Indian J. Plant Physiol., Vol. 14, No. 1, (N.S.) pp. 23-27 (Jan.-March, 2009)

IN VITRO DETECTION OF ANTIOXIDANTS IN DIFFERENT SOLVENT FRACTIONS OF GINGER (*ZINGIBER OFFICINALE* ROSC.)

MALAY BHATTACHARYA, PALASH MANDAL AND ARNAB SEN*

Department of Botany, North Bengal University, Siliguri-734 013, West Bengal, India

Received on 8 July, 2008, Revised on 5 March, 2009

SUMMARY

Free radicals are constantly generated and quenched by an efficient antioxidant network in the body. Many plant extracts and phytochemicals have antioxidant/free radical scavenging properties. Ginger is valued as medicine and antioxidant. Investigations were done to find out the free radical, hydroxyl radical and nitric oxide scavenging activity along with lipid peroxidation capability of different ginger fractions. Out of the 34 fractions studied, 10 fractions showed free radical scavenging activity ranging from 5.88% to 80%. Five different peaks were obtained. Diethyl ether and ethyl acetate (1:1) showed the maximum inhibition percent (80%) of antiradical activity. The chloroform fraction showed maximum hydroxyl radical scavenging activity followed by hexane fraction and diethyl ether and ethyl acetate (1:3) fraction. The maximum NO generation activity was observed in the benzene fraction (27.27%). The liver protective function was maximum (38.46% at 3000 mg/ml) in diethyl ether & ethyl acetate (1:1) fraction. The bioactive fraction of diethyl ether & ethyl acetate (1:1) showed the reactivity against natural product-polyethylene glycol reagent (NP/PEG) from which it may be concluded that zinger flavonoids have some contributory roles in scavenging free radical activity.

Key words: **Antioxidant, column chromatography, ginger, solvent fraction, soxhalation.**

INTRODUCTION

Oxidation is a redox chemical reaction that transfers electrons from a substance to an oxidizing agent and can involve the production of free radicals, which can form dangerous chain reactions. Antioxidants are molecules that slow or prevent the oxidation of other chemicals and can terminate these chain reactions by removing radical intermediates and can inhibit other oxidation reactions by themselves being oxidized. Free radicals of different forms are constantly generated for specific metabolic requirements and quenched by an efficient antioxidant network in the body (Bagul *et al.* 2005). When the network is in balance, organisms enjoy good health, but when it fails, the balance is disturbed leading to disharmony and disease. Free radicals have been implicated as the cause of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes etc and compounds that can scavenge free radicals have great potential in ameliorating these disease processes (Wilson 1988). Increased oxygen free radical activity can initiate peroxidation of lipids, which in turn stimulates glycosylation of protein, inactivation of enzymes and alteration in the structure and function of collagen, basement and other membranes. Many plant extracts and phytochemicals have been shown to have antioxidant/free radical scavenging properties (Larson 1988, Tripathi *et al.* 1996). Although food industries have used effective synthetic antioxidants, consumers of food, however, prefer natural antioxidants to synthetic antioxidants on the basis of the assumption that natural compounds are safe.

*Corresponding author, E-mail: senarnab_nbu@hotmail.com

Ginger, one of the oldest and most important spices, has been cultivated in tropical Asia for over 3000 years and is a rhizomatous spice used all over the world in alternative systems of medicine (Purseglove 1975). The whole part of the plant is aromatic, but it is the underground rhizome, fresh or preserved, that are the valuable commodities (Bhattacharya and Sen 2006). The rhizomes of this herbaceous plant are used as spice, in herbal medicine and as raw material in food, beverage and pharmaceutical industries. The plant is rich in secondary metabolites like oleoresin, essential oil and different polyphenols (Baghyalaxmi and Singh 1988). Ginger is valued as medicine as a carminative and stimulant of gastro-intestinal tract (Kumar *et al.* 1997).

Though detection of antioxidant in the crude extracts have been performed by several workers (Ahmed *et al.* 2000, Kikuzaki and Nakatani 1993), but adequate information on the antioxidant properties of different fractions in polar and non polar solvents are still lacking. Therefore, the present investigations were done to find out the free radical, hydroxyl radical and nitric oxide scavenging activity along with antilipid peroxidative activity of different ginger fractions.

MATERIALS AND METHODS

Rhizomes of *Zingiber officinale* Rosc cv. Gorubathan were collected from the fields of Gorubathan (West Bengal) just after harvest and were maintained in green house condition. Thirty grams of rhizome was crushed in a mechanical grinder. The crushed rhizome was subjected to soxhalation and exhaustively extracted with methanol for 48 hours. The solvent was evaporated at low temperature, to a final volume of 3 ml. The extract was subjected to silica gel 60[Merck, 200-400 mesh] column chromatography. A series of non polar to polar solvents like hexane, benzene, chloroform, diethyl ether, ethyl acetate, acetone, ethanol, methanol and water in 25, 50, 75 and 100% were passed through the silica column to obtain the various fractions. The fractions were vacuum evaporated at low temperature, dried and dissolved in 10 ml methanol. The methanol insoluble hexane fraction was dissolved in hexane.

Free radical scavenging activity: Free radical scavenging activities of different fractions were tested against DPPH, hydroxyl radical and nitric oxide. Effect on lipid peroxidation was evaluated in goat liver homogenate. The reaction mixtures of different assays are given below.

Assay of DPPH scavenging: Antiradical activity was measured by a decrease in absorbance at 517 nm of methanolic solution of coloured DPPH brought about by the sample (Vanni *et al.* 1997, Ravishankara *et al.* 2002). A stock solution of DPPH (100µM) in methanol was prepared. 100 µl of the fraction (300 mg/l) were added to 2900 µl of DPPH solution. Decreases in the absorbance in presence of the different fractions were noted after 30 min. The fraction showing maximum antiradical responses were diluted to different concentrations and their antiradical activities were observed. Percentages of DPPH scavenging activity were calculated as {1-(optical density of sample/optical density of control) x 100}. IC_{50} was calculated from graphical presentation of concentration verses radical scavenging activity.

Assay of hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the test compounds for hydroxyl radical generated from the Fe^{+3}/ascorbate/EDTA/H_2O_2 system. The hydroxyl radical attacks deoxyribose, which results in thiobarbituric acid reacting substance (TBARS) formation[13]. The reaction mixture contained deoxyribose (2.8 mM), $FeCl_3$ (0.1mM), EDTA (0.1mM), H_2O_2(1mM), ascorbic acid (0.1mM), KH_2PO_4 – KOH buffer (20 mM) and various concentrations of extract in a final volume of 1 ml. The reaction mixtures were incubated for 1 hour at 37°C. Deoxyribose degradation was measured as TBARS at 532 nm and percentage inhibition was calculated.

Assay of nitric oxide scavenging activity: Sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations of samples dissolved in methanol and incubated at room temperature for 150

minutes. Some reaction mixture without the sample but equivalent amount of solvent served as control. After incubation, 0.5 ml Griess reagent (1% sulphanilamide, 2% H_3PO_4 ad 0.1% naphthylene diamine dihydrochloride) was added. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylene diamine was read at 546 nm.

Determination of lipid peroxidation activity: Reaction mixture containing goat liver homogenate (0.1ml, 25% w/v) in Tris HCl buffer (40 mM, pH -7), KCl (30mM), ferrous iron (0.16 mM) and ascorbic acid (0.06 mM) were incubated for 1 hour at 37°C in presence and absence of extracts. The lipid peroxide formed was measured by TBARS formation. Incubation mixtures (0.4 ml) were treated with sodium dodecyl sulphate (SDS-8.1%, 0.2 ml), thiobarbituric acid (TBA-0.1%, 1.5 ml) and acetic acid (20%, 1.5 ml, pH -3.5). The total volume was made up to 4 ml with distilled water and kept in water bath at 100°C for 1 hour. After cooling, 1 ml of distilled water and 5 ml of mixture of n-butanol and pyridine (15:1 v/v) were added and shaken vigorously. After centrifugation, the absorbance of the organic layer was measured at 532 nm. The percentage inhibition of lipid peroxidation was determined by comparing the result of the test compounds with those of controls not treated with the extracts.

Detection of compounds: The bioactive fractions were analyzed on Silica Gel 60 F_{254} –precoated TLC plates (0.25 mm thickness). 1 mg. of component was dissolved in 1 ml. methanol; 10 µl was used for TLC. Compounds were separated through hexane-diethyl ether (40:60) solvent and detected under UV light (254 nm) and ammonia vapour. For spraying, Bartons reagent, Berlin blue reagent, Iodine reagent, Natural Product Polyethylene Glycol reagent (NP/PEG) and Phenol reagents were used in the experiment (Wagner and Bladt 1996).

RESULTS AND DISCUSSION

Free radicals are implicated in many disease conditions. Plant extracts containing free radical scavengers are well known for their therapeutic activities. Out of the 34 fractions obtained by silica gel chromatography of the methanolic extract, 33 were dissolved in methanol. The hexane fraction had both methanol soluble and methanol insoluble parts. The methanol insoluble part was dissolved in hexane. The solvent fractions exhibited different levels of free radical scavenging activities. Out of the 34 fractions studied, 10 fractions [Hexane (dissolved in hexane), Benzene, Chloroform, Chloroform : diethyl ether (1:1), Chloroform : diethyl ether (1:3), Diethyl ether, Diethyl ether : ethyl acetate (3:1), Diethyl ether : ethyl acetate (1:1), Diethyl ether : ethyl acetate (1:3), Ethyl acetate and Ethyl acetate : acetone (1:1)] showed DPPH scavenging activity ranging from 5.88% to 80%. Five different peaks were obtained when the inhibition percent of different fractions were plotted (Fig 1). Diethyl ether and ethyl acetate (1:1) showed the maximum inhibition percent (80%) of antiradical scavenging activity. This fraction

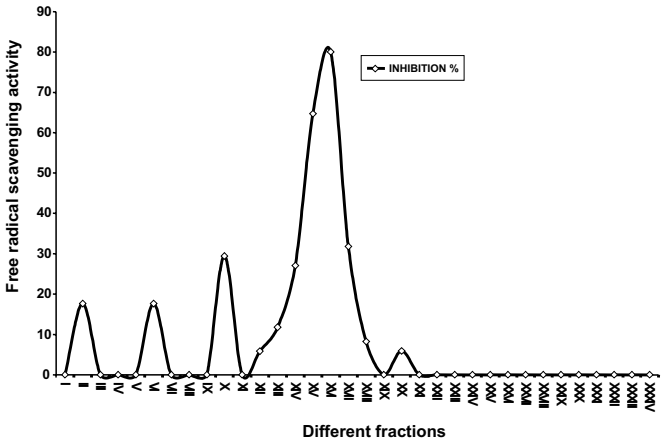

Fig. 1. Free radical scavenging activities (%) of different solvent fractions of ginger: I-Hexane, II-Hexane*, III-Hexane: benzene (3:1), IV-Hexane : benzene (1:1), V-Hexane : benzene (1:3), VI-Benzene, VII-Benzene : chloroform (3:1), VIII-Benzene : chloroform (1:1), IX-Benzene : chloroform (1:3), X-Chloroform, XI-Chloroform : diethyl ether (3:1), XII-Chloroform : diethyl ether (1:1), XIII-Chloroform : diethyl ether (1:3), XIV-Diethyl ether, XV-Diethyl ether : ethyl acetate (3:1), XVI-Diethyl ether : ethyl acetate (1:1), XVII-Diethyl ether : ethyl acetate (1:3), XVIII-Ethyl acetate, XIX-Ethyl acetate : acetone (3:1), XX-Ethyl acetate : acetone (1:1), XXI-Ethyl acetate : acetone (1:3), XXII-Acetone, XXIII-Acetone : ethanol (3:1), XXIV-Acetone : ethanol (1:1), XXV-Acetone : ethanol (1:3), XXVI-Ethanol, XXVII-Ethanol : methanol (3:1), XXVIII-Ethanol : methanol (1:1), XXIX-Ethanol : methanol (1:3), XXX-Methanol, XXXI-Methanol : water (3:1), XXXII-Methanol : water (1:1), XXXIII-Methanol water (1:3) and XXXIV-Water.
***Dissolved in hexane.**

was diluted to different concentrations (0.5 mg/ml – 10 mg/ml) and was subjected to DPPH assay for antiradical activity. The bioactive fraction showed a concentration dependent DPPH radical scavenging activity with IC_{50} of 8 mg/ml fresh weight basis.

Degradation of deoxy ribose mediated by hydroxyl radicals generated by the Fe^{3+}/ ascorbate/EDTA/H_2O_2 system was also inhibited by the six bioactive fractions concentrations dependent hydroxyl radical scavenging activity was observed (Fig 2). The chloroform fractions showed maximum hydroxyl radical scavenging activity followed by hexane fraction insoluble in methanol and diethyl ether & ethyl acetate:(1:3) fraction. The scavenging activities of all the bioactive fractions were more at higher concentrations and gradually decreased at lower concentrations. The inhibition percent recorded in the bioactive chloroform fraction was 43.75 at a concentration of 3000 and 300 mg/ml.

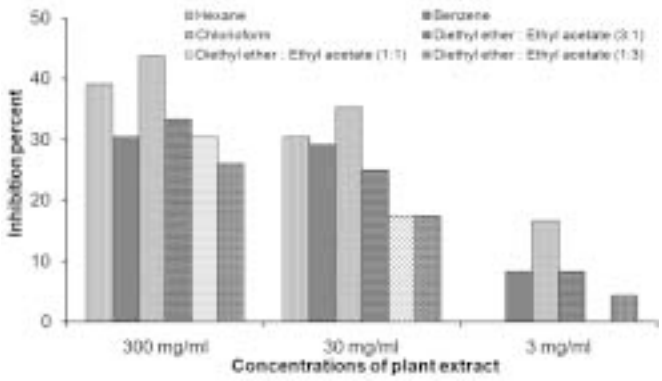

Fig. 2. Hydroxyl radical scavenging activities of the bioactive solvent fractions of ginger

Results indicate that ginger has nitric oxide scavenging activity (Fig.3). The hexane fraction was most active in NO scavenging activity. Maximum scavenging activity observed in the bioactive hexane fraction was 47.72% at a concentration of 3 mg/ ml. The scavenging activity increased with decrease in concentrations. The chloroform and diethyl ether and ethyl acetate fractions showed lower level of NO scavenging activities than hexane and benzene fractions.

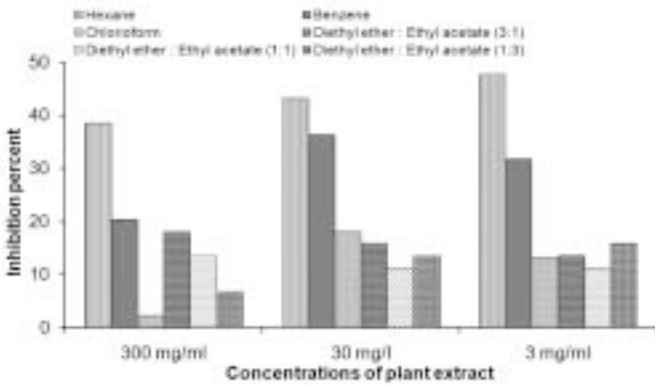

Fig. 3. Nitric oxide generating and scavenging activities of the bioactive solvent fractions of ginger

The different ginger fractions protected hepatocytes from damage due to lipid peroxidation induced in goat liver homogenate by ferric-ADP and ascorbate in a dose dependent manner (Fig.4). The maximum protective function was recorded in chloroform fraction at 300 mg/ ml (30.76%). Diethyl ether: ethyl acetate (1:3) showed similar hepatocyte protective activity in all the concentrations. The anti lipid peroxidative activity increases with increase of concentration of ginger.

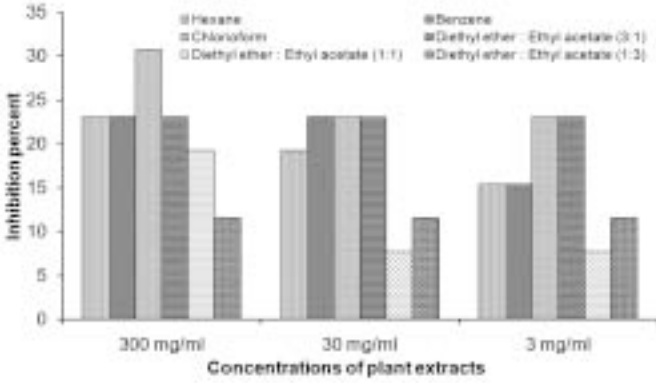

Fig. 4. Lipid peroxidation inhibition activities of the bioactive solvent fractions of ginger

When the distribution of polyphenols was analyzed in different fractions, it was observed that phenolic compounds were distributed from chloroform to aqueous fraction whereas gingeroles and arbutin related compounds were mainly restricted in diethyl ether: ethyl

acetate fractions. The bioactive fraction, diethyl ether & ethyl acetate (1:1) showed the sensitivity against NP/ PEG from which it may be concluded that zinger flavonoids have some contributory roles in scavenging antiradical activity. Polyphenols and flavonoids have already been reported to act as potent antioxidants in *Alpinia nutans* (Habsah *et al*. 2003), *Galium fissurense* (Delioram and Novel 2003) and many other medicinal plants.

In conclusion, ensemble of results indicates that more than one compounds are responsible for the antioxidant activity of zinger. It may happen that specific group of compounds are dedicated for scavenging specific type of free radicals. Understanding the dynamics of those antioxidant molecules in mammalian organs and their interaction kinetics with free radicals is a real challenge for formulating the nutraceuticals and safeguarding human health. Work is still going on with these isolated antioxidant molecules for developing appropriate pharmacological applications of medicinal components of zinger.

REFERENCES

Ahmed, R.S., Seth, V. and Banerjee, B.D. (2000). Influence of dietary ginger (*Zingiber officinales* Rosc) on antioxidant defense system in rat: comparison with ascorbic acid. *Indian J. Exp. Biol.* **38:** 604-606.

Baghyalaxmi and Singh, N.S. (1988). Meristem culture and micropropagation of a variety of ginger (*Zingiber officinale* Rosc.) with high yield of oleoresin. *J. Hort. Sci.* **63:** 321-327.

Bagul, M.S., Kanaki, S.N. and Rajani, M. (2005). Evaluation of free radical scavenging properties of two classical polyhedral formulations. *Indian J. Exp. Biol.* **43:** 732-736

Bhattacharya, M. and Sen, A. (2006). Rapid *in vitro* multiplication of disease-free *Zingiber officinale* Rosc. *Indian J. Plant Physiol.* **11:** 379-384.

Delioram, O.D. (2003). Novel flavanone glucoside with free radical scavenging properties from *Galium fissurense*. *Pharmaceutical Biol*. **41:** 475-240.

Elizabeth, K. and Rao, M.N.A. (1990). Oxygen radical scavenging activity of curcumin. *Int. J. Pharmacogl.* **58:** 237-240.

Habsah, M., Nordin, L.H., Ali, A.M., Sukari, M.A., Hin, Y.Y., Kikuzaki, H. and Nakatani, N. (2003). The antioxidative components from *Alpinia nutans*. *Pharmaceutical Biol.* **41:** 7-9.

Kikuzaki, H. and Nakatani, N. (1993). Antioxidant effects of some ginger constituents. *J. Food Sci.* **58:** 1407-1410.

Kumar, N., Kader, A., Rangaswami, P. and Irulappan (1997). Spices, Plantation Crops, Medicinal and Aromatic Plants. Oxford & IBH publishing Co. Pvt. Ltd. New Delhi.

Larson, R.A. (1988). The antioxidants of higher plants. *Phytochemistry* **27:** 969-.973

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Annal. Biochem.* **95:** 351-358.

Purseglove, J.W. (1975). Tropical Crops Monocotyledons. Longman, London.

Ravishankara, M.N., Shrivastava, L., Padh, H. and Rajani, M. (2002). Evaluation of antioxidant properties of root bark of *Hemidesmus indicus*. *Phytomedicine* **9:** 153-157.

Tripathi, Y.B., Chaurasia, S., Tripathi, E., Upadhay, A. and Dubey, G.P. (1996). *Bacopa monniera* Linn. as an antioxidant: Mechanism of action. *Indian J. Exp. Biol.* **34:** 523-528.

Vanni, T., Rajani, M., Sarkar, S. and Sishoo, C.J. (1997). Antioxidant properties of of the ayurvedic formulations Triphala and its constituents. *Int. J. Pharmacol.* **35:** 313-317.

Wagner, H. and Bladt, S. (1996). Plant Drug Analysis: A Thin Layer chromatography Atlas. Spinger-Verlag, Berlin.

Wilson, R.L. (1988). Free radical and tissue damage, mechanistic evidence from radiation studies. In: M.G. Simiec (ed.), Biochemical Mechanisms of Liver injury, pp. 123. Academic Press, New York.