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Research Article

EVALUATION OF ANTIOXIDANT POTENTIAL OF RHIZOME EXTRACTS OF TWO SPECIES OF *ALPINIA* ROXB. (ZINGIBERACEAE)

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ABSTRACT
Objective of the present study was to investigate the *in vitro* antioxidant activities of methanolic extracts of the rhizomes of *A. smithiae* and *A. vittata*. Both the extracts exhibited their scavenging effects in a concentration dependent manner. The assays were carried out against superoxide anion radical, hydroxyl radical, nitric oxide radical and inhibition of lipid peroxidation. Superoxide as well as hydroxyl radical scavenging activities and inhibition of lipid peroxidation was found to be more with *A. smithiae* extract while that of *A. vittata* was found to be more effective in scavenging nitric oxide radicals. The results obtained in the present study indicate that the rhizome extracts of both the species studied can be a potential source of natural antioxidants.

KEYWORDS: *Alpinia vittata*, *Alpinia smithiae*, Zingiberaceae, antioxidant, free radical, lipid peroxidation.

INTRODUCTION

Free radicals are often generated as byproducts of oxidative damage to DNA molecules, lipids and proteins[1]. This damage could lead to several human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, anemia, asthma, inflammation and neurodegenerative disorders [2, 3, 4]. Several plant derived compounds have been reported for their antioxidant properties. Zingiberaceae is a well known plant family and many of its species are being used in traditional medicine in the treatment of several diseases. The medicinal properties of the rhizomes of Zingiberaceous plants have been widely discussed and accepted worldwide. These plants contain many essential oils which have been reported for their antioxidant, antiinflammatory and antimicrobial properties.

Alpinia species is the largest and most widespread taxonomical genus in Zingiberaceae comprising about 230 species occurring throughout tropical and subtropical Asia. Many species of *Alpinia* are known for their medicinal values. The rhizomes of Zingiberaceae possess diverse biological activities like antimicrobial[5], antiulcer[6], antinflammatory, antioxidant[7], cytotoxic and antitumor[8, 9]) activities. In the present study the *in vitro* antioxidant activities of *A. vittata* and *A. smithiae* were evaluated.

MATERIALS AND METHODS

Plant materials

A. vittata W. Bull and *A. smithiae* M. Sabu *et* Mangaly, collected from Calicut University Herbal Garden were used for the present study. Voucher specimens of *A. vittata* (CALI-78810) and *A. smithiae* (CALI-17563) were deposited at the Herbarium of Department of Botany, University of Calicut.

Extraction

The tubers were separated from each plant, washed and sliced into small pieces. These were dried and powdered before extraction. 100g powder from each sample was separately extracted with methanol using a Soxhlet apparatus. The extracts thus obtained were filtered and evaporated. These extracts were then dissolved in minimum volume of dimethyl sulfoxide (DMSO) and made-up to desired volume with distilled water. The extracts at concentrations 20, 40, 60, 80, 100 µg/ml were used for the antioxidant assays.

Superoxide radical scavenging activity

The reaction mixture contained 3µg NaCN dissolved in EDTA (6µM), riboflavin (2µM), NBT (50µM) and various concentrations (10 - 1000µg/ml) of the extract and phosphate buffer in a final volume of 3ml. The tubes containing the reaction mixture were uniformly illuminated with an incandescent lamp for 15min and the absorbances were measured at 530 nm before and after the illumination[10]. Percent inhibition of superoxide radical was calculated using the equation,

$$\frac{\text{(Absorbance of control - Absorbance of extract)}}{\text{Absorbance of control}} \times 100$$

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extract was measured by studying the competition between deoxyribose and test compounds for the hydroxyl radicals generated from Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radicals attack deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances[11]. The reaction mixture contained deoxyribose (2.8mM), ferric chloride (0.1mM), EDTA (0.1mM), H_2O_2 (1mM), ascorbate (0.1mM), KH_2PO_4-KOH buffer (20mM, pH 7.4) and various concentrations of the sample in a final volume of 1ml was incubated for 1hr at 37^0C. Deoxyribose degradation was measured as thiobarbituric acid reactive substrate by the method of Ohkawa et al[12]. The inhibition produced by different concentrations was calculated and compared with that of control. Percent inhibition of hydroxyl radical was calculated using the equation,

$$\frac{\text{(Absorbance of control - Absorbance of extract)}}{\text{Absorbance of control}} \times 100$$

Nitric oxide radical scavenging activity

Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions, which were detected by Griess reaction[13]. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and different concentrations of the extract (1-100 µg/ml) was incubated at 25^OC for 150 minutes. After incubation, 0.5 ml

of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. The percentage of inhibition was calculated by,

$$\frac{\text{(Absorbance of control - Absorbance of extract)}}{\text{Absorbance of control}} \times 100$$

Lipid peroxidation assay

The level of lipid peroxidation was measured by the method of Ohkawa et al[12]. About 10 - 1000µg/ml of extract was incubated with 0.1ml rat liver homogenate (25%) containing 30 mM KCl, Tris-HCl buffer (0.04 M, pH 7.0), ascorbic acid (0.06 mM) and ferrous ion (0.16 mM) in a total volume of 0.5ml was incubated at 37°C for 1hr. After incubation, 0.4ml of reaction mixture was treated with 0.2ml SDS (8.1%), 1.5 ml TBA (0.8%) and 1.5 ml acetic acid (20%, pH 3.5) and was made up to 4ml by adding distilled water and kept for 1hr in a boiling water bath at 95^0C. The reaction mixture was cooled and 1ml distilled water and 5ml of pyridine : butanol (15:1) was added, mixed thoroughly and centrifuged at 3000 rpm for 10 min. Absorbance of the clear supernatant was measured at 532 nm against pyridine : butanol. Percent inhibition of lipid peroxidation was calculated using the equation,

$$\frac{\text{(Absorbance of control - Absorbance of extract)}}{\text{Absorbance of control}} \times 100$$

RESULTS

The antioxidant capability of *A. vittata* (Table 1) and *A. smithiae* (Table 2), were measured using superoxide radical, hydroxyl radical, nitric oxide radical scavenging and lipid peroxidation assay. The result indicates the antioxidant activity of both the extracts at five different concentrations 20, 40, 60, 80 and 100 µg/ml. Both the extracts showed a dose dependent increase in their antioxidant properties.

The superoxide radical scavenging activity measured by the decrease in absorbance at 560 nm with the extracts indicates the consumption of superoxide anion derived from riboflavin. *A. smithiae* showed 81.53% inhibition of superoxide radical with 100 µg/ml. On the other hand *A. vittata* produced only 76.88%. Both the extracts were found to be more effective in scavenging the hydroxyl radical produced in the reaction mixture. Of these *A. smithiae* showed more activity with an inhibition percentage of 57.05 with 100 µg/ml treatment, while *A. vittata* showed a lesser activity of 49.40 µg/ml with 100 µg/ml. Nitric oxide radical generated from sodium nitroprusside at physiological pH was inhibited by both the extracts. Among them *A. vittata* showed more inhibition of nitric oxide radical (63.45%) and *A. smithiae* showed only 60.12% with 100 µg/ml. The capacity of both the extracts to prevent lipid peroxidation was assayed using incubation of rat liver homogenates with the oxidant chemical species Fe^{2+}. The *A. smithiae* extract had the greatest activity in reducing lipid peroxidation with an inhibition percentage of 60.89, but *A. vittata* produced only 39.26 % with 100 µg/ml.

DISCUSSION

Free radicals (Reactive oxygen species) are low molecular weight metabolites reactive enough to damage vital biomolecules including DNA. The antioxidants can inhibit the free radicals by decreasing the localized O_2 concentration, thereby reducing molecular oxygen's oxidation potential, metabolising lipid peroxides to non-radical products. In light of the results obtained we can conclude that the rhizome extracts of *Alpinia* showed considerable antioxidant potential. A dose dependent increase in the antioxidant activity *of A. smithiae* and *A. vittata* with superoxide, hydroxyl radical, nitric oxide radical scavenging activities and inhibition of lipid peroxidation by the extracts was observed. The activity was high enough for these plants to be used as sources of potent antioxidant substances for use as natural additives in food and pharmaceutical industries. The GC- MS analysis of the rhizome essential oils of these plants showed the presence of various phytochemicals. The extract of *A. smithiae* contained 1,8-cineole, α -terpineol, trans-caryophyllene, caryophyllene oxide, β– fenchyl acetate, borneol, fenchyl alcohol, α-humulene, myrcene, α-terpenine, geranyl actetate and α – pinene. Likewise *A. vittata* contained zerumbone, limonene, β- caryophyllene, germacrene A and D, valencene, allo-aromadendrene, camphene, β- elemene etc. The compounds such as 1, 8-cineole and myrcene were reported to possess high antioxidant properties. Thus the ability of both the plant extracts in scavenging the free radicals and inhibiting lipid peroxidation may be due to the presence of these phytochemicals. The free radical scavenging activity and inhibition of lipid peroxidation by methanolic extract of *A. smithiae* and *A. vittata* suggests their antioxidant capability.

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REFERENCES

1. Farber JL. Mechanisms of cell injury by activated oxygen species. Environ Health Perspect 1994; 102: 17-24.
2. Polterat O. Antioxidants and free radical scavengers of natural origin. Current Org Chem 1997; 1: 415-440.
3. Olinski R, Gackowski D, Foksinski M, Rozalski, R, Roszkowski K, Jaruga P. Oxidative DNA damage: assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. Free Radical Bio Med 2002; 33: 192–200.
4. Halliwell B, Aruoma OI. DNA damage by oxygen derived species. Its mechanism and measurement in mammalian systems. FEBS Lett 1991; 281: 9–19.
5. Yamada Y, Kikuzaki H, Nakatani N. Identification of antimicrobial gingerols from ginger (*Zingiber officinale* Roscoe). J Antibact Antifung Agents 1992; 20: 309-311.
6. Matsuda H, Pongpiri YY, Morikawa T, Ochi M, Yoshikawa M. Gastroprotective effect of phenylpropanoids from the rhizomes of *Alpinia galanga* in rats: Structural requirements and mode of action. Eur J Pharmacol 2003; 471: 59-67.
7. Selvam R, Subramaniam L, Gayathri R, Angayakanni N. The antioxidant activity of turmeric (*Curcuma longa*). J Ethnopharmacol 1995; 47: 59-67.
8. Itokawa H, Morita H, Sumitomo T, Totsuka N, Takeya K. Antitumor principles from *Alpinia galanga*. Planta Med 1987; 53: 32-33.
9. Pal S, Choudhuri T, Chattopadhyay S, Bhattacharya A, Datta GK, Das T, Sa G. Mechanism of curcumin induced apoptosis of Ehrlich's Ascites Carcinoma cells. Biochem Biophys Res Commun 2001; 288: 658-665.
10. McCord JM, Fridovich I. Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244: 6049 - 6055.
11. Elizabeth K, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm 1990; 58: 237-240.
12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Annal Biochem 1979; 95: 351-358.
13. Green LC, Wagner DA, Glogowski J, Skipper PC, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and 15^N in biological fluids. Annal Biochem 1982; 126: 131.

TABLE 1. EFFECT OF *ALPINIA VITTATA* IN SCAVENGING SUPEROXIDE, HYDROXYL, NITRIC OXIDE RADICALS AND INHIBITION OF LIPID PEROXIDATION

Extract concentration(µg/ml)	Superoxide radical (in %)	Hydroxyl radical (in %)	Nitric oxide radical (in %)	Inhibitiion of lipid peroxidation (in %)
20	28.50	25.26	29.94	04.68
40	54.90	30.37	34.77	10.36
60	59.19	33.87	46.17	15.45
80	64.71	40.32	60.40	23.38
100	76.88	49.40	63.45	39.26

TABLE 2. EFFECT OF *ALPINIA SMITHIAE* IN SCAVENGING SUPEROXIDE, HYDROXYL, NITRIC OXIDE RADICALS AND INHIBITION OF LIPID PEROXIDATION

Extract concentration(µg/ml)	Superoxide radical (in %)	Hydroxyl radical (in %)	Nitric oxide radical (in %)	Inhibitiion of lipid peroxidation (in %)
20	42.92	23.79	03.42	07.35
40	44.96	38.50	17.60	18.50
60	57.07	51.61	56.23	54.70
80	81.17	54.03	58.67	58.40
100	81.53	57.05	60.12	60.89

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