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Antioxidant and radio-protective activities of lemon grass and star anise extracts

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ABSTRACT

Aqueous extracts of Lemon grass (*Cymbopogon citratus*) and Star-anise (*Illicium verum*) were prepared and evaluated for antioxidant activity using various in vitro assay systems. The extracts, even at low concentrations, worked as efficient scavengers of DPPH (IC_{50} of 72–90 μg/ml), and superoxide radicals (77–87% scavenging). The antioxidant activity coefficient as determined by β-carotene bleaching assay was high (600–800). Both the extracts exhibited high reducing power indicating good antioxidant potential. Lipid peroxidation, monitored as thiobarbituric acid-reactive substance (TBARS) were significantly lower ($p<0.05$) in irradiated meat containing these extracts. Antioxidant potential of Star anise extract was better than Lemon grass extract. The extracts were able to protect against radiation induced DNA damage in pBR322 plasmid.

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1. Introduction

The use of biologically active compounds from various plants as functional ingredients in food, beverage and cosmetic applications is gaining interest, mainly for providing naturally derived ingredients. These bioactive compounds commonly found in plants have been shown to have possible health benefits with anticarcinogenic, antihypertensive, antimutagenic, antimicrobial and antioxidant activities (Yen, Duh, & Tsai, 2002; John & Shahidi, 2010). The search for safe and effective naturally occurring antioxidants is now focused on edible plants, especially spices and herbs. Crude extracts of plant materials rich in phenolics are of importance in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Natural antioxidants present in plants are being used because of their safety and synthetic antioxidants are being gradually reduced from many food applications. In addition to imparting characteristic flavors, many herbs and spices prolong the storage life of foods by preventing rancidity through their antioxidant activity or through bacteriostatic or bactericidal activity (Beuchat & Golden, 1989).

Lemon grass (*Cymbopogon citratus*) is a widely used herb in tropical countries, especially in Southeast Asia and Africa. In India it is grown in several states and the oil from the plant is used in cosmetics, detergents/soap and beverages. Lemon grass leaves are used as a flavoring ingredient in herbal teas, soups, stews and curries. Star-anise (*Illicium verum*) is a commonly used spice native to southern China and northern Vietnam. The essential oil of star anise is used as a flavoring agent in confectionary. Star anise is an ingredient of the traditional five-spice powder of Chinese cooking and is also used in different Indian curry powders. It is the industrial source of shikimic acid, a primary ingredient used to prepare the anti-flu drug (Tamiflu), which mitigates the severity of the influenza virus (Ohira, Torii, Aida, Watanabe, & Smith,

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2009). The aim of the present study was to investigate the antioxidant and radioprotective potential of aqueous Lemon grass and Star anise extract and to test its efficacy in minimizing oxidative rancidity in chicken meat.

2. Materials and methods

2.1. Chemicals

Agarose, β-carotene, linoleic acid, nitroblue tetrazolium (NBT), butylated hydroxytoluene (BHT), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and catechin were purchased from Sigma Chemical Co. (St. Louis, MO). Thiobarbituric acid (TBA) was obtained from BDH Chemicals Ltd. (Poole, England). Phenazine methosulphate (PMS), deoxyribose, nicotinamide adenine dinucleotide (NADH) disodium salt and ferrozine were purchased from HiMedia (Mumbai, India). $CsCl_2$ purified plasmid DNA of pBR322 was obtained from Banglore Genei Pvt. Ltd, India. All other reagents used were of analytical grade and procured from Qualigens Fine Chemicals (Mumbai, India) or Sisco Research Lab (Mumbai, India).

2.2. Bacterial cultures

Escherichia coli JM109, *Pseudomonas fluorescens* ost5 (16s rRNA gene sequence submitted to Genbank, Accession no. DQ439976) a laboratory isolate, *Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* MTCC 470, were stored in 20% glycerol (v/v) at −20 °C. Before the start of experiments, the cultures were grown on nutrient agar. The isolates were subcultured twice before inoculation.

2.3. Preparation of lemon grass leaf (LGE) and star anise extract (SAE)

Lemon grass (*C. citratus*) and Star anise (*I. verum*) were purchased from a local market. Washed Lemon grass leaves/Star anise (100 g) were refluxed with 1 l distilled water for 1 h. Using cheesecloth, the filtrate was separated and the residue was then re-extracted with an additional 1 l of distilled water. The extracts were then pooled and centrifuged at 2500 × *g* for 20 min. The supernatant was filtered and concentrated using a rotary evaporator (Buchi Rotavapor, Flawil, Switzerland). The concentrates were then lyophilized to form LGE and SAE powders and stored at 4 °C until analyzed.

2.4. Determination of total phenolics and flavonoids

The amount of total phenolics in the extracts was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) using catechin as a standard. The total phenolic content was expressed as catechin equivalents. The flavonoid content of the extracts was estimated according to the colorimetric assay of Kim, Jeong, and Lee (2003). To 1 ml of diluted LGE/SAE, 4 ml of distilled water was added followed by 5% sodium nitrite solution (0.3 ml) and 10% aluminium chloride solution (0.3 ml). Tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1 M sodium hydroxide was added. The volume of reaction mixture was made to 10 ml with distilled water. The mixture was vortexed and the absorbance of the pink colour developed was determined at 510 nm. The results were expressed as catechin equivalents.

2.5. Radical scavenging activity

2.5.1. DPPH radical scavenging activity

DPPH assay was carried out according to the method of Yamaguchi, Takamura, Matoba, and Terao (1998). Appropriately diluted LGE/SAE was mixed with DPPH. The tubes were then vortexed and incubated at room temperature for 20 min (in dark) and the absorbance was measured at 517 nm. Percent DPPH-scavenging activity was calculated as:

[(Control absorbance − Extract absorbance)/(Control absorbance)] × 100.

2.5.2. Superoxide radical scavenging activity

Superoxide radical scavenging activity of LGE/SAE was estimated according to the method of Liu, Ooi, and Chang (1997). The reaction mixture consisted of 1 ml of NBT, 1.0 ml of NADH and 0.5 ml of an appropriately diluted sample. The reaction was initiated by addition of PMS to the mixture. The tubes were incubated at room temperature for 5 min and the absorbance was measured at 560 nm. Decreased absorbance of the reaction mixture indicated increased superoxide anion-scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_o - A_s)/A_o] \times 100,$$

where A_o is absorbance of the control and A_s is absorbance of the sample.

2.6. β-Carotene bleaching assay

The antioxidant capacity was estimated by β-carotene bleaching assay, as described by Velioglu, Mazza, Gao, and Oomah (1998) with some modifications. An emulsion was prepared by mixing β-carotene, linoleic acid, Tween 80 and distilled water. Aliquots of this emulsion were transferred into a series of tubes containing LGE/SAE. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. The tubes were then incubated in a water bath at 50 °C to induce autoxidation. After 60 min the absorbance at 470 nm was recorded. Antioxidant activity coefficient (AAC) was calculated as:

$$\text{AAC} = \frac{A_{S(60)} - A_{C(60)}}{A_{C(0)} - A_{C(60)}} \times 1000,$$

where $A_{S(60)}$ was absorbance of the antioxidant mixture at t=60 min., $A_{C(60)}$ the absorbance of the control at t=60 min., and $A_{C(0)}$ the absorbance of the control at t=0 min.

2.7. Reducing power

The reducing power was determined by the method of Oyaizu (1986). The extracts were mixed with sodium phosphate

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