-autoanalyzer. For control sample no test sample was added. DPPH radical scavenging was calculating using the following formuala DPPH radical scavenging activity (%) = $A_{control}$- $A_{test}/A_{control}\times100$.[19] The experiments were done in triplicates.

4.6 Nitric oxide scavenging (NOS) activity

At physiological pH, sodium niroprusside in aqueous solution spontaneously generates nitric oxide which on interacting with oxygen produces nitric ions that can be measured by using Griess reagent. Nitric oxide scavengers compete with oxygen culminating in diminished production of nitric oxide.[19, 20] 200 µl of 10 mM sodium nitroprusside and 200 ml of test solution / reference standard of various concentrations were incubated at room temperature for 150 minutes. 500 µl of Griess reagent was added followed by incubation at room temperature for 10 minutes.[21] For control buffer solution was used instead of test substances and absorbance was measured at 546nm.[21] All the experiments were repeated trice and percentage inhibition of nitric oxide was calculated using the below equation.[19-21]

(% inhibition) = $A_{control}$- $A_{test}/A_{control}\times100$.

Statistical analysis

All data were presented as mean ± S.D. and the graphpad prism 5.0 software was used to calculate the IC50 values.

5. RESULTS AND DISCUSSION

Thin layer chromatography of crude methanolic extract showed four distinct bands

TLC of crude methanolic extract portrayed four different bands with retention factors 0.4, 0.15, 0.43 and 0.63 respectively. The details of these bands are provided in Table 1 and Figure1.

Figure 1: TLC chromatogram of crude methanolic extract of ginger at three different light wavelengths. First one visible light, second 254 nm, third one 336 nm.

Table 1: TLC characteristics of crude methanolic extract of ginger under different wavelengths.

TLC Band	Retention Factor	TLC Profile characteristics		
		Visible light (Figure 1)	Shortwave UV 254 nm (Figure 2)	Longwave UV 366 nm (Figure 3)
1	0.4	Light Grey	Light pink	Greenish yellow
2	0.15	NV	Light pink	Greenish yellow
3	0.43	Light Grey	Light pink	Greenish yellow
4	0.60	NV	Light pink	Greenish yellow

Fourth fraction showed the presence of gingerol like compound

Different solvent systems as described in methodology were used for purifying the compound from crude extract of ginger. The extract was fractionated into seven fractions. Fractions 1 to 3 showed no bands but from fourth fraction onwards bands were obtained Table 2 and Figure 2. The fourth fraction showed presence of gingerol like compound.

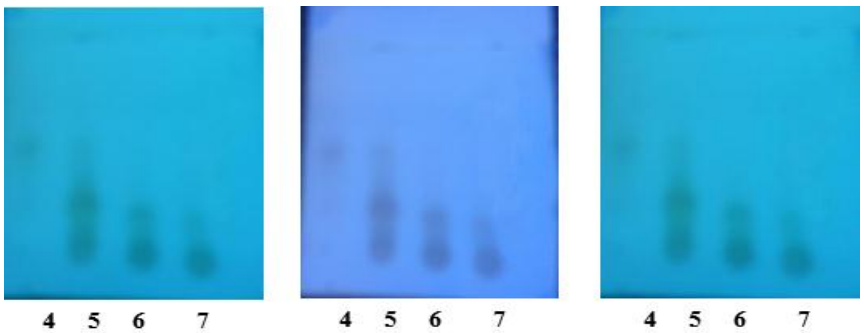

Figure 2: TLC chromatogram of different fractions 4th to 7th of ginger. First one visible light, second one 254 nm, third one 336 nm.

Table 2: TLC profile of fourth fraction of methanolic extract.

TLC Band	Retention Factor	TLC Profile characteristics		
		Visible light (Figure 1)	Shortwave UV 254 nm (Figure 2)	Longwave UV 366 nm (Figure 3)
1	0.43	Greenish Grey	Brick Red	Greyish Yellow

Ginger extract shows promising antioxidant activity

It is well established that the antioxidant activity of plant extracts is due to presence of hydrogen atoms or electrons and the ability to scavenge free radicals. DPPH test is frequently used to prove the capacity of donating hydrogen atoms.[22, 23] In the current study, extract of ginger showed a promising antioxidant activity as determined by DPPH assay in a dose dependent fashion. The defined extract showed maximum % inhibition 68.6 at the concentration of 100 µg/mL. Methanolic extract of ginger showed an IC_{50} of 27.85 µg/mL compared to standard antioxidant quercetin showing an IC_{50} of 5.304 (Figure 3 and Table 3). These results are in consistent with the previous studies reporting the antioxidant activity of ginger extract.[22]

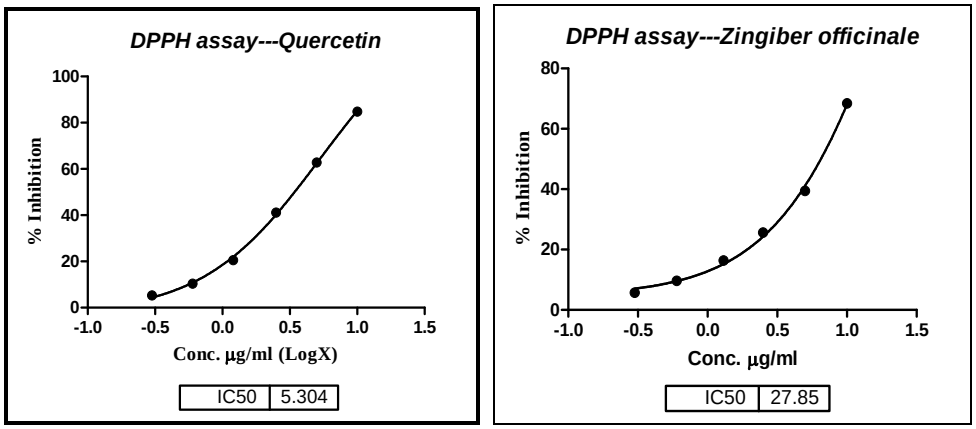

Figure 3: Antioxidant activity of ginger extract as determined by DPPH assay. Quercetin as positive standard and ginger as experimental.

Table 3: Free radical scavenging activity of ginger extract as determined by DPPH assay.

Plants Name	Concentration (µg/ml)	Absorbance Trail 1	Absorbance Trail 2	Absorbance Trail 3	Absorbance 590nm	% Inhibition	IC_{50}
Control	0.0	0.596	0.585	0.563	0.581	0.0	
Standard (Quercitin)	0.3	0.567	0.546	0.538	0.550	5.3	
	0.6	0.533	0.505	0.526	0.521	10.3	
	1.3	0.487	0.429	0.470	0.462	20.5	5.304µg/ml
	2.5	0.353	0.351	0.323	0.342	41.1	
	5.0	0.229	0.199	0.221	0.216	62.8	
	10.0	0.092	0.103	0.068	0.088	84.9	
	0.0	0.596	0.585	0.563	0.581	0.0	
Methanolic fraction	3.1	0.529	0.582	0.568	0.560	3.7	
	6.3	0.511	0.576	0.523	0.537	7.7	
	12.5	0.468	0.511	0.479	0.486	16.4	
	25.0	0.426	0.465	0.408	0.433	25.5	27.85µg/ml
	50.0	0.313	0.357	0.316	0.329	43.5	
	100.0	0.159	0.171	0.218	0.183	68.6	

Ginger extract shows potent nitric oxide radical scavenging activity

Nitric oxide radical inhibition study proved that the methanolic extract of ginger has vibrant nitric oxide scavenging activity, generated from sodium nitroprusside and reacting with oxygen to form nitrite.[24, 25] Our in vitro study showed that ginger has promising nitrogen oxide radical scavenging activity. This effect of ginger extract was found to be concentration dependent as shown in figure. Maximum percentage inhibition (56.52) was recorded at when the concentration of extract was 100µg/mL. The defined ginger extract showed an IC_{50} of 59.79µg/ml compared to curcumin (IC_{50} = 39.55µg/ml taken as standard as shown in Table. Our results align well with the previous findings where ginger extract has been reported to inhibit the production of nitric oxide and other inflammatory mediators, thereby supporting our results.[8]

Table 4: Free radical scavenging activity of methanolic extract of ginger as determined by nitric oxide scavenging assay.

Plants Name	Concentration (µg/ml)	Absorbance Trail 1	Absorbance Trail 2	Absorbance Trail 3	Absorbance Average	% Inhibition	IC_{50}
Control	0.0	0.526	0.537	0.563	0.542	0.00	
Standard (Curcumin)	2.5	0.515	0.525	0.548	0.529	2.34	39.55µg /ml
	5	0.459	0.513	0.509	0.494	8.92	
	10	0.415	0.439	0.492	0.449	17.22	
	20	0.372	0.341	0.446	0.386	28.72	
	40	0.269	0.234	0.219	0.241	55.60	
	80	0.184	0.149	0.198	0.177	67.34	
	0.0	0.526	0.537	0.563	0.542	0.00	
Methanolic fraction (Zingiber officinale)	3.1	0.519	0.535	0.547	0.534	1.54	59.79µg /ml
	6.3	0.494	0.508	0.531	0.511	5.72	
	12.5	0.476	0.473	0.524	0.491	9.41	
	25.0	0.428	0.391	0.437	0.419	22.76	
	50.0	0.271	0.306	0.302	0.293	45.94	
	100.0	0.216	0.232	0.259	0.236	56.52	

6. CONCLUSION

In the current study we evaluated the antioxidant activity of ginger extract using in vitro studies. Our findings established that ginger has potent antioxidant activity. These findings were determined by using DPPH assay and nitric oxide scavenging activity of ginger extract using quercetin and curcumin as standards respectively. IC_{50} of 27.85µg/mL was found for methanolic extract of ginger in case of DPPH assay in comparison to quercetin (IC_{50} 5.304). In nitric oxide scavenging assay, an IC_{50} of 59.79µg/ml was seen for ginger extract in comparison to positive standard curcumin (IC_{50} = 39.55µg/ml. Thus our studies showed that ginger has potent antioxidant activity and should be used as a remedy for disorders arising due to oxidative stress like neurodegeneration and cancer.

Conflict of Interest

The authors have no conflict of interest to declare.

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