Estimation of Phenol Content, Antioxidant Ability and Antibacterial Activity of Two Ginger *Zingiber officinale* Varieties

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Abstract: Ginger *Zingiber officinale* has been used as a medicine since ancient times. This study has been concentrated on identification of Indian and Chinese ginger rhizome methanolic extract properties. The objectives were: 1) Estimation of polyphenols, flavonone, dihydroflavonole, flavone and flavonole contents of two variety extracts; 2) Determination of antioxidant activities of the two variety extracts and 3) Finding out the antibacterial abilities of the two extracts. Results showed that the Indian variety contained higher significant polyphenols expressed as gallic acid equivalent than the Chinese variety. Also, the Indian variety demonstrated higher significant values than Chinese variety for the flavonoid group of flavones and flavonols measured as quercetine and flavonoid group of flavanone and dihydroflavonol measured as Naringenin. Also, the concentrations of flavones and flavonols were lower than concentrations of flavanone and dihydroflavonol in both extracts. In addition, it was observed that the Indian variety had higher significant DPPH scavenging ability (antioxidant) than that of the Chinese variety. As for antibacterial activity, Indian extract showed higher antibacterial activity than Chinese extract with gram positive and negative bacteria. Finally, it was concluded that ginger extracts contain considerable amounts of phenolic compounds which are responsible for the observed antimicrobial and antioxidant activities.
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1. Introduction

Oxidation reactions are crucial for life, however; they can also be damaging. Oxidation reactions can produce free radicals or reactive oxygen species (ROS). They can initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxide, which is related to aging and other diseases. Free radicals and reactive species attack lipids, proteins and DNA and induce their oxidation, which may result in oxidative damage (Sang et al., 2002). However, humans have evolved complex systems of multiple types of antioxidants enzymatic and nonenzymatic to protect cells and organs (Sadaf et al., 2012). The function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level (Rhee, 2006). Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress. Oxidative stress is associated with an elevated risk of chronic diseases, such as cardiovascular morbidity and cancer (Ninfali et al., 2005). Therefore, under conditions which promote oxidative stress, it is important to obtain antioxidant exogenously as a part of a diet or as a dietary supplements (Sadaf., et al., 2012). Exogenous antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction (Catherin and Rice-Eva, 2003; Bergman et al., 2001)). They can protect human being from oxidative damage by adsorbing and neutralizing free radical, quenching singlet and triplet oxygen, or decomposing peroxide by their redox properties (Sang et al., 2002). Apart from their role to benefit health, antioxidants are added to foods to prevent or delay food oxidation initiated by free radicals formed during their exposure to some environmental factors such as air, light and temperature. Food plants, such as fruits, vegetables, nuts and spices are the primary sources of naturally occurring antioxidants for human (Sang et al., 2002). The most important groups are polyphenolics, such as flavonoids (comprising flavonols, isoflavones, and anthocyans). Also some pigments such as chlorophyllins, phytosterines and allysulfides were found to have antioxidant activities. Many of these compounds such as ginger are currently marketed as food supplements and are used for the production of functional foods (Hoelzl et al., 2005). Ginger (Zingiber officinale) is cultivated in temperate zone or tropical zone countries (Nakatani et al., 2001). It is one of the most widely used spices in the world. It is one of these traditional folk medicinal plants that have been used for over 2000 years by Polynesia for treating diabetes, high blood pressure, cancer, fitness and many other illnesses (Tepe et al., 2006).

In most countries, only ginger rhizomes are consumed as a food and it is known as a resource with higher phenolic components (Rozanida et al.,

2006; Tang and Zhao, 2001). The components in ginger include: volatile oils, extractable oleoresins, many fats, carbohydrates, vitamins, minerals, medicine compounds such as: antioxidant, flavonoids and anticancer (Shukla et al., 2007). The volatile essential oils contributing to the characteristic flavour of ginger, varies from 1.0-3.0%. While the oleoresin, responsible for the pungent flavour of ginger, varies from 4.0-7.5% and also possesses substantial antioxidant activity (Balachandran et al., 2006). The oleoresins and essential oils determine the quality of ginger rhizome in the world trade. Kim et al. (2007) and Schwertner and Rios (2007) reported that the main phenolic components of ginger are 6-gingerol, 6-shogaol, 8-gingerol and 10- gingerol and these constituents had exhibited strong antioxidative activity.

Finally, the spread of drug resistant pathogens is one of the most threats to successful treatment of microbial diseases. In addition, the consumption of food contaminated with food-borne microorganisms can pose a serious threat to human health. The existence of microorganisms causes spoilage and results in reduction of the quality and quantity of possessed food (Anwar et al., 2009). Therefore, there has been a growing considerable interest to identify new sources of safe and inexpensive antioxidant and antimicrobial potential of natural origin (Abd El-Bakry and El-Baroty, 2008). Ginger varieties have been reported as a good potential source for anti-microbial (Gosh et al., 2011). Ginger compounds are active against specific type of diarrhea which is leading to cause death in infant. Moreover, it has been found that ginger is effective in treating nausea caused by sea sickness, morning sickness and chemotherapy, though it was found superior over a place for post-operative nausea (Sebiomi et al., 2011).

In most counteries, ginger is used as common beverages like tea (in tea-bags form) among many people in particular in winter season, due to their protective effect and curative remedy for numerous disorders. Therefore, the specific objectives of this study were: 1) Estimation of polyphenols, flavonone, dihydroflavonole, flavone and flavonole contents of two local ginger variety extracts; 2) Determination of antioxidant activities of the two ginger extracts and 3) Finding out the antibacterial abilities of the two extracts.

2. Materials and Methods

2.1. Plant materials

Fresh plant ginger rhizomes (*Zingiber officinale*) of Indian and Chinese varieties were purchased from local market. Rhizomes were washed thoroughly, chopped into small pieces, freeze dried, grinded into powder. Five grams of each sample was weighed accurately and suspended in 50 ml of methanol 80%. Each sample was shaken for 24 h in an electronic shaker at room temperature and filtered with Whatman No. 1 filter paper. Supernatants were kept at – 4 °C for further analysis.

2.2. Microorganisms

Antimicrobial activity of methanol extracts of two ginger varieties were evaluated against two strains of Gram-positive bacteria *Bacillus subtilis*, and Micrococcus sp., and one strain of Gram-negative bacterium *Escherichia coli*

2.3. Chemicals

Folin-Ciocalteu's phenol reagent, sodium carbonate, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4-Dinitrophenol (DNP), aluminium chloride, sulphuric acid and potassium hydroxide were purshaesd from Sigma-Aldrich. Also, Three chemical standards quercetine, galangin and naringenin were purchased from Sigma-Aldrich and were used as standards. Also, analytical grade of methanol was used for extraction.

2.4. Total polyphenolics

The total phenolic content of two varieties extracts were determined by using Folin-Ciocalteu assay. The procedure of (Popova *et al*., 2004) was employed using a reference of gallic acid standard solutions (20, 40, 60 80 and 100 $\mu g.mL^{-1}$) for calibration. Briefly, 1 mL of the standard solutions or two varieties extracts were transferred to a 50 mL volumetric flask containing 15 mL distilled water. Four mL of Folin–Ciocalteu reagent and 6 mL of a 20% sodium carbonate solution (w/v) were added. The volume was made up with distilled water to 50 mL. Samples were left for 2 hr and the absorbance was measured at 760 nm (Fahmi *et al*., 2011).

2.5. Flavone and flavonol

Flavones and flavonols in two varieties extracts were expressed as quercetine equivalent. Quercetine was used to make the calibration curve (standard solutions of 6.25, 12.5, 25, 50, 80 and 100 $\mu g.mL^{-1}$ in 80% methanol (v/v)). An aliquot (2 mL) of the standard solutions or two varieties extracts, 20 mL methanol and 1 mL of 5% aluminium chloride in methanol (w/v) were mixed in a volumetric flask and the volume was made up to 50 mL with methanol. The mixture was left for 30 min and the absorbance at 425 nm was measured (Kosalec *et al*., 2004).

2.6. Flavonone and dihydroflavonol

Flavanone and dihydroflavonol in two varieties extracts were expressed as naringenin equivalent. Naringenin was used to make the calibration curve (standard solutions of 0.125, 0.25, 0.3, 0.5, 1 $mg.mL^{-1}$) in methanol (v/v). An aliquot (1 mL) of the standard solutions or two varieties extracts and 2 mL of DNP solution (1 g DNP in 2 mL 96% sulphuric

acid, diluted to 100 mL with methanol in volumetric flask) were heated at 50°C for 50 min (water bath). After cooling to room temperature, the mixture was made up to 10 mL with 10% potassium hydroxide in methanol (w/v). A sample (1 mL) of the resulting solution was added to 10 mL ethanol and diluted to 50 mL with methanol. Absorbance was measured at 486 nm (Popova *et al*., 2004; Kosalec *et al*., 2004**).**

2.7. DPPH scavenging activity

Scavenging effect of two varieties extracts samples corresponding of the quenching intensity of 1,1-diphenlyl-2-picrylhidrazyl was carried out as described by (Fahmi *et al*., 2013). Sample solution of each tested material (1 ml) was mixed with the same volume of 6 uM of DPPH solution and was allowed at dark for 30 min. at room temperature. The absorbance was measured at 517 nm. The percentage of scavenging effect was determined by comparing the absorbance solution containing the test sample to that of blank sample as follows:

% DPPH Scavenging activity = $(A_0-A_1)/A_0$ x100%

Where,

A_0 measurement of the blank

A_1 measurement of the sample

2.8. Antibacterial assay

A spectrophotometric assay with some modification was conducted as described by (Akujobi *et al*., 2010). Briefly, into tubes containing 10 ml liquid medium, 10 µl of one type of ginger variety extract was introduced under sterile conditions. After a good agitation, each tube was inoculated with a pure culture of a different type of bacteria. Incubation was carried out at 37°C for 24 hours. After incubation, a measurement of growth density in each tube was carried out against a blank. The reading was made by spectrophotometer at 625 nm. The percentage of bacterial growth inhibition for each ginger variety was measured by the following formula:

Growth Inhibition% = (A control – A test) / A control x 100

A control: optical density at 625nm of control with 80% methanol.

2.9. Statistical analysis

One-way ANOVA followed by Duncan's multiple range test DMRT was used to assess the statistical significance of changes in all indices with the level of significant difference set at $p < 0.05$. Statistical analysis software (SPSS 16.0.0 release; SPSS Inc., Chicago, IL) was used for all analyses.

3. Results and Discussion

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the **deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols,** flavonoids and phenolic compounds. In this study, methanol solvent was used to extract phenolic compound from ginger rhizomes because different studies showed that methanol solvent was most effective in extracting phenolic components than that extracted by other solvents (Siddhuraju and Becker, 2003; Sun 2005; Turkmen *et al*., 2006; Sultana *et al*., 2007; Ghasemzadeh *et al*., 2011). The efficiency of methanol as a solvent to get better and much quantity of phenolic contents may be due to high reagent polarity (Sattar *et al*., 2013).

3.1. Phenolic contents

The medicinal properties of *Zingiber officinale* are due to the presence of certain bioactive compounds having antioxidant activities (Bak *et al*., 2012). Total phenols were determined using the Folin-Ciocalteu reagent and expressed as gallic acid equivalents (GAE). The Follin-Ciocalteu method was selected because of its high intensity, quick results and low interference (Sultana *et al*., 2010). This method is based on oxidation-reduction reactions in which phenolic compounds are oxidized and show maximum absorbance in the wavelength region between 725 and 765 nm (Fahmi *et al*., 2013). The level of phenolic compounds in extracts of the rhizomes in the two varieties of *Zingiber officinale* are presented in Table 1. Comparing the varieties, it was found that Indian ginger had higher contents of total phenols than Chinese variety. Differences between the varieties were significant (p _ 0.05). This result indicated that the total content of phenolics is influenced by the ginger variety. This high level of total phenolic compounds was determined in ginger rihzomes by different studies (Ghasemzadeh *et al*., 2010; Maizura *et al*., 2011). In fact, many medicinal plants contain large amount of antioxidants such as polyphenols.

Table 1. Total phenol (galic acid equivalent), flavone and flavonole (quercetine equivalent), and flavonone and dihydroflavonole (naringenin equivalent) contents of the methanolic extracts of two varieties of *Zingiber officinale*

Variety	Total phenol (g/100g DW)	Flavone and flavonole (ug/100mg DW)	Flavonone and dihydroflavonole (ug/100mg DW)
Chinese	$264^{a} \pm 1$	$0.040^{a} \pm 0.01$	$40^{a} \pm 1$
Indian	$388^{b} \pm 1$	$0.045^{b} \pm 0.01$	$50^{b} \pm 1$

All analyses were the mean of triplicate measurements ± standard deviation; Values with the same superscript letter within each column are not significant different (p>0.05).

The biological activity of ginger depends on compounds from the polyphenolic fraction, mainly flavonoids. Flavonoids, one of the main groups of phenolic compounds in ginger, are the key compounds for estimation of ginger quality. Therefore, main groups of flavonoids were estimated in the methanolic extract of ginger varieties. Flavonoids were determined by two independent colorimetric methods, one for deterimination of flavones and flavonols and the other for determination of flavanones. The group of flavones and flavonols was measured as quercetine and the group of flavanone and dihydroflavonol was measured as Naringenin. Comparison of the concentrations of two groups of flavonoids in ginger extracts from the two varieties showed that there were significant differences between the two varieties. The Indian variety demonstrated higher values than Chinese variety for both groups. However, results of this study showed that the concentrations of flavones and flavonols were lower than concentrations of flavanone and dihydroflavonol in both varieties extract. Previous studies have shown that some flavonoids components such as quercetin had anticancer activities and were able to inhibit cancer cell growth (Elattar and Virji, 2000). Also, flavonoids were 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 (Sohi *et al*., 2003). It has been postulated that galic acid 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 of its pharmacological effects could be attributed to the presence of these valuable constituents.

3.2. DPPH scavenging activity

DPPH assay is one of methods used to examine the efficiency of the antioxidants. DPPH is a stable free radical with violet color. When DPPH is mixed with a phenolic compound that can donate a hydrogen atom, it will transfer into a reduced form with loss of color. The radical form of DPPH is purple and has maximum absorbance at 517 nm. When it reacts with an antioxidant, a reduced DPPH is formed, and the absorbance intensity decreases. The stronger the antioxidant is, the more the intensity decrease will occur. Based on the decrease of the absorbance intensity, we can judge the potential of the antioxidant. It was observed that methanolic extracts of the Indian variety rhizome had higher significant activity than that of the Chinese variety (Table 2). This study showed that ginger methanolic extracts have good free radical scavenging ability and can be used as a radical inhibitor or scavenger, acting possibly as a primary antioxidant. Also, in this study the antioxidant activity is found to be linearly proporational with phenolic contents for both ginger varieties. A close to linear correlation between DPPH radical-scavenging activity and concentrations of polyphenolic compounds in various vegetable and fruits has been reported (Pyo *et al*., 2004). Oktay *et al*. (2003) reported a strong positive relationship between total phenolic contents and antioxidant activity, which appears to be the trend in many plant species. Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds. Also, based on the results obtained, it is possible that several phenolic compounds of different polarities may contribute to the antioxidative properties of ginger rhizome extracts. Kikuzaki and Nakatani (1993) reported that methanolic extracts may include phenolic and hydrox-phenolic compounds with acid, alcohol, sugar or glycoside. Part of the antioxidative activity may be due to these components or flavonoids. In addition, antioxidative activities observed in ginger varieties could be the synergistic effect of more than two compounds that may be present in the plant. Finally, this antioxidant activity is may be due to the phenolic compounds redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

Table 2. DPPH scavenging activities of the methanolic extracts of two varieties of *Zingiber officinale.*

Variety	**Inhibition %***
Chinese	$25.25^{a} \pm 0.01$
Indian	$31.81^{b} \pm 0.01$

* Results expressed in percent of free radical inhibition.

All analyses were the mean of triplicate measurements ± standard deviation; Values with the same superscript letter within each column are not significant different ($p>0.05$).

3.3. Antibacterial activity

The *in vitro* antimicrobial activity of the two varieties extracts against the tested bacteria was assessed. The antimicrobial activities for *Zingiber officinale* rhizomes extract of both varieties are shown in Table 3. Both ginger extracts exhibited different degrees of antibacterial activity. Gram-positive bacteria; *Basillus subtilis* and *Micrococus* sp. showed sensitivity to methanol extract. *Basillus subtilis* indicated growth inhibition percentage ranged from 39.71 to 41.71 and *Micrococus* sp. growth inhibition percentage ranged between 9.8 to 29.6. While, for Gram negative bacteria; *Escherichia coli* showed sensitivity to ginger extracts with inhibition

percentage ranged from 3.17 to 16.46. Also, a close to linear correlation between antibacterial activity and concentrations of polyphenolic compounds in different varieties extracts. The results indicated that both extracts were more effective against the Gram-positive bacteria than to the Gram-negative ones. The higher resistance of the Gram-negative bacteria could be due to the complexity of the cell wall of this group of microorganisms. Indeed, the external membrane of Gram-negative bacteria renders highly hydrophilic surfaces whereas the negative charge of the surface of the Gram-positive wall may reduce their resistance to antibacterial compounds (Elaissi *et al*., 2011). Since the main mechanism by which will produce its antimicrobial action through the disruption of bacteria membrane Integrity (Deba *et al*., 2008), then the antimicrobial potency of ginger mainly caused by the presence of oxygenated mono- and sesquiterpenes, phenolic compounds (shogaol, gingerol) (Hossain *et al*., 2011), which are lipid-soluble phenol compounds primarily isolated from the root of ginger (Liu, 2011). Bajpai *et al*. (2009) and his colleague also support our explanation that as a result of the presence of mono- and sesquiterpenoids within plant extract, which consider main cause for their antimicrobial mode of action. Since these compounds have different ways of effect since these compounds not only attack cell walls and cell membranes i.e., affecting their permeability and release of intracellular constituents (e.g. ribose, Na glutamate) but they also interfere with membrane functions (electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity). Thus, these compounds might have several invasive targets which could lead to the inhibition of bacterial pathogens. Since, both varieties of ginger extracts have exhibited moderate to significant antimicrobial properties, hence, they can be used in the treatment of many bacterial diseases as well as a naturally food additives and preservatives which considered in new applications of food technology. However, Indian variety ginger extract showed higher antibacterial (growth inhibition) than Chinese ginger extract with gram positive and negative bacteria. These findings are in agreement with findings by Singh *et al*. (2008) and Bellik (2014).

Table 3 Inhibition growth % of ginger against the selected bacterial species

Variety	*E. coli*	*Basillus subtilis*	*Micrococus* sp.
Chinese	$3.17^{a} \pm 0.01$	$39.71^{a} \pm 0.01$	$9.80^{a} \pm 0.01$
Indian	$16.46^{b} \pm 0.01$	$41.71^{b} \pm 0.01$	$29.60^{b} \pm 0.01$

All analyses were the mean of triplicate measurements ± standard deviation; Values with the same superscript letter within each column are not significant different ($p>0.05$).

4. Conclusion

It can be concluded that ginger extracts contain considerable amounts of phenolic compounds which are responsible for the observed antimicrobial and antioxidant potency. Also, it can be suggested that ginger extracts can be used in the treatment of many bacteria and as well as a naturally food additives and preservative**s.**

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