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Factors influencing antioxidant activities and total phenolic content of guava leaf extract

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ABSTRACT

The aim of this study is to investigate the influence of certain factors on the yield, antioxidant activity (AA) and total phenolic content (TPC) of guava leaf extract. The effects of pretreatment of leaf sample prior to extract, extraction method, and the leaf age were investigated. Folin–Ciocalteau was used to determine the TPC. Trolox equivalent antioxidant capacity (TEAC) and equilibrium concentration (EC) were used for evaluation of AA. Results indicated that ultrasonication is the most suitable method for guava leaf extraction as it yielded the extract with the significantly highest TPC and AA. Blanching followed by ice water cooling (BCD) was suggested for the pretreatment process of guava leaves. The study of leaf maturity demonstrated that the highest activity was from the young leaves. Hot water was the best solvent to extract the active principles. The extract of BCD pretreated young leaves, extracted by hot water exhibited the highest TPC and AA with the TEAC and EC values of 24.30 ± 0.50 and 20.41 ± 0.67 mM/mg, respectively. These values are 1.88 and 8.72 times higher than the synthetic antioxidant butylated hydroxy toluene (BHT) and 1.75 and 1.21 times higher than vitamin E, respectively. It was concluded that pretreatment and drying process, method of extraction and leaf maturity played important roles on the bioactive compounds and their antioxidant power of guava leaf extract.

1. Introduction

Antioxidants which are used nowadays are obtained mainly from two major routes; chemical synthesis and natural living source extraction. According to scientific research, severe toxicity caused by chemical synthetic antioxidants such as genotoxicity, carcinogenicity (Ito et al., 1986; Williams, Iatropoulos, & Whysner, 1999), or hepatotoxicity (Safer & Al-Nughamish, 1999) has been increasingly reported. Hence, the use of synthetic antioxidants is tending to decrease and needs replacement with other safer compounds. Meanwhile, natural antioxidants, derived mostly from their plants have been reported for high potential in prophylacsis and treatment of many degenerative diseases caused by chain oxidative reactions such as atherosclerosis, coronary heart disease, aging and cancer (Finkel & Holbrook, 2000). An inverse relationship has been reported between consumption of natural antioxidants and mortality from such degenerative diseases (Govindarajan, Vijayakumar, & Pushpangadan, 2005). Therefore, the search for non-toxic high potential natural antioxidants is of increasing interest. Many authors present their work about antioxidant activities from different plant sources (Cai, Luo, Sun, & Corke, 2004; Okonogi, Duangrat, Anuchpreeda, Tachakittirungrod, & Chowwanapoonpohn, 2007). Guava (*Psidium guajava*), one of the most effective edible plants, has long been used as a traditional medicine. It has been demonstrated to have several biological activities such as antidiabetic (Oh et al., 2005), anticough, antibacterial (Jaiarj et al., 1999) and antispasmodic actions (Lozoya et al., 2002). Recently, it has been reported to possess high potential for antioxidant activity (Guo et al., 2003). Besides the plant cultivar, other factors have been revealed to influence the quality of plant extracts. The use of high temperature in the drying process or in the extraction of the plant sample is generally expected in order to prevent the compounds from deteriorating due to moisture content or existing enzyme, resulting in extended shelf life or higher activity of the active compounds. For example lycopene, a heat-stable natural antioxidant found in tomato fruits (Nicoli, Anese, & Parpinel, 1999; Stahl & Sies, 1992) demonstrated a higher antioxidant activity through thermal treatments of tomato fruits (Chen, Wu, Tsai, & Liu, 2000). However, enzymatic and/or non-enzymatic processes that may occur during the drying process may lead to significant changes in the composition of photochemicals (Capecka, Marcezeek, & Leja, 2005). In addition, the high temperature may accelerate the degradation

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reaction of certain compounds (Burg & Fraile, 1995; Tomaino et al., 2005), resulting in decreasing shelf life or activity of the active principles. Several vitamins, for example, show rapid degradation with the increasing temperature. The age of the plant materials is also one of important factors that should be considered. Klavsen and Madsen (2008) revealed that some mechanisms and photosysthesis in *Littorella uniflora* were depended on the leaf age of plant. Hence, to obtain plant extracts with the highest potential, there is a need to understand precisely those factors which might affect the interested activity. Although guava has been reported by many authors for its promising antioxidant activity, no research has been carried out to investigate the factors which affect antioxidant activity in guava. This study was the first to determine the influence of parameters in the extraction process on the antioxidant activity and total phenolic content of guava. Those parameters were involved mainly in the difference between extraction methods, the pretreatment and drying process. Since our previous study found that the leaf was the most effective part of this plant (Tachakittirungrod, Okonogi, & Chowwanapoonpohn, 2007), in this study the influence of leaf maturity was also investigated as well as the polarity of solvent used in the extraction process.

2. Materials and methods

2.1. Chemicals

Gallic acid, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), ferric chloride ($FeCl_3 \cdot 6H_2O$), and potassium persulfate were from Sigma (MO, USA). Trolox was obtained from Aldrich Chemical Company (Steinheim, Germany) and 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ) was from Fluka Chemicals (Buchs, Switzerland). Ethanol, ethyl acetate and hydrochloric acid were from Merck (Darmstadt, Germany). All other reagents were of the highest quality grade available.

2.2. Plant materials

Plants generally exhibit chemical composition change according to the cultivar and environmental conditions such as climate and soil (Thaipong, Boonprakob, Cisneros-Zevallos, & Byrne, 2005). To fix the variations of cultivar, only one flesh clone of guava (*P. guajava)* harvested from Sopa garden (Chiang Mai, Thailand) was used throughout this study. The harvesting time was also limited to the second half of July, 2007 in order to overcome the variations due to the environmental condition.

2.3. Extract preparation

2.3.1. Effect of pretreatment

In this study, the middle age leaf (ML) was used according to its location majority in the plant and its stage of maturity. The sample was divided into three groups and each was treated before drying as followed: blanching in boiling water for 30 s and after that immersion in ice water for 15 min (BCD); blanching in boiling water for 30 s and then exposure to 30 °C for 15 min (BD); and no treatment group (FD). After that each group was divided into two categories according to the drying conditions of 30 °C for 72 h (drying #1) and 50 °C for 20 h (drying #2). Each dried leaf sample was pulverized into fine powder then subjected to extraction process by ultrasonication for 10 min × 3 using ethanol as the extraction solvent. The solvent was removed by using rotary evaporation under vacuum at 45 °C. The obtained extracts were kept in light-protected containers at 4 °C until total phenolic content and antioxidant activity were analyzed.

Fig. 1. Macroscopic characters of guava leaves in different stage of maturity; young age leaf (a), middle age leaf (b) and old age leaf (c).

2.3.2. Effect of extraction method

In this study, the extraction solvent used was ethanol and the guava leaf sample was the BCD with drying #2 pretreated leaf powder of ML sample. Four different extraction techniques were used: maceration at ambient temperature for 24 h × 3 without stirring; maceration at ambient temperature for 24 h × 3 with 200 rpm stirring; ultrasonication at ambient temperature for 10 min × 3; soxhlet extraction at the boiling point of ethanol for 4 h. The crude extract was obtained by evaporation of the solvent under vacuum at 45 °C. The extracts were kept in light-protected