http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 6 (24): 1996-2000, 2003
ISSN 1028-8880

Superoxide Scavenging and Tyrosinase Inhibitory Active Compound in Ginger (*Zingiber officinale* Roscoe)

[1]F. Khanom, H. Kayahara, M. Hirota and K. Tadasa
Faculty of Agriculture, Shinshu University, 8304, Minamiminowa-mura,
Kamiina-gun, Nagano-Ken 399-4598, Japan
Production Department (Herbal), The ACME Laboratories Ltd., Dhammai, Dhaka, Bangladesh

Abstract: The superoxide-scavenger and tyrosinase inhibitory active material was extracted by methanol from ginger (*Zingiber officinale* Roscoe) and was partitioned into hexane, aqueous and ethyl acetate parts. The highly active hexane soluble part was subjected to fractioned by silica gel column chromatography and was obtained in eight fractions. All most all fractions gave a remarkable amount of superoxide-scavenging and tyrosinase inhibitory activities. The highest superoxide-scavenging and tyrosinase activities were found in the fraction five (58.2 and 42.7%, respectively) and its purity was confirmed by thin layer chromatography (TLC). This fraction was amounting as 274.3 mg and characterized by nuclear magnetic resonance (NMR), infrared (IR) and mass spectrometer. The compound was gingerol and its structure was confirmed as 3-decanone, 5-hydroxy-1 (4-hydroxy-3-methoxy phenyl) -3-one on the basis of spectral evidence.

Key words: Ginger, gingerol, superoxide, scavenger, tyrosinase, inhibition

INTRODUCTION

In vitro bioassay systems have been extensively used to monitor the biological activities of extracts, fractions and isolated compounds during the isolation process of bioactive constituents in the plant materials. The involvement of free radicals and other oxidants in aging and in several diseases has been investigated (Hassan, 1981 and Niki, 1995). For example, active oxygen damages the skin directly and produce lipid peroxides which result in the formation of insoluble pigments such as eumelanins and phaeomelanins (Miao *et al*., 1997). Much physiological damage may be directly imputable to the hydroxyl radical because it is highly reactive, and any hydroxyl radical produced *in vivo* would react at or close to its site of formation (Anbar, 1967 and Scholes, 1983). Superoxide and its precursor usually reduce the production and reactivity as well of the hydroxyl radicals (Halliwell, 1977). On the other hand, tyrosinase is one of the multifunctional enzymes containing copper that is found in fungi, plant materials and animal tissues (Lerner and Fitzpatrick, 1950) and is responsible for melanin biosynthesis (Mason, 1948; Lerner and Fitzpatrick, 1950). Its activity on phenolic substrates has been reported (Madhusingh and Sundberg, 1974).

Ginger is one of the most important spices throughout the world, its tuber have been utilized for treating headache, nausea, stimulant, stomachache and colds as a traditional medicine (Shoji *et al*., 1982). Chemical studies of ginger have been carried out by a large number of investigators (Kikuzaki *et al*., 1991; Kiuchi *et al*., 1992; Kikuzaki and Nakatani, 1993 and Kikuzaki *et al*., 1994). There are some reports on superoxide-scavenger (Miao *et al*., 1997 and Khanom *et al*., 2000a) and tyrosinase inhibitory activity (Sung and Cho, 1992 and Khanom *et al*., 2000b) of ginger but the compound related to superoxide-scavenging and tyrosinase-inhibitory activities not reported yet. Therefore, the aim of this study is to identify the superoxide-scavenging and tyrosinase-inhibitory active compound in ginger.

MATERIALS AND METHODS

Extraction: The fresh slice of 4 kg ginger was exhaustively extracted with 6 liter of methanol at room temperature for one week. The extract was filtrated, and the filtrate was collected, evaporated and lyophilized. The yield, lyophilized sample of 62 g was stored in refrigerator at 2-5°C for subsequent analysis.

Superoxide-scavenging activity tests: The 20 mg lyophilized sample was dissolved in 10% methanol in a concentration of 1 mg ml^{-1} and used for superoxide-scavenging test. The superoxide-scavenging activity was

done in cytochrome c (cyt. c) method (McCord and Fridovich, 1969) as described in our previous report (Khanom *et al*., 2000a). In this method, superoxide anion was generated by a xanthine and xanthine oxidase system. The superoxide-scavenging activity was calculated by measuring the reduction rate of ferricytochrome c. Briefly, the assay was performed in 3 ml of reaction mixture in a 1 cm cuvette maintained at 25°C. The reaction mixture contained 0.3 ml of a sample solution, 0.5 ml of ferricytochrome c (1_~10^{-5} M), 0.5 ml of xanthine (5_~10^{-5} M), and 1.0 ml DW and 0.5 ml of a KH_2PO_4/K_2HPO_4 buffer (50 mM; pH 7.8) containing EDTA (10^{-4} M). A solution of 0.2 ml of xanthine oxidase (0.5_~10^{-8} M) was added to the reaction mixture to produce a rate of reduction of ferricytochrome c at 550 nm by using 0.025-absorbance unit per 1.5 min and to make 3 ml of the reaction mixture. Data on the resulting straight line were taken for calculation by the following formula

$$\text{Inhibitory rate (Inb. r \%)} = \frac{\Delta b - \Delta s}{\Delta b} \times 100\%$$

Here, Inb. r (%)=the inhibition rate of sample solution against the radicals. Δ b=the difference min^{-1} of the blank's (without the addition of sample solution replaced by water) optical density value. Δs=the difference min^{-1} of the optical density value of the sample in the part of straight line.

Tyrosinase inhibitory activity tests: For tyrosinase inhibitory activity test 20 mg of lyophilized sample was dissolved in 10% dimethyl sulfoxide (DMSO) in a concentration of 1 mg ml^{-1} and used for tyrosinase-inhibitory test for the raw extract. This test was performed according to the method of Kobayashi *et al.* (1995) as described in our previous report (Khanom *et al*., 2000b). Briefly, 1.0 ml of a McIlvaine buffer solution (pH 6.8), 1.0 ml of DW containing 0.3 mg ml^{-1} of L-tyrosine and 0.9 ml of the sample solution, which had been dissolved in 10% DMSO/DW, were taken in a test tube, mixed thoroughly and preincubated at 30°C for 10 min. Then, 0.1 ml of 480 units ml^{-1} of tyrosinase (from *Agricus biporus*; Sigma Chemical Co) was added and incubation continued at 30°C for another 10 min. Finally, 0.1 ml of 1 M sodium azide was added to stop the reaction, and the absorbance was measured at 475 nm by a UV-160A UV-visible recording spectrophotometer (Shimadzu, Kyoto, Japan). A control test was run with a 10% DMSO solution, and the inhibitory activity was calculated according to the following formula:

$$\text{Inhibitory activity (\%)} = \frac{(C-S)}{C} \times 100$$

Where, C is the absorbance of the control at 475 nm, and S is the absorbance of the sample at the same wavelength.

Partitioning of lyophilized sample: The lyophilized remaining sample (61.96 g) was successively partitioned in hexane and distilled water (DW) and then ethyl acetate and DW and collected in 3 parts: hexane soluble part, ethyl acetate soluble part and aqueous soluble part. The experimental protocol is shown in Fig. 1. The biological activities of these three parts were then performed as described previously.

Column chromatography and compound identification: The hexane soluble part was found more active both in superoxide-scavenging and tyrosinase inhibitory activities and this part was then subjected to silica gel column chromatography. An aliquot of this part (2 g) was chromatographed on silica gel with ethyl acetate/hexane mixtures (30% ethyl acetate) and successfully partitioned into eight fractions. The biological activity of each fraction was performed and the purity of these fractions was tested by thin layer chromatography (TLC). The fraction was found pure on TLC plate and showed higher biological activities were chosen for characterization by NMR, IR, and Mass spectrometry.

Instrumental: IR spectra were recorded as films by a Jasco Report-100 infrared spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded by a Bruker DR 500 FT-NMR spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. Mass spectra were recorded with a Jeol JMS 700 mass spectrometer, and Merck silica gel 60 (70-230 mesh) was used for column chromatography.

RESULTS AND DISCUSSION

The initial methanol extract of ginger was used for superoxide-scavenging and tyrosinase inhibitory activity tests and the values were found as 90.4 and 48.1%, respectively. Ginger has been widely using as a spice and it's importance as traditional medicine also been reported (Shoji *et al*., 1982; Kiuchi *et al*., 1992 and Khanom *et al*., 2000a). The antioxidative effects (Kiuchi *et al*., 1992 and kikuzaki *et al*., 1993), tyrosinase inhibitory activity (Khanom *et al*., 2000b) and constituents of ginger has also been reported (Kikuzaki *et al*., 1991 and Kikuzaki *et al*., 1994).

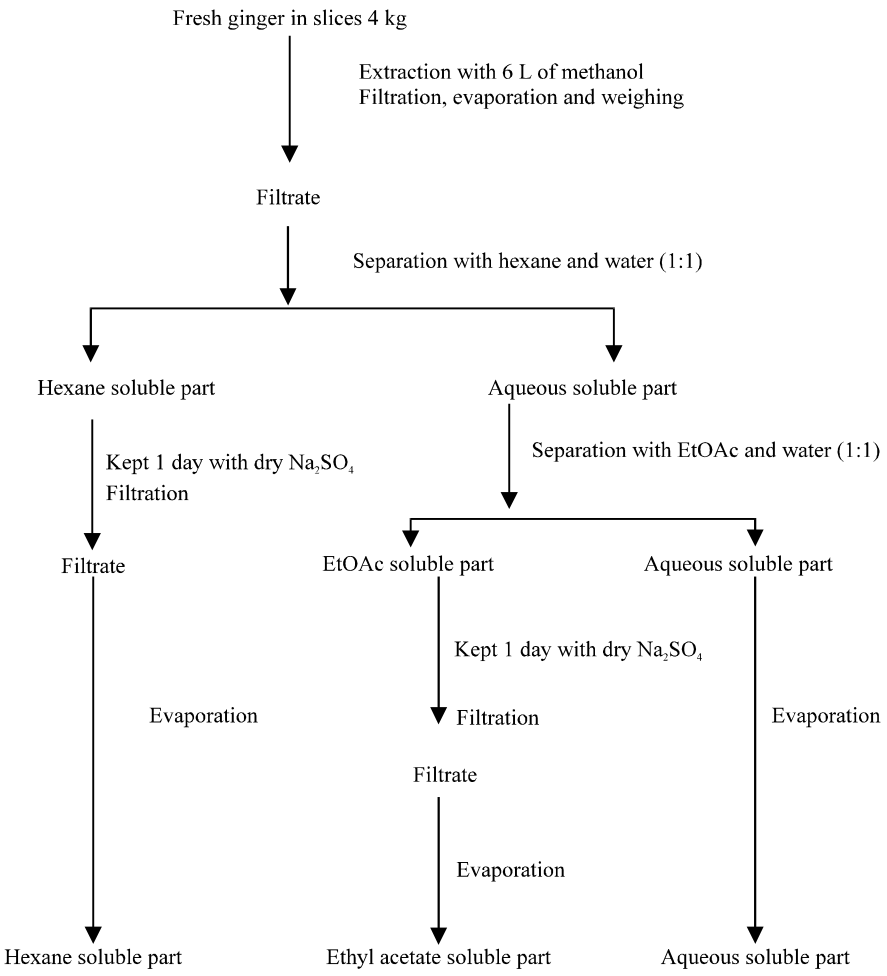

Fig. 1: Experimental protocol for separating in different parts of ginger methanol extract

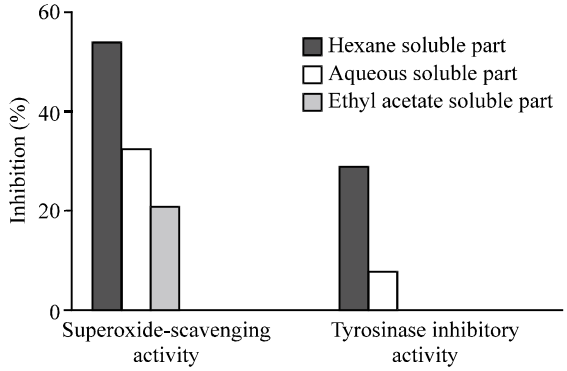

Fig. 2: Biological activities of three different parts of ginger extract

From 4 kg of raw ginger, 61.96 g of yellow color yield was obtained. The extract yield was then successfully partitioned into three parts and their bioassay results are summarized in Fig. 2. In hexane soluble part, superoxide-scavenging and tyrosinase inhibitory activities were found 52.8 and 28.3%, respectively. While in aqueous soluble part, they were obtained as 31.7 and 6.8%, respectively. On the other hand, in ethyl acetatesoluble part, superoxide-scavenging activity was 20.3% but the tyrosinase inhibitory activity was not found at all. The highly active hexane soluble part was subjected to silica gel column chromatography and eight fractions were eluted with 30% ethyl acetate in hexane. Though all most all fractions showed both of superoxide-scavenging and tyrosinase inhibitory activity but the highest superoxide-scavenging and tyrosinase inhibitory activities were found in fraction five (58.2 and 42.7%, respectively) as shown in Fig. 3. The fraction five was obtained in 274.3 mg and its purity was confirms by TLC (not shown). This fraction further subjected to characterize by NMR, IR, and Mass spectrometer.

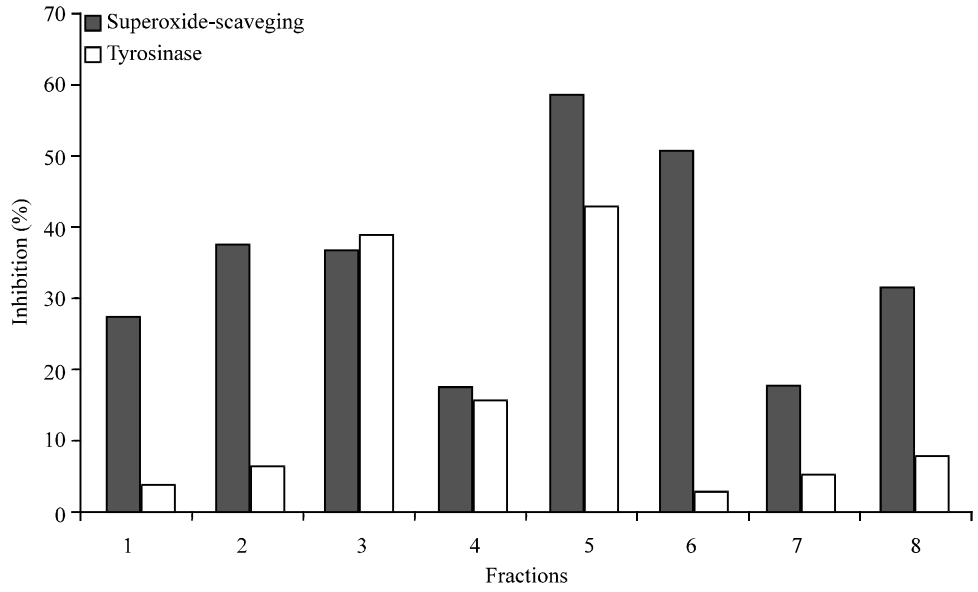

Fig. 3: Superoxide-scavenging and tyrosinase inhibitory activities in different fractions of ginger extract (hexane soluble part) separated by column chromatography

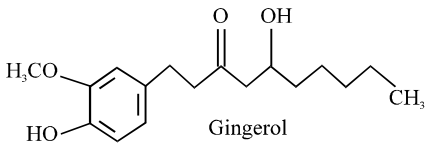

Fig. 4: Chemical structure of gingerol confirmed by NMR, IR and Mass spectrum

HR-EIMS m/z (M^+): calcd for $C_{17}H_{26}O_4$, 294.1832; found, 294.0.

IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2910 (-CH_2-), 1700 (-CO-); ^{1}H-NMR δ_H ($CDCl_3$): 0.89 (3H, t, J= 6.7 Hz), 1.27 (br, s, J= 4.1 Hz), 2.48 (H, dd. J= 8.9 Hz, 17.4 Hz), 2.55 (H, dd. J= 2.8 Hz, 17.3 Hz), 2.72 (2H, t, J= 7.4 Hz), 2.83 (2H, t, J= 7.4 Hz), 3.85 (3H, s, J= 0 Hz), 4.02 (H, s, J= 0 Hz), 5.55 (H, s, J= 0Hz), 6.65 (H, d, J= 8.0Hz), 6.67 (H, s, J= 0 Hz), 6.81 (H, d, J= 8.0 Hz); ^{13}C-NMR δ_c ($CDCl_3$). The ^{13}C-NMR spectrum of compound showed seventeen carbon signals, which were assigned as a six aromatic carbon, a methoxy carbon, a carbonyl, a hydroxyl methine, seven methylene carbon and one methyl carbon. The chemical structure of this compound was confirmed as 3-decanone, 5-hydroxy-1 (4-hydroxy-3-methoxy phenyl) -3-one as shown in Fig. 4.

The isolated bioactive compound had the structural characteristics of phenolic groups, which might be inhibit radical formation or quench a generated radical in endoperoxide reaction (Kiuchi *et al*., 1992). Further more, phenolic compounds such as, carotenoids, flavonoids and caffeic acid, ferulic acid were found to scavenge superoxide anion and inhibit lipid peroxidation induced by active oxygen species such as superoxide anion or hydroxyl radical, respectively (Toda *et al*., 1991 and Kiuchi *et al*., 1992). In conclusion, we may suggests that the structures of gingerol containing phenolic groups would be responsible for so-scavenger and tyrosinase inhibitory active factor as found in the present study.

ACKNOWLEDGMENTS

The first author, Firoza Khanom, wishes to thank "Rotary Yoneyama" Japan for providing financial assistance by a scholarship during the course of this study. We are also grateful to Dr. M.A.M. Yahia Khandoker, Associate Professor, Department of Animal Breeding and Genetics, Bangladesh Agricultural University for valuable suggestions and critical reading of the manuscript.

REFERENCES

Anbar, M. and P. Neta, 1967. A compilation of specific bimolecular rate constants for the reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals with inorganic and organic compounds in aqueous solution. Int. J. Appl. Radiat. Isot., 18: 493-523.

Halliwell, B., 1977. Generation of hydrogen peroxide, superoxide and hydroxyl radicals during the oxidation of dihydroxyfumaric acid by peroxidase. Biochem. J., 163: 441-448.

Hassan, H.M., 1981. Determination of microbial damage caused by oxygen free radicals, and the protective role of superoxide dismutase. In "Methods in Enzymology," Vol. 105, Academic Press, New York, pp: 404.

Khanom, F., H. Kayahara and K. Tadasa, 2000a. Superoxide-scavenging and prolylendopeptidase inhibitory activities of Bangladeshi indigenous medicinal plants. Biosci. Biotechnol. Biochem., 64: 837-840.

Khanom, F., H. Kayahara and K. Tadasa, 2000b. Tyrosinase inhibitory activity of Bangladeshi indigenous medicinal plants. Biosci. Biotechnol. Biochem., 64: 1967-1969.

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

Kikuzaki, H., Y. Kawasaki and N. Nakatani, 1994. Structure of antioxidative compounds in ginger. Ame. Chem. Soc., 24: 237-243.

Kikuzaki, H., J. Usuguchi and N. Nakatani, 1991. Constituents of Zingiberaceae. I. Diarylheptanoids from the rhizomes of ginger (*Zingiber officinale* Roscoe). Chem. Pharm. Bull., 39: 120-122.

Kiuchi, F., S. Iwakami, M. Shibuya, F. Hanaoka and U. Sankawa, 1992. Inhibition of prostaglandin and leukotriene biosynthesis by gingerols and diarylheptanoids. Chem. Pharm. Bull., 40: 387-391.

Kobayashi, Y., H. Kayahara, K. Tadasa, T. Nakamura and H. Tanaka, 1995. Synthesis of amino acid derivatives of kojic acid and their tyrosinase inhibitory activity. Biosci. Biotechnol. Biochem., 59: 1745-1746.

Lerner, A.B. and T.B. Fitzpatrick, 1950. Biochemistry of melanin formation. Physiol. Rev., 30: 91-126.

Mason, H.S., 1948. The chemistry of the melanin. III. Mechanism of the oxidation of dihydroxyphenylalanine by tyrosinase. J. Biol. Chem., 172: 83-99.

Madhusingh, C. and L. Sundberg, 1974. Purification and properties of tyrosinase inhibitor from mushroom. FEBS Letters, 49: 156-158.

McCord, J.M. and I.J. Fridovich, 1969. Superoxide dismutase. An enzymic function for erythrocuprein (Hemocuprein). J. Biol. Chem., 244: 6049-6055.

Miao, Z., H. Kayahara and K. Tadasa, 1997. Superoxide-scavenging and tyrosinase-inhibitory activities of the extracts of some Chinese medicines. Biosci. Biotechnol. Biochem., 61: 2106-2108.

Niki, E., 1995. In "Antioxidants-Free Radicals and Biological Defense," Eds. E. Niki, H. Shimasaki, and M. Mino, Japan Scientific Press, Tokyo (in Japanese), pp: 3-5.

Scholes, G., 1983. Radiation effects on DNA. The silvanus thompson memorial lecture. Br. J. Radiol., 56: 221-231.

Shoji, N., A. Iwasa, T. Takemoto, Y. Ishida and Y. Ohizumi, 1982. Cardiotonic principles of ginger (*Zingiber officinale* Roscoe). J. Pharm. Sci., 71: 1175-1176.

Sung, C-ki and S-Hye, Cho, 1992. The purification and characterictics of tyrosinase from ginger (*Zingiber officinale* Rosc). Korean Biochem. J. (Korean language), 25: 564-572.

Toda, S., M. Umura and M. Ohnishi, 1991. Effects of phenolcarboxylic acids of superoxide anion and lipid peroxidation induced by superoxide anion. Planta Medica, 57: 8-10.