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Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts

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Abstract

The antimicrobial and antioxidant activities of Indian gooseberry (*Phyllanthus emblica* Linn.) and galangal (*Alpinia galanga*) extracts were investigated. Two different methods (disc diffusion and agar dilution methods) were employed to evaluate the antimicrobial activities of plant extracts against *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) values of Indian gooseberry and galangal extracts were found to be 13.97 and 0.78 mg/ml and the minimum biocidal concentration (MBC) values were 13.97 and 2.34 mg/ml, respectively. The antioxidant activities of Indian gooseberry and galangal extracts, which were evaluated by the β-carotene bleaching method, were 86.4% and 70.3%, respectively. The total phenolic contents of Indian gooseberry and galangal extracts, as determined by the Folin–Ciocalteu method, were 290.4 ± 0.7 and 40.9 ± 0.2 mg/g plant extract (in GAE), respectively. The GC–MS analysis showed that the main compounds of galangal extract are 1,8-cineole (20.95%), β-bisabolene (13.16%), β-caryophyllene (17.95%) and β-selinene (10.56%). On the other hand, the use of high-performance liquid chromatography (HPLC) with UV detection indicated many compounds within the Indian gooseberry extract.

Keywords: GC–MS; Minimum inhibitory concentration; Natural antioxidants; *Staphylococcus aureus*; Total phenolic contents; UV-HPLC

1. Introduction

Herbs and spices are known for their antimicrobial and antioxidative properties. Due to an increasing demand for natural food additives, herbs and spices have emerged as popular ingredients and have a tendency of replacing synthetic antimicrobial and antioxidant agents. Generally, essential oils of spices possess strong antibacterial properties against foodborne pathogens and contain high concentrations of phenolic compounds (Burt, 2004; Delaquis, Stanich, Girard, & Mazza, 2002; Nevas, Korhonen, Lindström, Turkki, & Korkeala, 2004). These compounds exhibit a wide range of biological effects, including antioxidant properties.

Indian gooseberry (*Phyllanthus emblica* Linn.) or "Makhaam Pom" in Thai or "Amla" in Hindi is one of the most often used herbs and is widely available in most tropical and subtropical countries. Its fruit is reputed to probably have the highest content of vitamin C compared with any other naturally occurring substances in nature. Active extracts of *P. emblica* have been shown to possess several pharmacological properties, e.g., analgesic, anti-inflammatory, antioxidant and chemoprotective activities (Khan et al., 2002).

Galangal (*Alpinia galanga*) or "Kha" in Thai has traditionally been used as spice in Thai foods. This spice is, like other spices, rich in phenolic compounds such as flavonoids and phenolic acids. In Thailand, galangal is used for medical purposes such as carminative, stomachic, antispasmodic, antiphlogistic and antibacterial drugs. Galangal is also readily available and inexpensive in Thailand. Among the predominant bacteria involved in food-borne diseases, *Staphylococcus aureus* is one of the leading causes of gastroenteritis resulting from the consumption of contaminated foods. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins in foods. *S. aureus* is an important pathogen due to a combination of toxin-mediated virulence,

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invasiveness and antibiotic resistance (Jablonski & Bohach, 2001).

Since food processors and consumers have expressed a desire to reduce the use of synthetic chemicals in food preservation, common culinary herbs and spices that exhibit antimicrobial and antioxidant activities could be a source of natural alternatives. Although herbs and spices in Thailand, including Indian gooseberry and galangal, contain potent antimicrobials and antioxidants, they have not been sufficiently tested for their activities. The aims of the present investigation were therefore to assess the antimicrobial and antioxidant activities of Indian gooseberry and galangal, which are common plants of Thailand. From our preliminary study, it was found that Indian gooseberry and galangal juices exhibited very low antimicrobial and antioxidant activities, hence only the ethanolic extracts of these plants were investigated in the present study. The antimicrobial activities of the extracts were determined by the disc diffusion and agar dilution methods, while the antioxidant activities were determined by the β-carotene bleaching method. The chemical composition of the galangal extract and of its fractions was studied using gas chromatography–mass spectrometry (GC–MS). The composition of the Indian gooseberry extract was determined by the UV-high performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Chemicals

Ascorbic acid was purchased from Riedel-de-Haën (Seelze, Germany). Linoleic acid, β-carotene and gallic acid were obtained from Fluka (Buchs, Switzerland). Folin–Ciocalteu reagent, orthophosphoric acid, sodium carbonate and absolute ethyl alcohol were purchased from Carlo Erba (Vigevano, Italy) while polyoxyethylene (20) sorbitan monolaurate (Tween 20) was obtained from BDH (Dorset, England). Methanol and acetronitrile were of HPLC grade and were purchased from Lab-Scan Analytical Sciences (Bangkok, Thailand). For antimicrobial tests Tryptic Soy Agar (TSA), Tryptic Soy Broth (TSB), Mueller Hinton agar (MHA) and buffer peptone water were purchased from Difco (Detroit, USA).

2.2. Materials

Indian gooseberry fruits (*P. emblica* Linn.) and galangal (*A. galanga*) rhizomes were purchased from a local market. The Indian gooseberry fruits were first washed thoroughly to remove impurities. After washing the fruits were cut into small pieces and dried overnight in a tray dryer at 40 °C. They were then ground with a blender (Waring, model HGB2WT, Torrington, CT) to make a powder. For preparation of galangal, the fresh rhizomes were cleaned, washed with water, cut into small pieces and dried in a tray dryer at 40 °C, after which they were ground in a blender to make a powder.

2.3. Extraction procedures

To prepare Indian gooseberry extract, the dried Indian gooseberry powder (10 g dry basis) was extracted with 50 ml of 95% ethanol (Ahmad, Mehmood, & Mohammad, 1998). The extract was filtered through a filter paper (Ø110 mm, Cat. no. 1001 110, Schleicher and Schuell GmbH, Dassel, Germany); the filtrate was collected and concentrated in a rotary evaporator (Resona Technics, Labo Rota 300, Gossau, Switzerland) at 40 °C for 10 min and kept at 4 °C until its use.

The dried galangal powder (10 g dry matter) was extracted with 100 ml of 95% ethanol (Oonmetta-aree, Suzuki, Gasaluck, & Eumkeb, 2006) and left at room temperature overnight. The extract was filtered through a filter paper (Ø110 mm, Cat. no. 1001 110, Schleicher and Schuell GmbH, Dassel, Germany); the filtrate was collected and concentrated by the rotary evaporator at 40 °C for 15 min and kept at 4 °C until its use.

2.4. Antimicrobial activity evaluation

2.4.1. Microbial culture

S. aureus (ATCC 25923) was obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. The microorganism was maintained in TSA at 5 °C. Stock culture of *S. aureus* was grown in TSB at 37 °C for 18 h at 160 rpm (cell in early stationary phase). The maximum level of the microorganism was 10^{10} CFU/ml; the concentration was subsequently adjusted to 10^{8} CFU/ml using buffer peptone water.

2.4.2. Disc diffusion method

The agar diffusion method was employed for screening of the antimicrobial activities of the extracts. Briefly, a suspension of the tested microorganism (0.1 ml of 10^{8} CFU/ml) was spread on the MHA. Filter paper discs (6 mm in diameter) were soaked with 15 µl of the extracts and placed on the inoculated plates (Vardar-Ünlü et al., 2003). After being kept at 4 °C for 2 h, the plates were incubated at 37 °C for 24 h. The diameters of the inhibition zones were then measured in millimeters. All experiments were performed in duplicate.

2.4.3. Agar dilution method

A series of two-fold dilutions of each extract, ranging from 20 to 0.1 ml/l, were prepared in MHA with 5 ml/l Tween 20. Plates were dried at 37 °C for 30 min prior to inoculation with 1–2 µl spots containing approximately 10^{4} CFU of microorganism (Hammer, Carson, & Riley, 1999). MHA, with 5 ml/l Tween 20 but with no extracts, was used as a positive growth control. Inoculated plates were incubated at 37 °C. The minimum inhibitory concentration (MIC) was determined as the lowest concentration