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Bangladesh J. Sci. Ind. Res. 48(2), 115-118, 2013

**BANGLADESH JOURNAL
OF SCIENTIFIC AND
INDUSTRIAL RESEARCH**

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Antioxidant activities of *Z. officinale* Roscoe and *A. allughas* Roscoe (Zingiberaceae) Rhizomes

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Abstract

Ginger (*Zingiber officinale* Roscoe) and Lachii (*Alpinia allughas* Roscoe) are well known and widely used herbs. Ethanol, acetone, methanol and n-hexane extracts of *Zingiber officinale* Roscoe and *Alpinia allughas* Roscoe roots were screened for their antioxidant activities in an effort to compare and validate the medicinal potential of their subterranean part. Total phenol (mg gallic acid/g dry matter) and flavonoid contents (catechin equivalents/g dry matter) were estimated by Folin-Ciocalteu and aluminium chloride colorimetric tests respectively. Radical scavenging activity by DPPH methods was expressed as percent inhibition. Total phenolic contents varied from 10 ± 0.12 to 14 ± 0.03 mg gallic acid/g for *Z. officinale* and 5.46 ± 0.02 to 12.9 ± 0.06 mg gallic acid/g for *A. allughas*. Total flavonoids were 5.33 ± 0.75 to 8.34 ± 2.1 mg catechin/g for *Zingiber officinale* and 1.50 ± 0.447 to 9.92 ± 2.5 mg catechin/g for *Alpinia allughas* in four solvents. Maximum phenolic and flavonoid contents were observed in methanol extracts. The antioxidant activity (percent inhibition) of *Zingiber officinale* and *Alpinia allughas* ranged from 26.8 to 68.3 and 14.3 to 58.5 respectively in different solvents. Overall, the findings indicate that the both spices are good sources of phytochemicals which could be exploited as great potentials for drugs and/or nutritional supplements.

Keywords: *Zingiber officinale*; *Alpinia allughas*; Phenols; Flavonoids; Rhizomes

Introduction

Free radicals and reactive oxygen species generated during metabolic functions can damage DNA, lipids and proteins. The efficacy of antioxidant defense repair systems to scavenge and minimize the formation of reactive oxygen species can be enhanced by plant derived antioxidants. Phytotherapy is of significant attention as natural antioxidants could be promising agents for management of oxidative stress-related diseases (Kar *et al.*, 1999).

Numerous species of *Zingiberaceae* family exhibit antioxidant, antimicrobial and cytotoxic activities and are subjected to pharmacological research (Ghasemzadeh *et al.*, 2010; Priya *et al.*, 2011; Lu *et al.*, 2012; Rani *et al.*, 2012). Among these, most interesting are *Zingiber officinale* and *Alpinia allughas* whose wild rhizomes are used as traditional medicines and spices by the local population. *Zingiber officinale* (ginger) rhizome is used as a spice. *Alpinia allughas* (Lachii) rhizome has numerous essential oils and is a therapeutic supplement in folk medicine for gout and colic diseases (Prakash *et al.*, 2007; Nanasombat *et al.*, 2009; Sharma *et al.*, 2011). In present study, antioxidant compo-

nents and activities of *Zingiber officinale* and *Alpinia allughas* rhizomes (root) in four solvent were assessed.

Material and method

Plant material and extract preparation

All the plants were collected from the local retail markets of Faisalabad, Pakistan. About 250 g of the root parts of the plants were cut into smaller pieces and air-dried under the shade. The materials were then extracted with ethanol, acetone, methanol and n-hexane. Extracts of each solvent were evaporated under reduced pressure and the final residues were used for the bioassays.

Determination of Total Phenolic Contents

Total phenol contents (TP) were estimated using Folin-Ciocalteu assay as described previously (Duarte-Almeida *et al.*, 2006) with slight modifications. Briefly, extract was dissolved in 1 mL dimethyl sulfoxide (DMSO) and 1 mL of 10% dilution of Folin-Ciocalteu reagent. After 3 minutes, 3 mL of Na_2CO_3 (1% w/v) was added and the resulting mix-

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ture was incubated at room temperature for 2 hours. The absorbance of all the samples was measured at 760 nm and results were expressed in terms of gallic acid equivalent (mg gallic acid/g dry matter).

Determination of Total Flavonoid Contents

Aluminium chloride colorimetric technique was used for total flavonoid contents (TF) estimation. Each extract (0.5 mL of 1:10 g/mL) was mixed with 1.5 mL of respective solvent, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The absorbance was measured at 510 nm and TF concentrations were articulated as catechin equivalents/g dry matter (Siddique *et al.* 2010).

Determination of Antioxidant activities

Radical scavenging activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl)

The DPPH assay was carried out as described by Souri *et al.* (2008). The antioxidant activity of extracts was assessed by measuring their scavenging abilities to 2, 2-diphenyl 1-1-picrylhydrazyl stable radical. 50 μ L aliquot of various concentrations of the samples was added to 5 mL of a 0.004 % methanol solution of DPPH. After 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm. Percent inhibition was calculated as;

$$I\% = (A \text{ blank} - A \text{ sample} / A \text{ blank}) \times 100$$

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

Statistical analysis

All data were expressed as mean \pm S.D of triplicate measurement. To find the optimum antioxidant activity and significant difference between variables, Students t-test, tukey B test and ANOVA were performed using the Statistical Package for the Social Sciences (SPSS Inc. Chicago, IL,

USA) software, (version 15.0). A *p*-value of less than 0.05 is considered statistically significant.

Results and discussion

Total Phenolic Contents

The medicinal properties of Zingiberaceae family are due to the presence of certain bioactive compounds having antioxidant activities (Bak *et al.*, 2012). The levels of phenolic compounds in various solvents are presented in Table I. TP were determined using the Folin-Ciocalteu reagent and expressed as gallic acid equivalents (GAE) per gram. The Follin-Ciocalteu method was selected because of its high intensity, quick results and low interference (Sultana *et al.*, 2010).

Antioxidant activities of plant extracts are usually linked to their phenolic content. The high potential of phenolics to scavenge free radicals may be due to many phenolic hydroxyl groups they possess (Atmani *et al.*, 2009). Hinneburg *et al.* (2006) found the total phenolic content of aqueous ginger extract to be 23.5 mg gallic acid/g of sample. In our study, TP varied from 10 ± 0.12 to 14 ± 0.03 mg GAE per gram for *Z. officinale*. These results were in accordance with previously reported 10.22 ± 0.87 - 13.5 ± 2.26 mg GAE per gram TPCs in Malaysian ginger varieties (Ghasemzadeh *et al.*, 2010). Shirin and Prakash (2010) determined 510 ± 2.2 (methanol), 565 ± 4.1 (ethanol) and 325 ± 1.9 (acetone) TP as mg of Tannic acid equivalents (TAE) /100 g of sample in ginger roots. TP in *Z. officinale* were higher than *A. allughas*. For *A. allughas*, TP in present study varied from 5.46 ± 0.02 to 12.9 ± 0.06 mg GAE per gram in four solvents. Literature reports no studies on antioxidant potential of *A. allughas*.

The difference in TP of *Z. officinale* and *A. allughas* was significant ($p < 0.05$) in acetone extracts and maximum TP were observed in methanol extracts as compared to all other solvents. Methanol has been proven most effective solvent to determine antioxidants. The efficiency of methanol as solvent to get better and much quantity of phenolic contents

Table I. Antioxidant components of *Z. officinale* and *A. allughas* rhizomes in different solvent extracts

Antioxidant components	Specie	Methanol	Ethanol	Acetone	n-Hexane
Total Phenolic Contents (TP)	<i>Z. officinale</i>	14 ± 0.03	11.2 ± 0.07	10 ± 0.12	13.5 ± 0.05
	<i>A. allughas</i>	12.9 ± 0.06	10.3 ± 0.06	5.46 ± 0.02	11.0 ± 0.06
Total Flavonoid Contents (TF)	<i>Z. officinale</i>	8.34 ± 2.1	5.33 ± 0.75	5.95 ± 0.46	7.81 ± 0.56
	<i>A. allughas</i>	8.20 ± 5.7	1.92 ± 0.014	1.50 ± 0.447	9.92 ± 2.5

All analyses are the mean of triplicate measurements \pm standard deviation. TP expressed as mg gallic acid/g dry material; TF expressed as catechin equivalents/g dry material

may be due to different reasons; changed climate, reagent efficacy, manual error and different quantity in reaction media. The methanol and n-hexane were found to be most effective solvents as compared to ethanol and acetone for total phenolics. The trend of phenolic contents in decreasing order was methanol > n-hexane > ethanol > acetone. Applying tukey B test; there was no significance difference in TP contents among n-hexane, acetone and ethanol.

Total Flavonoid Contents

The flavonoids function as metal chelators, reducing agents, chain-breaking antioxidants and quenchers of the formation of singlet oxygen (Ghimeray *et al.*, 2009). Flavonoids with hydroxyl groups are responsible for radical scavenging effect in plants (Ebrahimpzadeh *et al.*, 2008). Total flavonoid contents were determined using the aluminium chloride colorimetric technique and expressed as expressed as mg quercetin/g of dry plant material. The TF varied from 5.33 ± 0.75 to 8.34 ± 2.1 mg catechin/g for *Zingiber officinale* and 1.50 ± 0.447 to 9.92 ± 2.5 mg catechin/g for *Alpinia allughas* (Table I). Similar to our results, 3.66 ± 0.45 and 4.21 ± 0.98 mg quercetin/g plant materials TF were observed in some varieties of *Zingiber officinale* Roscoe by Ghasemzadeh *et al.* (2010). However, Shirin *et al.* (2010) observed 0.249 ± 0.002 - 0.685 ± 0.005 (g quercetin equivalents/100 g plant material) TF in *Zingiber officinale* roots.

The difference of TF in *Zingiber officinale* and *Alpinia allughas* was significant in all the solvents. Similar to the phenolics results, maximum concentrations of total flavonoid in *Z. officinale* and *A. allughas* rhizomes were observed in methanol extracts as compared to all other solvents. It can be due to higher solubility of phenols and flavonoids in methanol. The trend of flavonoid contents in decreasing order was methanol > n-hexane > acetone > ethanol.

Antioxidant Activities

The DDPH assay was carried out as described by Souri *et al.* (2008). The antioxidant activity of extracts was assessed by measuring their scavenging abilities to 2, 2-diphenyl 1-1-picrylhydrazyl stable radical. The DPPH is very stable free radical along deep violet color which gives maximum absorption within range of 515 to 528nm. When it loses its chromophore, its color becomes yellow. As the degree of hydroxylation of phenolic contents or the amount of phenolic contents elevated, the DPPH free radical scavenging ability is also enhanced (Nenadis *et al.*, 2002). Degree of discoloration indicates scavenging potential of the antioxidant extract which is due to the hydrogen donating or radical scavenging ability (Ajila *et al.*, 2007).

Table II. Antioxidant activity of *Z. officinale* and *A. allughas* rhizomes in different solvent extracts

Specie	Inhibition (%)			
	Methanol	Ethanol	Acetone	n-Hexane
<i>Z. officinale</i>	68.3	27.2	26.8	49.2
<i>A. allughas</i>	58.5	14.3	20.2	36.5

All 6 are the mean of triplicate measurements. Radical scavenging activity is expressed as percent of free radical inhibition

The antioxidant activity (percent inhibition) of *Zingiber officinale* and *Alpinia allughas* ranged from 26.8 to 68.3 and 14.3 to 58.5 respectively in different solvents (Table II). The difference in antioxidant activity between *Zingiber officinale* and *Alpinia allughas* was significant in all the solvents. Earlier, 51.41-58.22 % inhibition was observed in methanolic extracts of *Zingiber officinale* rhizomes by Ghasemzadeh *et al.* (2010).

The comparison between the solvents indicated quiet significant difference in antioxidant activity of methanol and n-hexane. The trend of scavenging activity to the free radicals for *Zingiber officinale* was methanol > n-hexane > ethanol > acetone. The trend in DPPH scavenging activity of *A. allughas* was methanol > n-hexane > acetone > ethanol.

The activity of the extracts in the DPPH assay indicate their hydrogen-donating ability as the free radical are known to cause auto-oxidation of the unsaturated lipids in foods (Singh *et al.*, 2007). Oktay *et al.* (2003) reported strong antioxidant activity of water and ethanol extracts of fennel seeds. The combined effect of solvents and different plants on radical scavenging activity is significant. As evident from the present data, methanol is most suitable solvent for getting maximum activity of scavenging free radicals.

Conclusion

The present study has provided some comparative information on the phytochemistry of *Z. officinale* Roscoe. and *A. allughas* Roscoe. available in local Pakistani markets. The investigation also indicates that the *A. allughas*, a less studied plant is one of the best sources for natural antioxidant. Further, there is a need to isolate and identify these natural antioxidant compounds present in *Z. officinale* and *A. allughas*.

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Received: 23 January 2013; Revised: 13 March 2013;
Accepted: 21 May 2013.