International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **3(11),** 54-58, November **(2014)** *Int. Res. J. Biological Sci.*

Evaluation of Phytochemical Constituents in Various Heat Treatments of *Zingiber officinale* Roscoe and *in vitro* Antioxidant Assay Systems

Rubila. S and T.V.Ranganathan
Department of Food Processing and Engineering, School of Biotechnology and Health Sciences, Karunya University, TN, INDIA

Available online at: www.isca.in, www.isca.me
Received 5th August 2014, revised 6th October 2014, accepted 9th November 2014

Abstract

The present work investigate the various heat treatment of ginger, like cutting (27ºC), Drying (60ºC), Roasting (70ºC) and Sauting (80ºC) on the phytochemical availability. The results showed that flavonoids, steroids, tannin and phenols were found in all temperatures. But at temperatures 70ºC and 80ºC, the alkaloids, free amino acid, glycosides, saponin, and phytosterols were destroyed, because most of the aromatic components were affected by heat. The Antioxidant activity the ginger was measured using (DPPH) assay, Phosphomolybdenum assay and metal chelating assay. High radical scavenging activity and metal chelating activity was observed at IC_{50} 64.4µg/ml and 67.64 µg/ml respectively.

Keywords: *Zingiber officinale*, Phytochemicals, Soxhlet and Antioxidant activity.

Introduction

Zingiber officinale Roscoe (Ginger) belongs to the family Zingiberacea. It is a perennial slender, and the maximum height is 3 feet from underground rhizomes. Ginger is also an important medicinal plant in Ayurveda and Unani system of medication because of its therapeutic properties[1]. It is cultivated in equatorial and semitropical countries like Australia, Africa, India, Jamaica, Mexico and china. *Zingiber officinale* has grass like leaves and also white or yellowish green color flower[2]. It contains starch, mucilage 2% of essential oil and 8% tarry matter. The essential oil consists of mixture of monoterpenes and sesquiterpenes[3]. Now a day's most of the researches focus on medicinal plants because of high pharmacological activity and low toxicity[4].

It is mostly used in folklore medicine. The most active component of oleoresin is gingerol and shogaols[5]. The rhizome has to undergo a series of pretreatment during sample preparation to ensure a high yield and quality of the oleoresin and essential oil[6].

The secondary metabolites of ginger have been studied by many authors[7-11]. The present work describes evaluation of phytochemicals constituents of ginger during various heat treatments.

Material and Methods

Plant materials: The fresh rhizomes (*zingiber officinale)* were procured from local market. Cutting: Ginger was cut into small pieces and kept at 27ºC for 5 minutes. After 5 minutes the sample was transferred into high purity cellulose thimble for extraction. Drying: Rhizome was dried at 60ºC for 6 hours. After drying the ginger was ground by blender mixer. The powdered sample was (0.104mm size particle) used for extraction. Roasting: The Sample was roasted at 70°C. After roasting the sample was transferred into high purity cellulose thimble for extraction. Sauting: Ginger was sauted with 1ml edible oil at 80ºC. After sauting, the sample was kept in a high purity cellulose thimble for extraction.

Phytochemical analysis: 1 gm of sample was dissolved in 100ml of carbinol to get a stock standard of 1% (W/V). Phytochemical analysis was carried out by the described elsewhere[12,13].

Test for Alkaloids: During this test 5 ml of the extract was mixed with 2 ml of Hydrochloric acid. Then, 1ml of Dragendroff's reagent was added in the prepared solution. An orange or red precipitate produced indicates the presence of alkaloids.

Test for Free Amino acids: In this test, 1 ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple color shows the presence of free amino acids.

Test for Anthraquinones: 5 ml of the extract solution was hydrolyzed with sulphuric acid extracted with benzene. 1ml of dilute Ammonia was added to it. Formation of Pink color denotes the presence of anthraquinones.

Test for Flavonoids: 1 ml of the extract, few drops of dilute sodium hydroxide was added. An intense yellow color was produced in the plant extract, which become colorless on addition of a few drops of dilute acid indicates the presence of flavonoids.

Test for Glycosides: To 2ml of extract, glacial acetic acid, 1 drop 5% Ferric chloride and concentrated sulphuric acid was

added. Reddish Brown color appeared at junction of 2 liquid layers and upper layer turned bluish green indicating the presence of glycosides.

Test for Phytosterol: The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drops of diluted acetic acid; 3ml of acetic anhydride was added followed by few drops of concentrated sulphuric acid. Appearance of bluish green color showed the presence of phytosterol.

Test for Saponin: The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of Saponin.

Test for Steroids: 1ml of the extracts was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and Sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

Test for Tannins: 5ml of the extract and a few drops of 1% lead acetate solutions were added. A yellow precipitate was formed, indicates the presence of tannins.

Test for Triterpenoids: 10mg of the extract was dissolving with 1ml of Chloroform, 1 ml of acetic anhydride and 2 ml of concentrated sulphuricacid was added. Formation of reddish violet color indicates the presence of triterpenoids.

***In vitro* Antioxidant Assay, Phosphomolybdenum assay:** Antioxidant activity was evaluated by according to the method[14]. 0.1 ml of sample or ascorbic acid in 1 mM dimethyl sulphoxide (standard) or distilled water (blank) was added with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a test tube. The test tubes were covered with foil and incubated in a water bath at 95°C for 90 minutes. After the samples had cooled at ambient temperature, the absorbance of the mixture was measured at 695 nm against the blank.

DPPH Assay: Antioxidant activity was measured by according to the method[15]. Different concentrations of sample were taken and the volume was adjusted with 0.1ml methanol. About 5 ml of a 0.1 mM methanol solution of DPPH was added to the aliquots of samples and standards (Butylated Hydroxytoluene) and shaken vigorously. Negative control was prepared by adding 100µl of methanol in 5 ml of 0.1 mM methanol solution DPPH. The tubes were allowed to stand for 20 minutes at 27°C. The absorbance of the sample was measured at 517 nm against the blank (methanol). Radical scavenging activity of the samples was expressed as IC50 which is the concentration of the sample required to inhibit 50% of DPPH concentration.

Metal chelating activity: The chelating activity was determined by the method[16]. 0.1ml of samples and Butylated Hydroxytoluene were added to 50µL solution of 2 mM Ferric chloride. The reaction was stimulated by the addition of 0.2ml of 5 mM ferrozine and the mixture was shaken vigorously and left standing at ambient temperature for 10 minutes. Absorbance of the solution was measured at 562 nm against the blank. Metal chelating capacity (%) = [(A0-A1) / A0] X 100, where, A0 is the absorbance of the control, and A1 is the absorbance of the sample / standard.

Results and Discussion

The photochemical screening is mandatory for drug synthesis and formulation, latter phytochemical test is essential for qualitative and quantitative determination of pharmacologically bioactive compounds[17]. Oleoresin is extracted from ginger by soxhlet method. The viscous nature of the sample is aromatic and black in color.

Table - 1
Characteristics of Defatted Methanolic extract

S.No	Characteristics	Methanolic Extract
1.	Physical Appearance	Viscous
2.	Color	Black
3.	Odor	Aromatic
4.	Taste	Bitter

The rhizomes had the maximum amount of active components. The product of active component is also varied in the different method of solvent extraction[18,19].

Here the following phytochemicals like Flavonoids, tannins, phenols, steroids present in 27 ºC, 60ºC, 70ºC and 80ºC temperatures. It has increase the quality of the medicinal plants. But free amino acid, anthraquinones, and phytosterols were absent in four temperatures. Because most of the aromatic components affected by heat[20]. Commonly alkaloids have antimicrobial properties[21]. Flavonoids and tannins are phenol compounds acts as primary antioxidant and free radical scavenging activity. Flavonoids have the anti-tumoral, anti allergic and anti inflammatory activities[22-24]. Tannin has anti-ischemic effect and endothelium dependent vasorelaxant activity[25]. Steroids and triterpenoid had the analgesic properties[26, 27].

Phosphomolybdenum Assay: Molybdenum (V1) is converted into Molybdenum (V) by antioxidant compound, followed by the formation green phosphate (MoV) complex, maximum absorbed at 695nm. The methanol extracts ginger showed highest antioxidant activity. Here positive and negative charged molecule move from antioxidant to molybdenum (VI) complex[28].

Table- 2
Analysis of Phytochemicals by carbinol extract of *Zingiber officinale*

S.No	Phytochemicals Test	Cutting 27°c	Drying 60 °c	Roasting 70 °c	Sauting 80 °c
1.	Test for Alkaloids	+	+	-	-
2.	Test for Free Amino acids	-	-	-	-
3.	Test for Flavonoids [with NaOH Test]	+	+	+	+
4.	Test for Steroids[Salkowski Test]	+	+	+	+
5.	Test for Tannins [Lead Acetate Test]	+	+	+	+
6.	Test for Saponin [Foam Test]	-	+	-	-
7.	Test for Triterpenoids	+	-	+	-
8.	Test for Glycosides	-	-	-	
9.	Test for Anthrquinones	-	-	+	+
10.	Test for Phytosterol	-	-	-	-
11.	Test for Phenol	+	+	+	+

+ = Presence; - =Absence

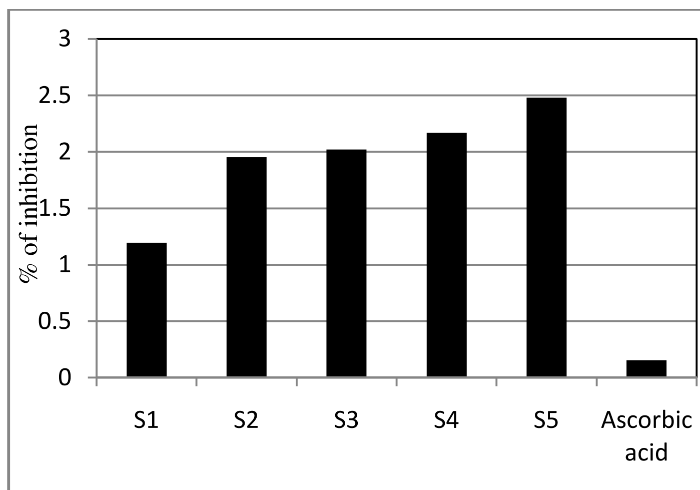

Figure-1
Phosphomolybdunem Assay

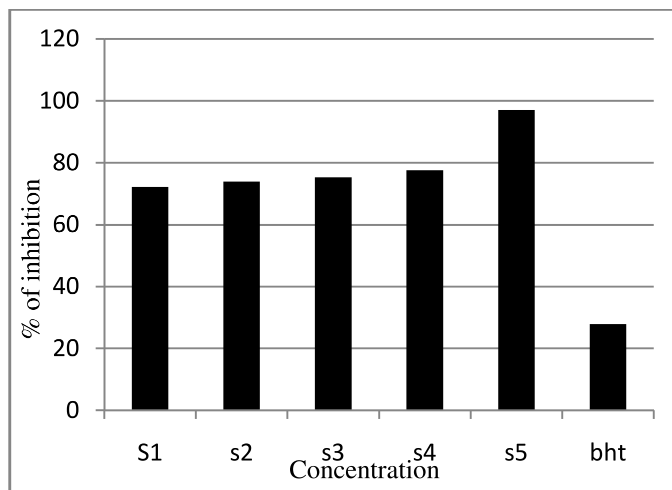

Figure-2
Dpph radical scavenging activity

DPPH Assay: DPPH radical scavenging activities are mainly used for assessing antioxidant activity in plant materials. The result of DPPH assay was expressed in IC_{50} values or % of inhibition. Different concentration of sample initially decreases the IC $_{5o}$ value. So that the less value of IC_{50} indicates a high amount of antioxidant activity.

All concentrations of ginger showed excellent DPPH radical scavenging activity. High radical scavenging activity was observed at (IC_{50} 64.4µg/ml) which is compared with Butylated hydroxy toluene.

Antiradical activity is directly linked with phenol compound to contribute the transformation of electron. Mostly this reaction occurs in the plant samples [29, 30]. Results showed that extract contain high inhibitng compounds, as well as a primary antioxidants.

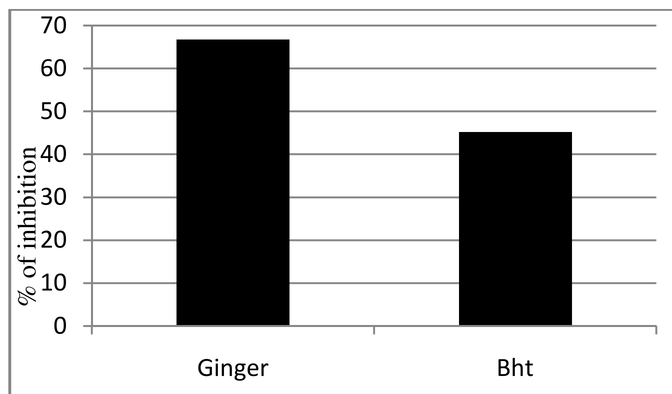

Figure-3
Metal Chelating Activity

Iron is important for transport of oxygen, respiration and enzyme activities. The mechanism of oxidation such as Fenton reaction, where the transition of metal ions plays an important role. Various ROS may be generated and different target structures such as lipids, proteins and carbohydrates can be affected. So it's very important to study the antioxidant activity.

The methanol extracts of ginger showed higher activity (67.64%). It prevents the formation of insoluble metal complex. So that antioxidant directly related to iron binding capacity.

Conclusion

This results revealed that most of the biologically active phytochemical were found in the 27ºC (Cutting) and 60ºC (Drying) only. But 70ºC (Roasting) and 80ºC (Sauting) almost all the alkaloids, free amino acid, glycosides, saponin, and phytosterols were destroyed because most of the aromatic components affected by heat. The methanolic extracts of ginger showed high metal chelating activity (67.64%), ginger had the excellent antioxidant activity.

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