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Antioxidant Properties of Alpha Asarone

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ABSTRACT

Plant phenolics have been recognized largely as beneficial antioxidants that can scavenge harmful active oxygen species. In plant systems, phytophenolics can act as antioxidants. Alpha asarone is one of the phytochemical constituent of essential oil of *Acorus calamus* and bark of *Guatteria gaumeri* which exhibits many biological activities. In the present study antioxidant activity of alpha asarone was evaluated using scavenging assays of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals, hydroxyl radicals, superoxide anion radicals, chelating ability of ferrous ion, reducing power and inhibition capability of Fe (II)-induced lipid peroxidation. Alpha asarone exhibited strongest antioxidant activities in scavenging DPPH radicals and highest reducing power. Alpha asarone also exhibited best performance in chelating metal ion and inhibiting lipid peroxidation. The results obtained revealed that alpha asarone possess higher antioxidant activity.

Key words: Alpha-asarone, DPPH, ferrous ion, *Acorus calamus*

INTRODUCTION

Alpha asarone (trans-isomer of 2, 4, 5-trimethoxy-l-propenylbenzene) is one of the phytochemical compound in the rhizome oil of *A. calamus*. Alpha asarone was found to be a potential antithrombotic, antimicrobial, insecticidal and nematicidal and antifeedant agent. Alpha asarone is also a good natural radio protective agent with therapeutic implications (Divyasree and Nair, 2010). Antioxidant phytochemicals of plants exert their effect by neutralizing highly reactive radicals. Among thousands of phytochemicals found in our diets or traditional medicines, polyphenols and carotenoids stand out as the two most important groups of natural antioxidants. However, collectively these phytochemicals are good antioxidants; the roles and effect of individual compounds are often not well known. Thousands of polyphenols have been identified from various plants and their antioxidant properties were proved by many researchers. Manikandan and Devi (2005) have reported the antioxidant activity of alpha asarone in noise-induced rat brain but the *in vitro* antioxidant activity of alpha asarone has not been studied so far. So the alpha asarone was chosen for this study.

MATERIALS AND METHODS

Alpha asarone, DPPH, Deoxyribose, Quercetin were purchased from Sigma-Aldrich, USA. Other chemicals used for study was purchased from SRL, India.

Determination of DPPH radical-scavenging activity: The DPPH radical-scavenging activity of alpha asarone was measured by previously published methods of Yang *et al.* (2006). The alpha asarone was mixed with methanol to get various concentrations: 100, 80, 60, 40, 20 and 10 μg mL^{-1}. From each concentration, 2 mL of alpha asarone was mixed with 1 mL of methanolic solution containing DPPH radicals, with final concentration of 0.2 mM DPPH. The contents were mixed vigorously and incubated in the dark for 30 min. Absorbance was measured at 517 nm. Absorbance of the control was determined by replacing the sample with methanol. Quercetin was used as standard. The scavenging activity was calculated using the equation:

$$\text{Scavenging activity (\%)} = \frac{A_{517} \text{ of control-} A_{517} \text{ of sample}}{A_{517} \text{ of control}} \times 100$$

Determination of reducing power: The reducing power was determined as described by Oyaizu (1988). The 2 mL of alpha asarone mixed in methanol was taken in various concentrations such as 20, 40, 60, 80, 100 μg and mixed with 2 mL of 0.2 M phosphate buffer (pH 6.6) and 2 mL of potassium ferricyanide (10 mg mL^{-1}). This solution was incubated for 20 min at 50°C. After incubation 2 mL of trichloroacetic acid (100 mg mL^{-1}) was added. The 2 mL of the above mixture was diluted by adding 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride. After 10 min of reaction the absorbance was measured at 700 nm. Quercetin was used as a standard to compare the reducing power of alpha asarone.

Determination of superoxide anion radical-scavenging activity: Superoxide anion scavenging activity was determined using riboflavin-light NBT system (Mehrotra *et al.*, 2003). It was done by mixing 0.5 mL of 50 mM phosphate buffer (pH 7.6), 0.3 mL of 50 μM riboflavin, 0.25 mL of 20 mM PMS and 0.1 mL of 0.5 mM NBT. To this mixture 1 mL of various concentration of alpha asarone (10, 20, 40, 60, 80, 100 μg) dissolved in methanol was added. The mixture was incubated for 20 min under fluorescence lamp. After 20 min absorbance was measured at 560 nm. The percent of scavenging activity was calculated using the equation:

$$\text{Scavenging activity (\%)} = \frac{A_{560} \text{ of control-} A_{560} \text{ of sample}}{A_{560} \text{ of control}} \times 100$$

Quercetin was used as a standard.

Ability of chelating ferrous ions: The chelating ability of ferrous ion of alpha asarone was determined by ferrous ion-ferrozine complex method (Meyar and Isaksen, 1995). Various concentration of alpha asarone (10, 8, 6, 4 and 2 mg mL^{-1}) in methanol was prepared. From each concentration 0.8 mL of extract was mixed with 50 μL of 2 mM FeCl2 and 200 μL of 5 mM ferrozine. It was incubated for 10 min at 25±2°C and absorbance was measured at 562 nm using against methanol as blank. Activity of alpha asarone to chelate ferrous ion was calculated using the equation:

$$\text{Chelating ferrousion (\%)} = \frac{A_{562} \text{ of control-} A_{562} \text{ of sample}}{A_{562} \text{ of control}} \times 100$$

Determination of hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity of the asarone extracts was measured by the method of Halliwell and Gutteridge (1981). It was determined by mixing 500 μL of 2-deoxyribose (2.8 mM) in 50 mM potassium phosphate buffer (pH 7.4), 200 μL of premixed ferric chloride (100 μM) and (100 μM) EDTA solution (1:1;v/v), 100 μL of 200 μM H_2O_2 in different concentration of alpha asarone solution (100 μL). To initiate the reaction 100 μL of ascorbate (300 μM) was added and incubated for 1hour at 37°C. After incubation, a solution of TBA in 1 mL (1%; w/v) of 50 mM NaOH and 1 mL of 2.8% (w/v; aqueous solution) TCA was added to the mixture and incubated in boiling water bath for 15 min and cooled. Absorbance was measured at 532 nm. Quercetin used as a standard. Methanol was used as control and its absorbance was measured. The scavenging activity on hydroxyl radical was calculated as:

$$\text{Scavenging activity (\%)} = \frac{\text{1-A}_{532}\text{ of sample}}{\text{A}_{532}\text{ of control}} \times 100$$

Inhibition of lipid peroxide formation induced by Fe^{2}+-ascorbate system: Lipid peroxidation assay of rat microsomal was carried out by using the method of Sabu and Kuttan (2002). Lipid peroxidation inhibition of alpha asarone was determined by mixing 100 μL of rat liver extract in 40 mM Tris HCl buffer 25% w/v, 100 μL of 30 mM KCl, 0.1 mL of 0.16 mM ferrous ion and 0.1 mL of 0.06 mM ascorbic acid in 0.1 mL of various concentration of alpha asarone. The 0.5 mL of mixture was incubated at 37°C for 1 h and absorbance was measured at 532 nm in spectrophotometer. The percent inhibition of lipid peroxidation was determined by comparing the results of the test compounds with that of control without alpha asarone solution. Quercetin was used as positive control.

RESULTS

Dpph radical-scavenging activity: Alpha asarone displayed striking DPPH radical scavenging activities (Fig. 1). DPPH is a stable free radical that can donate a hydrogen atom when it reacts

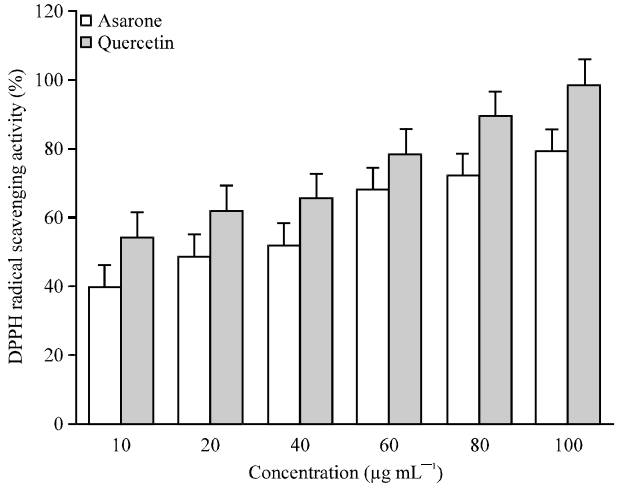

Fig. 1: DPPH radical scavenging activity of alpha asarone

with the phenolic compound present in the samples. Radical scavenging potential of alpha asarone was dose-dependent (10-100 μg mL^{-1}). The higher concentration exhibited 79% (100 μg mL^{-1}) of antioxidant activity and lower concentration exhibited 39% (10 μg mL^{-1}) of antioxidant activity.

Reducing power: Reducing power of alpha asarone was displaying highest activity at 100 μg concentration and activity was found to be dose dependent (Fig. 2).

Superoxide radical scavenging activity: Superoxide radical scavenging activity of alpha asarone depends on the concentration (Fig. 3). The higher concentration exhibited 66% scavenging activity (100 μg) and lower concentration exhibited 37% activity (10 μg).

Ferrous ion-chelating activity: Ferrous ion activity is shown in Fig. 4. The metal chelating ability of alpha asarone was determined at the concentration range from

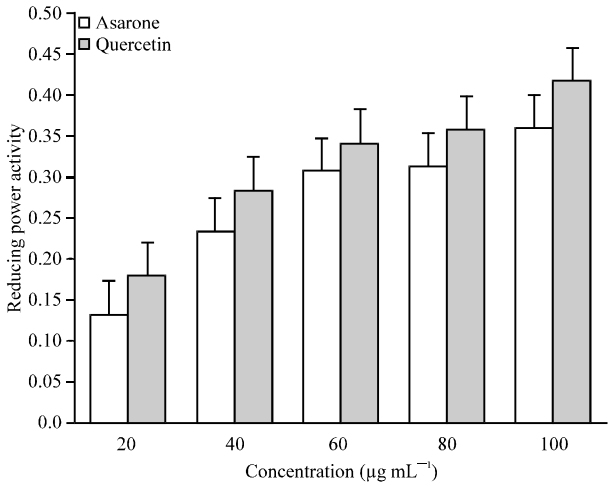

Fig. 2: Reducing power of alpha asarone

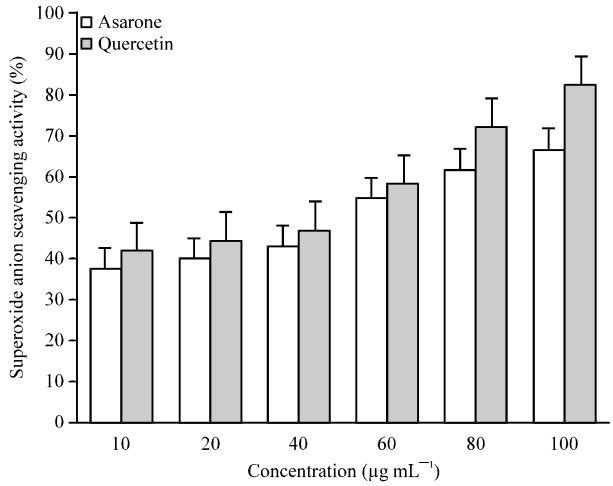

Fig. 3: Superoxide radical scavenging activity of alpha asarone

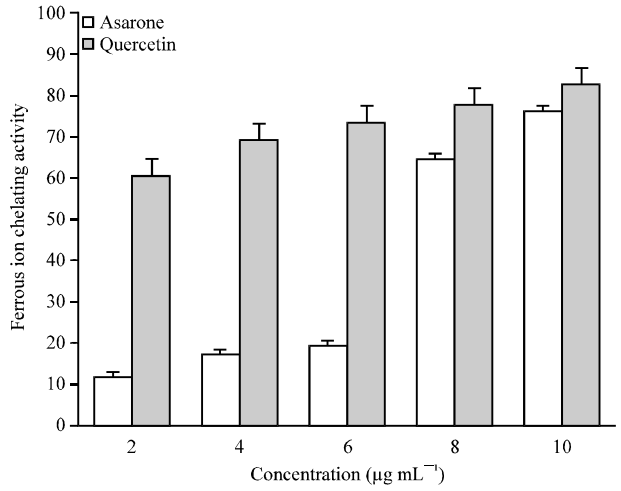

Fig. 4: Ferrous ion-chelating ability of alpha asarone

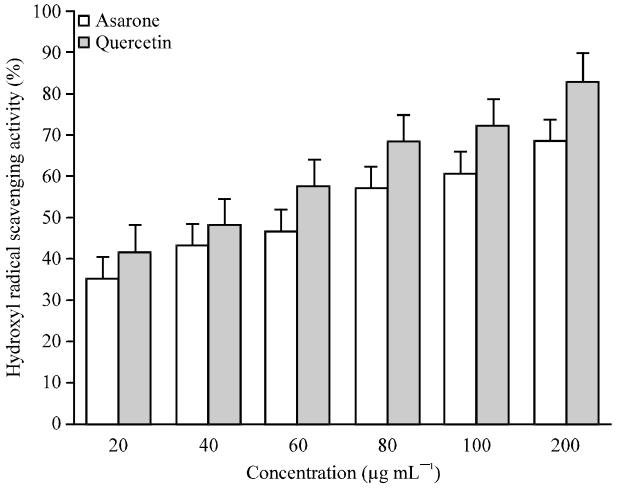

Fig. 5: Hydroxyl radical scavenging activity of alpha asarone

2-10 mg mL^{-1}. Least activity about 19% was observed until 6 mg mL^{-1} concentration but it rapidly increases to 76% with respect to 10 mg mL^{-1} concentration.

Hydroxyl radical scavenging activity: The hydroxyl radical is an extremely reactive in biological systems and has been implicated as highly damaging species in free radical pathology and also damaging bio molecules of the living cells. As in Fig. 5, the hydroxyl radical scavenging activity was higher with respect to higher concentration of alpha asarone present in the sample.

Determination of capability of lipid peroxidation inhibition: The cellular components inactivation and oxidative stress of biological systems take place due to lipid peroxidation. Peroxidation produces several toxic by products that damage the bio molecules. Alpha asarone inhibited lipid peroxidation about 55% at 100 µg concentration (Fig. 6).

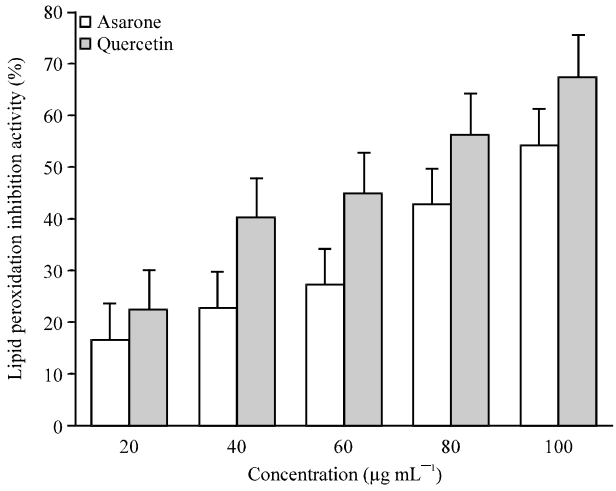

Fig. 6: Capabilities of lipid peroxidation of methanolic extract of alpha asarone

DISCUSSION

Manikandan and Devi (2005) studied an antioxidant property of alpha asarone against noise stress. The compound have shown effectively protective role by normalizing the increased superoxide dismutase and lipid peroxidation, decreased catalase, glutathioperoxidase, vitamin C and E, protein thiols in different regions of the rat brain due to noise exposure. The action of α-asarone against noise stress is attributed to its antioxidant property. Devi and Ganjewala (2011) have studied antioxidant potential of *A. calamus* leaf and rhizome extracts prepared with methanol. DPPH assay, superoxide anion radical scavenging assay was used to determine the antioxidant property. Both rhizome and leaves extract exhibited very strong activity. Our study on antioxidant property of alpha asarone correlates with antioxidant activity of rhizome and leaves extract of *Acorus calamus*. From the result we can suggest that antioxidant activity *Acorus calamus* is from alpha asarone which is one of the major photochemical compound of this plant.

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