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Evaluation of Phenolics Content, Flavonoids and Antioxidant activity of *Curcuma amada* (Mango Ginger) and *Zingiber officinale* (Ginger).

Sadhana Singh* and AK Gupta

Department of Chemistry, Sam Higgin Bottom Institute of Agriculture Technology and Sciences, Deemed University (AAI-DU), Allahabad, Uttar Pradesh, India

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For Correspondence:

Department of Chemistry, Sam Higgin Bottom Institute of Agriculture Technology and Sciences, Deemed University (AAI-DU), Allahabad, Uttar Pradesh, India

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ABSTRACT

The Zingiberaceae, the largest family in Zingiberales, comprises generally 300 genera and 1000 species, and is pantropical, concentrated mainly in the old world, chiefly in Indonesia. Members of the family yield spices, dyes, perfumes, medicines, and number of ornamental species are cultivated for their showy flowers. Recently this plant has acquired great importance in the present day world with its antiaging, anticancer, antioxidant, anti-alzheimer's diseases, and variety of other medicinal properties due to its significant potential. The present study aims at comparing the Phenolics content, Flavonoids and Antioxidant activity of *Curcuma amada* and *Zingiber officinale*. The antioxidant activity and phenolic contents of the leaves as determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and the total amounts of phenolics and flavonoids were higher than those of the rhizomes. *Zingiber officinale* had higher antioxidant activities as well as total contents of phenolic and flavonoid in comparison with *Curcuma amada*. This study validated the medicinal potential of the leaves and rhizome of *Zingiber officinale* and the positive relationship between total phenolics content and antioxidant activities in *Zingiber officinale*.

INTRODUCTION

The genus *Curcuma* under the family Zingiberaceae comprises of over 80 species of rhizomatous herbs. *Curcuma amada* Roxb., popularly known as mango-ginger is having characteristic odour similar to raw mangoes (*Mangifera indica* L.) and used as major ingredient in the pickles, candies, salads, sauces and chutneys, another species *Zingiber officinale* (L.) Rosc. (Ginger) has been used as a spice for over 2000 years. Its roots and the obtained extracts contain polyphenol compounds (6-zingiberol and its derivatives), which have a high antioxidant activity. *Zingiber officinale* is one of these traditional folk medicinal plants that have been used for over 2000 years by Polynesians for treating diabetes, high blood pressure, cancer, fitness and many other illnesses [1]. Although the digestion stimulating effect of this spice became known a long time ago, therefore, the present work was aimed to analyze the total phenol content, flavonoids and antioxidant potential of the Zingiberaceae family, *Zingiber officinale* in comparison with *Curcuma amada*.

Generation of free radicals or reactive oxygen species

(ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress^[2], which plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process^[3]. This concept is supported by increasing evidence indicating that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this, thus lowering the risk of disease^[4]. Antioxidants are substances that when present in low concentrations, compared to those of an oxidisable substrate significantly delay or prevent oxidation of that substance^[5]. Apart from their role as health benefactors, antioxidants are also added to food to prevent or delay its oxidation, normally initiated by free radicals formed during the food's exposure to environmental factors such as air, light and temperature^[6]. At present most of the antioxidants used for this are manufactured synthetically. The main disadvantage with the synthetic antioxidants is their side effects when taken in vivo^[7]. Strict governmental rules regarding the safety of the food has necessitated the search for alternatives as food preservatives^[8].

Plants are a potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are secondary metabolites of plants^[9]. Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols, etc. are among the antioxidants produced by plants for their own sustenance. Beta-carotene, ascorbic acid and alpha tocopherols are widely used antioxidants^[10]. *Zingiber officinale* contains a number of antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids, and polyphenols such as flavonoids, flavones glycosides, rutin, etc.^[11].

MATERIALS AND METHODS

The rhizomes of *C.amada* (mango ginger) and *Zingiber officinale* (ginger) were collected during May 2011 from the Local market, Allahabad, India, They were cut into small pieces (5 cm), shade dried and ground to fine powder. Known quantities of the ground rhizome. Materials were extracted with methanol using a soxhlet apparatus for 16h and the solvent was evaporated to dryness under reduced pressure. The residues were weighed and stored at 4 °C until use.

A modified method was used for this purpose/objective/part: diluted solution (1 mL) containing flavonoids, 5% (w/w) NaNO₂ (0.7 mL) and 30% (v/v) ethanol (10 mL) were mixed for 5 min, and then 10% AlCl₃ (w/w, 0.7 mL) was added and mixed altogether. Six minutes later, 1 mol/L NaOH (5 mL) was added. The solution was then diluted to 25 ml with 30% (v/v) ethanol. After standing for 10 min, the absorbance of the solution was measured at 430 nm with a spectrophotometer. A standard curve was plotted using quercetin as a standard. Different concentrations of quercetin were prepared in 80% ethanol and their absorbance was read at 430 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). The results were expressed in mg quercetin/g dry weight by comparison with the quercetin standard curve, which was made under the same condition^[12].

The total phenolic content was determined using Folin-Ciocalteu reagents with analytical grade gallic acid as the standard. 1 mL of extract or standard solution (0-500 mg/L) was added to deionized water (10 mL) and Folin-Ciocalteu phenol reagents (1.0 mL). After 5 minutes, 20% sodium carbonate (2.0 mL) was added to the mixture. After being kept in total darkness for 1 h, the absorbance was measured at 750 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Amounts of TP were calculated using gallic acid calibration curve. The results were expressed as gallic acid equivalents (GAE)g/g of dry plant matter^[13].

The evaluation of radical scavenging activity (antioxidant activity) was conducted by the method of (Brand-Williams et al.) with modifications. The 40 µg/mL was prepared. A stock solution of the sample was diluted. The concentration was tested in triplicate. The portion of sample solution (0.5ml) was mixed with 3.0ml of 0.1mM 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and allowed to stand at room temperature for 30 min. and light protection. The absorbance was measured at 517 nm. The scavenging activity of the samples at corresponded intensity of quenching DPPH. Lower the absorbance of the reaction mixture indicates the higher free radical scavenging activity. The different in absorbance between the rest and the control DPPH was calculated and expressed as (%) scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using following equation.

$$\text{Scavenging effect (\%)} = (1 - A_s/A_c) * 100$$

A_s is the absorbance of the sample at t=0 min.

A_c is the absorbance of the sample at t=30 min.

RESULTS AND DISCUSSION

In the DPPH test, antioxidants were typically characterized by their IC₅₀ value (Inhibition Concentration of Sample required to scavenge 50% of DPPH radicals). The results were obtained by linear regression analysis of the dose response curve plotted using % inhibition and concentration^[14].

The level of phenolic compounds in methanolic extracts of the leaves, rhizomes, and stems in the *Curcuma amada* and *Zingiber officinale*. Poly Phenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds^[15,16,17,18]. The activity is believed to be mainly due to their redox properties, which plays an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides^[19,20]. (Table.1)

In both, the total flavonoid and phenolic contents in the leaves were more than in the rhizomes. It is summarized in (Table .1) The total content of flavonoids and phenolics are influenced by the interaction between varieties and parts of plants. In fact, many medicinal plants contain large amount of antioxidants such as polyphenols. Previous studies have shown that some flavonoids components such as quercetin had anticancer activities and were able to inhibit cancer cell growth^[21,22]. Gallic acid was reported as a free radical scavenger and as an inducer of differentiation and apoptosis in leukemia, lung cancer, and colon adenocarcinoma cell lines, as well as in normal lymphocyte cells^[23,24]. It has been postulated that GA plays an important role in the prevention of malignant transformation and cancer development same as quercetin. Hence, the results of this research showed that flavonoids are important components of this plant, and some oits pharmacological effects could be attributed to the presence of these valuable constituents. Further work is required to establish if quercetin or any other flavonoids have any role in the prevention of this cancerous growth and development.

Table 1: Total phenolic and flavonoid contents of the methanolic extracts in different parts of *Curcuma amada* and *Zingiber officinale*.

		Leaves	Rhizomes
Total Flavonoids ^(a)	<i>Curcuma amada</i>	3.16 ± 0.33	2.14 ± 0.42
	<i>Zingiber officinale</i>	5.54 ± 1.83	3.66 ± 0.45
Total phenolics ^(b)	<i>Curcuma amada</i>	18.2 ± 0.52	9.2 ± 0.5
	<i>Zingiber officinale</i>	33.0 ± 1.13	10.22 ± 0.87

All analyses are the mean of triplicate measurements ± standard deviation;
a: Expressed as mg quercetin/g of dry plant material;
b: Expressed as mg gallic acid/g of dry plant material.

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

Leaves have higher activity than rhizomes. At a concentration of 40 ug/mL, the scavenging activity of the methanol extract of *Zingiber officinale* leaves and rhizome is higher than it was observed that methanolic extracts of the *Curcuma amada*

In this study, results showed that DPPH radical scavenging abilities of the extracts of plant parts were less than those of butylated hydroxytoluene (BHT) (83.7%) and α-tocopherol (92.3%) at 40 ug/mL [25]. (Table 2)

Table 2: DPPH scavenging activities of the methanolic extracts in different parts of *Curcuma amada* and *Zingiber officinale*. BHT and α-tocopherol were used as positive controls.

Plants	Extract source	Inhibition % ^(a)
<i>Curcuma amada</i>	leaves	47.64 ± 0.45
	rhizome	47.69 ± 0.34
<i>Zingiber officinale</i>	Leaves	51.12 ± 1.65
	rhizome	51.14 ± 0.51
Controls	BHT	96.21 ± 0.24
	α-Tocopherol	89.57 ± 1.12

All analyses were the mean of triplicate measurements ± standard deviation;
a: Results expressed in percent of free radical inhibition.

The present study aims at comparing the Phenolics content, Flavonoids and Antioxidant activity of *Curcuma amada* and *Zingiber officinale*. The antioxidant activity and phenolic contents of the leaves as determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and the total amounts of phenolics and flavonoids were higher than those of the rhizomes. *Zingiber officinale* had higher antioxidant activities as well as total contents of phenolic and flavonoid in comparison with *Curcuma amada*. This study validated the medicinal potential of the leaves and rhizome of *Zingiber officinale* and the positive relationship between total phenolics content and antioxidant activities in *Zingiber officinale*.

This study showed that ginger and mango ginger's methanolic extracts have good free radical scavenging ability and can be used as a radical inhibitor or scavenger, acting possibly as a primary antioxidant.

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