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Antioxidative properties and stability of ethanolic extracts of Holy basil and Galangal

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Abstract

The aims of this work were to assess the influence of concentration, heat treatment, and pH value on antioxidant activity of ethanolic extracts obtained from Holy basil (*Ocimum sanctum Linn*) and Galangal (*Alpinia galanga*). The antioxidative properties were evaluated. The ethanolic extracts of Holy basil and Galangal showed good heat stability (80 °C, 1 h). At neutral and acidic pH, Holy basil extracts had high antioxidative stability, whereas Galangal extracts showed higher antioxidative stability at neutral than at acidic pH ranges. Antioxidant activity of both extracts at neutral pH was higher than at acidic pH ranges. Holy basil and Galangal extracts exhibited strong superoxide anion scavenging activity, Fe^{2+} chelating activity, and reducing power in a concentration-dependent manner. Antioxidant activity of both extracts correlated well with reducing power. Furthermore, ethanolic extracts of Holy basil and Galangal acted as radical scavenger and also as lipoxygenase inhibitor.

Keywords: *Ocimum sanctum Linn*; *Alpinia galanga*; Antioxidant activity; Scavenging effect; Chelating effect; Reducing power; Lipoxygenase inhibitory activity

1. Introduction

Oxidative deterioration of fat components in foods is responsible for the rancid odors and flavors which decrease nutritional quality. The addition of antioxidants is required to preserve food quality. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ) and propyl gallate (PG) are widely used as antioxidants in the food industry.

Their safety, however, has been questioned. BHA was shown to be carcinogenic in animal experiments. At high doses, BHT may cause internal and external hemorrhaging, which contributes to death in some strain of mice and guinea pigs. This effect is due to the ability of BHT to reduce vitamin K-depending blood-clotting factor (Ito et al., 1986). Therefore, the importance of replacing synthetic antioxidants by natural ingredients from oil seeds, herbs and spices and other plant materials has increased due to health implications and increased functionality which improves solubility in both, oil and water.

It is well known that natural antioxidants extracted from herbs and spices (rosemary, oregano, thyme, etc.) have high antioxidant activity and are used in many food applications (Hirasa & Takemasa, 1998; Nakatani, 1997). A number of studies deal with the antioxidant activity of extracts from herbs and spices (Cuvelier, Berset, & Richard, 1994; Economou, Oreopoulou, & Thomopoulos, 1991; Kikuzaki & Nakatani, 1993; Lu & Yeap Foo, 2001).

Most of the antioxidative potential in herbs and spices is due to the redox properties of phenolic compounds which allow them to act as reducing agents,

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hydrogen donators and singlet oxygen quenchers (Caragay, 1992; Rice-Evans, Miller, & Paganga, 1997).

Currently, there is an increasing demand for new ethnic foods. New ethnic foods also include the emerging cuisines such as Thai, Vietnamese, Indian and Moroccan, which have strong flavors and aromas. Some of the popular ingredients for developing these foods include tamarind, cardamon, lemon grass, basil, galangal etc. (Cousminer & Hartman, 1996; Uhl, 1996). Galangal (*Alpinia galanga*), a rhizome closely related to the ginger family, is commonly used in stir-fries, curries and soups in Southeast Asia. In fact, it has been reported that galangal, which has gingery notes with slightly sour and peppery notes, is an essential component of Thai curry paste (Uhl, 1996). Several researchers have reported that galangal extract showed antioxidant activity in model system (Barik, Kunda, & Dey, 1987; Cheah & Abu Hasim, 2000; Wang, Chen, Liu, & Guo, 1997). Jitoe et al. (1992) observed that all tropical ginger extracts have antioxidant activities in alcohol/water system.

Basil has been traditionally used in Mediterranean and Southeast Asian foods. Types that are most commonly used in European and American cuisine are local sweet basil, lemon basil, purple ruffle and mintier Egyptian basil. Holy basil (*Ocimum sanctum Linn*), an annual herbaceous plant with slightly hairy, pale green leaves, is widely used as flavouring in Southeast Asian cuisine especially in Thai stirred fries. Holy basil leaves are spicy and have lemony notes (Uhl, 1996). Javanmardi, Stushnoff, Locke, and Vivanco (2003) reported that Iranian basils possess valuable antioxidant properties for culinary and possible medicinal use.

Main objectives of this work were to study the antioxidant properties of the ethanolic extracts from Holy basil and Galangal, including free radical scavenging activity, superoxide anion radical scavenging activity, Fe^{2+} chelating activity, lipoxygenase inhibitory activity, and reducing power. Effects of concentration, heat, and pH on the antioxidant activity of Holy basil and Galangal extracts were also determined.

2. Materials and methods

2.1. Chemicals

Nitroblue tetrazolium (NBT) [No. 2060-67-4], linoleic acid (99%) [No. 60-33-1], 2,2-diphenyl-2-picrylhydrazyl (DPPH) [No. 1898-66-4], 3-(2-Pyridyl)-5-6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine) [No. 2741-96-3], ferrous chloride [No. 10025-77-1], nicotin-amide adenine dinucleotide (NADH) [No. 2101-23-3], ethylenediaminetetraacetic acid (EDTA) [No. 13235-36-4], trichloracetic acid (TCA) [No. 2009-27-2], polyoxyethylenesorbitan monolaurate (Tween 20) [No. 905-64-5] and EC 1.13.11.12 lipoxygenase [No. 2328-53-1] were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents used were of analytical grade and were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany).

2.2. Materials

Fresh Holy basil (*Ocimum sanctum Linn*) leaves and Galangal (*Alpinia galanga*) rhizomes, imported from various locations in Thailand, were purchased from Asian supermarkets in Vienna, Austria. Samples were cleaned, washed with water, cut into small pieces, dried overnight in an air dryer (Memmert-GmbH + Co.KG, type UM 200–800, Germany) at 40 °C, ground to a particle size of 25 mesh by using a grinder (Moulinex, Type MCU 1A, France), and stored at −20 °C in an airtight container until used.

2.3. Extraction procedure

In pre-trials, antioxidative properties such as total phenolic content, reducing power, and antioxidant activity of Holy basil and Galangal were influenced by extraction conditions. Optimum conditions for extraction of antioxidants from Holy basil and Galangal in these trials were the following.

Dried Holy basil powder (4.50 ± 0.05 g dry basis) were extracted by stirring with 50 ml of ethanol and water (3:1, v/v) at 75 °C and 300 rpm for 30 min, whereas, dried Galangal powder (4.5 g dry basis) was extracted with 50 ml of ethanol and water (1:1, v/v) at 50 °C for 1 h. Each extract was then filtered through filter paper (595 1/2 folded filters, ø125 mm, Ref. No. 10311644, Schleicher and Schuell GmbH, Germany); the filtrate was collected and dried by a rotary evaporator (Büchi rotavapor (R), Switzerland) at 40 °C, filled in a plastic bottle and stored at −20 °C until used.

2.4. Properties of Holy basil and Galangal extracts

2.4.1. Effect of concentration

Different concentrations of ethanolic extracts (0.10, 0.25, 0.50, 0.75 and 1.0 mg/ml) were used and antioxidant activity was determined according to the method described by Taga, Miller, and Pratt (1984).

2.4.2. Effect of heat treatment

Dried ethanolic extract (1.00 ± 0.01 g) was placed in a 25 ml beaker and heated in an oven (Memmert-GmbH + Co.KG, type UM 200–800, Germany) at 80 °C for 30 and 60 min, cooled to room temperature, and dissolved in ethanol to produce a final concentration of 1.0 mg/ml. The solution was investigated for antioxidant activity using the β-carotene bleaching method (Taga et al., 1984).