Pak. J. Chem. 6(3-4): 38-41, 2016 Full Paper
ISSN (Print): 2220-2625
ISSN (Online): 2222-307X DOI: 10.15228/2016.v06.i03-4.p01

Assessment of Antioxidant and Antimicrobial Activities of Ginger

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ABSTRACT
Three solvent extracts of ginger were examined for their antioxidant and antimicrobial activities. The free radical scavenging antioxidant activity of ginger extracts was determined by DPPH (2, 2-Diphenyl-1-picrylhydrazyl) method. The antioxidant activity of methanolic extract of ginger was 80%, ethanolic extract 68% and with chloroform extract (67%). Antioxidant activity of ginger with methanolic extract was found higher than ethanolic and chloroform extracts. Phytochemicals *i.e.*, flavonoids, phenolic acid and tannins were determined from methanolic ethanolic and chloroform ginger extracts. Total phenolic content in methanolic extract was 70.5±0.02 mg/g, ethanolic extract 53±0.03 mg/g and from chloroform extract was 33±0.08 mg/g. Total phenolic content in methanolic extract was higher as compared to ethanolic and chloroform extracts. Antimicrobial activity with Methanolic ginger extract showed the highest antimicrobial effect against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* followed by ethanolic and chloroform extracts. Results indicated that ginger extract have remarkable benefits in medical field and can be used in the treatment of many bacterial and fungal diseases as well as a naturally food additives, preservatives and pharmaceutical industries.

Keywords: Ginger, antioxidant activity, Phytochemicals, antimicrobial activity.

1. INTRODUCTION
Spices are the most importance part for the human diet and have been used for thousands of years to improve the color, aroma and flavor of food. These are also known for their antioxidant, antimicrobial and other various therapeutic uses[1].

The antioxidant and antimicrobial properties of various extracts from medicinal plants are of great concern because of their potential use for the oxidation prevention, controlling toxin-producing microorganisms and various pathogens in the foods[2]. These antioxidants are polyphenol compounds, which are found in almost all parts of the plants[3]. Due to unstable nature and possible unfavorable side effects of synthetic antioxidants, the demand for natural antioxidant sources has been greatly increased[4,5]. Ginger has been found to inhibit lipid peroxidation and successfully scavenge superoxide anions[6]. It has been reported that the prominent sources of antibacterial activities of extracts are the total phenolic contents[7].

Ginger (*Zingiber officinale*) belongs to the family *Zingiberaceae*. The plant is extensively cultivated in India, Africa, China, Mexico, Hawaii and Jamaica for thousands of years as a spice and for medicinal purposes[8]. It has been found effective in the treatment of cataract, heart disease, migraines, struck amenorrhea, athletes foot, bursitis, chronic fatigue, cold, flu, coughs, depression, dizziness, fever, kidney stones. Powdered derived ginger roots are used to make capsules and sold in pharmacies for medicinal uses[9].

Ginger was found to have more than 70% antioxidant activity[10]. The obtained extracts of ginger roots are rich source of polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant and antimicrobial activity[11].

Objective of the present study was to examine the antioxidant and antimicrobial activities of three solvent extracts (extracted in ethanol, methanol, chloroform) of Ginger.

2. MATERIALS AND METHODS
2.1 Sample collection and preparation of extract
Ginger sample was collected from the local market. All samples were shadow dried, powdered and saved for further extraction. Three different solvent *i.e.* methanol, ethanol and chloroform were used for extraction. Ginger powder was weighed amount (90mg), dissolved in three different solvents separately and put on shaker for overnight. Solvent free extracts were then stored at room temperature for further analysis.

2.2 Determination of antioxidant activity
Antioxidant activity (Radical scavenging activity) of sample was determined by DPPH (2, 2-Diphenyl-1picrylhydrazyl) method[12]. 4.0 mg of ginger sample was dissolved in 1.0 ml of their respective solvents. Same procedure was repeated with all samples. Ascorbic acid was used as standard. 4.0 mg/g stock solution of ascorbic acid was prepared with different concentrations (50,100,150,200 and 250 µg/ml) of both standard and extract stock solution. 2.4 mg of DPPH was dissolved in

*Corresponding Author Received 8th December 2016, Accepted 20th December 2016

100 ml of methanol. Then add 2 µl of each extract and 2 ml DPPH, kept in darkness for 35 min. Absorbance was taken at 517 nm. Same procedure was repeated for standard ascorbic acid. This assay was performed in triplicate.

$$\text{Antioxidant activity in percentage} = Ac- As/Ac \times 100$$

Where Ac is absorbance of control and As is absorbance of sample.

2.3 Phytochemical analysis

2.3.1 Phenolic compounds determination

Phenolic in ginger samples was determined by using the FC (Folin-Ciocalteau) reagent[13]. 1.0 mg of extract and 4.0 mg of Gallic acid was dissolved in solvent to make the stock solution. Then different concentrations were prepared. 1.0 ml of FC reagent was diluted with 10.0 ml of water. Then 2 ml of sodium carbonate, 1.0 ml of FC reagent and 100 µl of extract was added in test tube and kept for 45 min in dark. Absorbance was taken at 765 nm. Results are expressed as mg/g.

2.3.2 Flavonoids determination

Photometric assay was used to determine the flavonoids in ginger given by Hanafi and Amrani[14] with small modification. 1.0 g of sample was dissolved in their respective solvents. 4.0 mg/ml solution was prepared by dissolving quercetin in 1ml of dimethyl sulfoxide. Then 1ml of 10% aluminum chloride was added in each test tube and kept for 20 min. Absorbance was taken at 430 nm. Different concentrations of quercetin (25µg-250µg) were also used to draw the standard curve. Assay was performed in triplicate.

2. 4 Screening for antimicrobial activity

2.4.1 Extract preparation

Ginger extracts were prepared in different solvents (chloroform, ethanol and methanol). 1.0 g of ginger sample was mixed with 1 ml of solvent. The same procedure was repeated with all the solvents.

2.4.2 Antibacterial assay

In vitro antibacterial activities of ginger extract were analyzed against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Agar well diffusion method[15] was used to determine this assay. Lauria-Bertini media (4.25 gm agar, 6.25 gm nutrient broth and distilled water) was used to prepare inoculums of all microbes. Autoclaved Lauria-Bertini media was poured into Petri plates under sterilized conditions. Standardized inoculums were introduced onto the surface of sterile agar plate, and evenly distributed in zigzag manner by using a sterile spreader. Each well was poured by extract of about 75 µl. At 37ºC, plates were incubated for overnight. Zone of inhibition was measured in mm.

2.4.3 Minimum Inhibitory Concentration (MIC)

Inhibitory effect of each ginger extract in mm was used to determine their minimum inhibitory concentration (MIC) according to Wilson *et al* (2005)[16]. An antibiotic penicillin was taken as standard.

2.5 Statistical Analysis

The data obtained were expressedas means ± standard deviation (SD) after triplicate analysis.

3. RESULTS AND DISCUSSIONS

3.1 Antioxidant activity determination

Oxidation of biological molecules induces different pathological diseases such as atherosclerosis or cancer. These damages are caused by due to the presence of free radicals. For that reason, the concepts of pharmacological supplements to defend against free radicals with antioxidants[17]. It is reported that [6]-gingerol, [6]-shogaol have displayed strong antioxidant activity in vitro. It is also known that the antioxidant activity of plant extracts containing polyphenol components are due to their capacity to be donors of hydrogen atoms or electrons to capture the free radicals[18]. DPPH analysis is one of the tests used to prove the ability of the components of the ginger extract to act as donors of hydrogen atoms[19].

Ginger possesses several therapeutic assets like anti-inflammatory, anticancer, antibacterial and antioxidant effects[20]. Observed value in antioxidant activity of methanolic extract was in the range of 23 to 80%, ethanolic extract was 20 to 68% and chloroform extract was in the range of 18 to 67% in the concentrations of 25 to 250 µg/ml. Methanolic extract of ginger showed highest antioxidant potential as compared to ethanolic and chloroform extracts as shown in Table. 1.

Table.1: DPPH Free Radical Scavenging Activity of different Ginger Extracts

Extract conc. µg/ml	Methanolic extract (%)	Ethanolic extract (%)	Chloroform extract (%)
25	23	20	18
50	38	26	29
100	47	37	36
150	58	48	45
200	69	59	55
250	80	68	67

3.2 Phytochemical Analysis of Ginger Extracts

Phytochemicals from ginger extracts (methanolic, ethanolic and chloroform) were analyzed for total phenolic, total flavonoids and tannins as shown in Table 2. Total phenolic content in methanolic extract was 70.5±0.02 mg/g, ethanolic extract 53±0.03 mg/g and with chloroform extract was 33±0.08 mg/g. When the polarity of solvent decreases, the concentration of total phenolic is also decreased. More polar is the solvent, more it is efficient to grab the bioactive components.

Results indicated that ginger could serve as an excellent source of phenolic, tannins and flavonoids. These bioactive secondary plant metabolites are not only useful in food industry but also in pharmaceutical industries.

Table: 2. Phytochemical Analysis of Ginger Extracts.

S.no	Phytochemicals	Methanolic extract (mg/g)	Ethanolic extract (mg/g)	Chlorofor m extract (mg/g)
1	**Phenolic**	70.5±0.02	53±0.03	33±0.08
2	**Flavonoids**	7.97±0.01	4.48±0.02	1.93±0.01
3	**Tannins**	124±0.01	106±0.02	74±0.02

3.3 Antimicrobial activity measurement

Ginger extracts exhibited different degrees of antibacterial activity. The strongest antibacterial effect on the methanol extract was observed against *Escherichia coli* followed by *Pseudomonas aeruginosa and Staphylococcus aureus*. Conversely, ethanol exerted the most effect on *Escherichia coli* followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while the antibacterial effect on the chloroform against *Escherichia coli* was strongest then followed by *Pseudomonas aeruginosa and Staphylococcus aureus.* The minimum inhibitory concentrations (MIC) in millimeter of all the extracts were in the range of Gentamycin values.

Table 3: Antibacterial Activity of Different Extracts of Ginger

Extracts	*E. coli* (MIC = mm)*	*S. aureus* (MIC = mm)*	*P. aeruginosa* (MIC = mm)*
Chloroform	15	10	14
Ethanol	17	18	19
Methanol	21	18	23
Gentamycin	17	16	22

***** minimum inhibitory concentrations in millimeter

4. CONCLUSION

Methanolic extract of ginger showed highest antioxidant potential as compared to ethanolic and chloroform extracts. Results indicated that ginger could serve as an excellent source of phenolic, tannins and flavonoids. These bioactive secondary plant metabolites are not only useful in food industry but also in pharmaceutical industries. Apart from this, the presence of phenolic signifies that it could be an important source for antimicrobial substance which might help against multi-drug resistant bacteria. It can be concluded that ginger extracts, mostly methanolic extract could be a promising alternative antioxidant activity. Since, they have exhibited moderate to significant antimicrobial properties, hence, they can be used in the treatment of many bacterial and fungal diseases as well as naturally food additives and preservatives.

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