Positive Relationship between Anthocyanin and Corosolic Acid Contents in Leaves of Lagerstroemia speciosa Pars.

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Abstract Corosolic acid content and distribution in Lagerstroemia speciosa Pars. were investigated, indicating that the red leaves contained more corosolic acid than the green leaves and other plant parts such as petals, roots and seeds. The leaf redness was derived from cyanidin 3-O-glucoside which was first identified in this species. The contents of corosolic acid and cyanidin 3-O-glucoside were found to be well correlated, supporting the traditional perception of local people of the Philippines that red leaf banaba tea exerts more pronounced medicinal effects.

Key words: Anthocyanin, Banaba, Diabetes, The Philippines, Triterpene

Introduction

Lagerstroemia speciosa Pars. is a semi-deciduous tree belonging to Lythraceae which is quite common throughout the Philippines (called “Banana” in Tagalog). It can reach a height of up to 20 m with hard and ovule-shaped leaves, 15-20 cm in length, and blooming beautiful pink or purple flowers which form a conical inflorescence.

In the Philippines, Banaba is well known as a traditional medicinal plant, especially effective for the treatment of diabetes, obesity, constipation and skin disorders (Garcia, 1940). Banaba has also been reported to prevent HIV infection in Bangladesh (Faruk et al., 2002). These medicinal effects of Banaba were attributed to the presence of corosolic acid (Fig. 1) or other water-soluble tannins (klein et al., 2007). Corosolic acid is considered to be one of the most effective ingredients of Banaba (Judy et al., 2003), which can be used as a medicine for metabolic syndromes. Therefore, there has been a high demand in the USA, Europe and Japan for both Banaba tea and corosolic acid recently.

Corosolic acid which is a triterpene acid (2 α-hydroxyursolic acid), identified in the methanol extract of L. speciosa has been reported to exert a significant glucose transport-stimulating activity (Murakami et al., 1993). This compound is also found in other plant species like Pyrus calleryana (El-Hawary et al., 2003), Monochaetum vulcanicum (Chaturvedula et al., 2004), Parthenocissus tricuspidata (Saleem et al., 2004), Prunus serrulata (Jung et al., 2004), Perilla frutescens (Banno et al., 2004), Hyssopus officinalis (Skrzypek and Wysokinska, 2003), and Chaenomeles sinensis (Gao et al., 2003). However, L. speciosa is the major plant among these plant species for the extraction of corosolic acid due to the presence of a large number of leaves that can be collected at once without serious damage to the plant itself.

It had empirically been recognized that high-quality Banaba tea can be prepared using red leaves, presumably due to their higher content of corosolic acid compared to green leaves, although such data had not been reported yet. In the present paper, therefore, attempts were made to elucidate the content of corosolic acid in green and red leaves, as well as in flower buds, petals, seeds and roots. Then we identified the anthocyanin of red Banaba leaf and petal, and finally analyzed the relationship between anthocyanin accumulation and corosolic acid content.

Materials and Methods

Distribution of corosolic acid in Banaba plant

Green leaf, red leaf, flower bud, petal, seed, and root of Banaba (fifty mg each) were collected at 10 o’clock...
in the morning during the period of 12-16 March, 2006 from 5-year-old trees grown in the garden of Use Techno Corporation Company, located in Angels city, Pampanga state, the Philippines. The samples were immediately put into a 750 µl methanol solution kept at 75°C and sonicated for 30 minutes. The extracted solutions were passed through a 0.22 µl disk filter, and then the filtered solutions (20 µl) were analyzed by Shimadzu HPLC system using a Wakopak ODS column with a UV detector. The extract was eluted with 85% MeOH at 1 ml/min and passed through a Wakosil II 5C18 column (I.D. 4.6×200mm: Wako Pure Chemical Industries, Ltd.) and detected using a UV detector at 210nm absorbance. Experiments were replicated three times.

**Identification of anthocyanins in Banaba leaf and petal**

Extraction and isolation were carried out as follows; fresh red leaves (5 g) and petals (5 g) of Banaba tree were collected on March 16, 2006 and extracted with FM (HCOOH/MeOH=8:92). After filtration and concentration, the anthocyanin was isolated by preparative paper chromatography (PPC) using solvent systems: BAW (n-BuOH/HOAc/H₂O=4:1:5, upper phase) and 15%HOAc. The isolated anthocyanin was purified by Sephadex LH-20 column chromatography using solvent systems: MeOH/HOAc/H₂O=70:2:25.

The isolated anthocyanins were identified by UV-visible spectroscopy, HPLC-ESI-MS (Mass spectrometry), acid hydrolysis, and HPLC comparison with authentic specimens of cyanidin 3-O-glucoside (Hattori and Hayashi, 1937), malvidin 3,5-di-O-glucoside (Takemura et al., 2005) and malvidin 3-O-glucoside (Takemura et al., 2008).

**Results and Discussion**

**Distribution of corosolic acid in Banaba plant**

The results are shown in Fig. 2, indicating that the distribution of corosolic acid in the Banaba plant was highest in red leaf, followed by petal, green leaf, flower bud and lowest in seed and root. Since red leaves contained almost twice the amount of corosolic acid as green leaves, red leaves were used for the production of high quality-Banaba tea rich in corosolic acid. For the first time, it was elucidated that the petals of Banaba contained a large amount of corosolic acid. As a result, it was found that corosolic acid displayed a high accumulation in red leaf and petal, which both contained high levels of anthocyanins.

**Identification of anthocyanins in Banaba leaf and petal**

In the present study, three anthocyanins, i.e., cyanidin 3-O-glucoside (A1), malvidin 3,5-di-O-glucoside (A2), and malvidin 3-O-glucoside (A3) were identified in Banaba leaves and petals. The distribution of each anthocyanin was investigated by HPLC-ESI-MS analysis. The contents of anthocyanins in different parts of the Banaba plant are shown in Fig. 2.

**Relationship between corosolic acid and anthocyanin contents in banaba leaves with various degrees of redness**

Banaba leaves with various degrees of redness were collected from Banaba trees in the garden of Use Techno Corporation Company located in Angels city, Pampanga state, the Philippines during the period of 12-16 March, 2006. Corosolic acid content was analyzed by the same method as that described above and qualitative HPLC analysis of anthocyanin was performed with Shimadzu HPLC systems using Senshu Pak, PEGASIL ODS column (I.D. 6.0×150 mm, Senshu Scientific Co., Ltd.), at a flow-rate of 1.0 ml min⁻¹, detection: 190-600 nm, and eluents: H₃PO₄/CH₃COOH/MeCN/H₂O = 3:8:6.83.

![Fig. 2 Corosolic acid distribution in Lagerstroemia speciosa](image-url)

Samples were collected at 10 o’clock in the morning during the period of 12-16 March 2006. Data were indicated in means (n=3) with SE (vertical bars). Different alphabetical letters show significant differences by Fisher’s LSD test (p<0.05).
(A2) and malvidin 3-O-glucoside (A3) were isolated and identified from *Lagerstroemia speciosa*. Compound A1 was isolated from the leaves and the latter two compounds from the petals. A2 has been identified from this species by Lowry (1976), while A1 and A3 were isolated from the plant for the first time. Chemical characteristics of these three compounds are as follows:

**Cyanidin 3-O-glucoside (Chrysanthemin, A1, Fig. 3).** UV λ max nm: 0.01%HCl-MeOH 281, 528; +AlCl3, 313, 569. HPLC-ESI-MS: m/z 449 [M+H]+ (cyanidin + 1 mol glucose), 287 [M-162+H]+ (cyanidin). HPLC: retention time 7.38 min. Acid hydrolysis: cyanidin and glucose.

**Malvidin 3,5-di-O-glucoside (Malvin, A2, Fig. 4).** UV λ max nm: 0.01%HCl-MeOH 276, 535; +AlCl3, 276, 535. HPLC-ESI-MS: m/z 655 [M+H]+ (malvidin + 2 mol glucose), 493 [M-162+H]+ (malvidin + 1 mol glucose), 331 [M-324+H]+ (malvidin). HPLC: retention time 10.15 min. Acid hydrolysis: malvidin and glucose.

**Malvidin 3-O-glucoside (Oenin, A3, Fig. 5).** UV λ max nm: 0.01%HCl-MeOH 277, 536; +AlCl3, 276, 536. HPLC-ESI-MS: m/z 493 [M+H]+ (malvidin + 1 mol glucose), 331 [M-162+H]+ (malvidin). HPLC: retention time 17.70 min.

Although Lowry (1976) had reported that peonidin 3,5-di-O-glucoside was also found in the flower of *Lagerstroemia speciosa* in addition to malvidin 3,5-di-O-glucoside, the former could not be detected in our observation.

**Determination of corosolic acid and anthocyanin contents in banana leaves with various degrees of redness**

The results (Fig. 6) revealed a high correlation between the corosolic acid content and anthocyanin accumulation (R=0.877). It was observed that the redder leaves contained a large amount of corosolic acid, supporting the common perception of local people of the Philippines that red leaf Banana tea exerts more pronounced medicinal effects. In addition, cyanidin 3-O-glucoside (A1), a foliar anthocyanin of Banana, has been reported to be a strong antioxidant (Tsuda et al., 1994).

The possible physiological roles of foliar anthocyanins in the leaves of tropical plants included temperature-raising effect (Smith, 1909), photoprotection under high light environment (Gould...
and Lister, 2006), screen effect against harmful UV-B radiation (Caldwell, 1981, Lee and Lowry, 1980), defense effect against herbivores through direct toxicity or through warning or cryptic coloration (Stone, 1979), as well as antifungal effect (Coley and Aide, 1989), while the role of corosolic acid in Banaba itself had not yet been described. It is difficult to elucidate the physiological effect of correlated accumulation of anthocyanin and corosolic acid. However, under some stressful conditions, chemical diversity of plants is known to shift to defense metabolism, so that the carbon fluxes from the primary to the secondary metabolic pathways are markedly stimulated (Iriti and Faoro, 2009). In practice, however, it appears that the use of red Banaba leaves enables to produce high-quality tea with a large amount of corosolic acid.

Because Banaba can grow in rather sterile lands, the plant can be used for phytoremediation in devastated areas such as those affected by the eruption of Mount Pinatubo in June 1991. Based on our study, Banaba plant can play a role in creating a new business for Aborigine tribes in the Philippines, including the export red Banaba leaves to developed countries to meet the demand for natural medicinal commodities to treat life-style-related diseases.

References


