

# Determination of Bioactive Compounds in Roots of Different Ages *Pueraria mirifica*, Airy Shaw *Suvatandhu* and *Butea superba*, Roxb. from Various Locations in Thailand

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## Abstract

**Bioactive compounds from roots of White Kwao Krua (*Pueraria mirifica*) and Red Kwao Krua (*Butea superba*) extracted by organic solvents were determined from HPLC fingerprints and compared with standard isoflavonoids (puerarin, daidzein and genistein). Roots of White Kwao Krua from Chiang Mai province harvested at the age of 6 y old gave the highest amounts of puerarin, daidzein and genistein at 290, 89 and 16 mg/kg of the dried root respectively. Roots that were younger or older than 6 y old appeared to contain less amount of active compounds. Red Kwao Krua from Chiang Mai had the highest amount of the active compounds. Puerarin contents in Red Kwao Krua were lower whereas daidzein and genistein were greater than those found in White Kwao Krua. The contents of puerarin, daidzein and genistein in Red Kwao Krua from Chiang Mai were 1.9, 37.2 and 4.5 mg/kg of the dried root respectively. For miroestrol contents, the highest amount of 45.0 mg/kg of the dried root was found in White Kwao Krua collected from Chiang Mai province at the age of 5.5 y old. No miroestrol could be determined from roots of Red Kwao Krua. This study suggested that both isoflavonoids and miroestrol contents in the two plants depended on ages and locations of cultivation in Thailand. Information from this study can be applied for the selection of sources and ages of the plants that contain high amounts of the bioactive compounds for herbal formulations.**

## INTRODUCTION

White Kwao Krua (*Pueraria mirifica*) and Red Kwao Krua (*Butea superba*) are Thai medicinal plants, widely used for generations by the native northern Thais. They have been used as tonics for longer life, rejuvenation, improving the muscle tone, and firming of the body. The juice or powder from sun-dried flesh of White Kwao Krua root are mixed with cow's milk or honey and prepared as pills in northern Thai folk medicines (Anusarnsunthorn, 1932). White Kwao Krua and Red Kwao Krua are two of the four Kwao Krua types, which have been included in the recipe of northern Thai traditional medicines for centuries. Previous research reported that at least three important groups of chemicals were found in White Kwao Krua (Ingham et al., 1986): coumarins, flavonoids (daidzein, daidzin, genistin, genistein, puerarin) and chromene (miroestrol). The claim of anti-aging and rejuvenation effects of this plant appears to be from these compounds, especially miroestrol which is a known potent estrogenic principle (Lakshnakara et al., 1952; Cain, 1960). Red Kwao Krua has been used traditionally for men as a phytoandrogen in many Thai folk medicinal recipes. It has been found to contain butenin and butin as well as other compounds that are also found in White Kwao Krua (Manosroi et al., 2002). The bioactivity of these two plants is correlated to the amounts of bioactive compounds, which depend on root ages and locations of cultivation. This study compared the contents of some bioactive compounds found in the roots of White Kwao Krua and Red Kwao Krua harvested at different ages from various locations in Thailand.

## MATERIALS AND METHODS

Eleven root samples of White Kwao Krua were collected from Chiang Mai, Chiang Rai and Kanjanaburi provinces in Thailand. The average ages of the plant were determined from the annual rings of at least three tuberous roots of the same plant. Two root samples of Red Kwao Krua were collected from Chiang Mai and Kanjanaburi provinces. The root samples were chopped into small pieces, dried at  $50\pm 2^\circ\text{C}$  and ground into powder, and extracted by methanol (AR grade) using a soxhlet apparatus. The extract was filtered through a filter paper (Whatman No. 42). and dried by rotary evaporator (Buchi, Rotavapor R-124, Switzerland). Percentage yields and physical characteristics of the dried crude extracts were examined. The fingerprint chromatogram of each crude extract was performed by HPLC (Thermo Separation Products, TSP, UV 1000/P2000). The HPLC conditions for puerarin were - mobile phase: methanol/water (2:3), flow rate 1.0 mL/min, column: Luna 10  $\mu$  C<sub>18</sub> 100° A (250 x 4.6 mm) and UV detector at 254 nm. For daidzein, genistein and miroestrol the conditions were - mobile phase: acetonitril/water (1:1), flow rate 1.0 mL/min, column: Luna 10  $\mu$  C<sub>18</sub> 100° A (250 x 4.6 nm) and UV detection at 254 nm. Amounts of puerarin (P5555), daidzein (D7802) and genistein (G6649) in each extract were analyzed by comparing the HPLC fingerprint chromatogram of extracts with standard isoflavonoids (Sigma, St. Louis, MO). Contents of these compounds were calculated in percentages of dried root. Purified miroestrol ( $\approx 85\%$  purity as determined by HPLC and identified by NMR) was prepared by the method previously described with some modifications (Ingham et al., 1986; Jones and Pope, 1961). The amounts of miroestrol in each extract were determined by comparing the HPLC fingerprint chromatogram with purified miroestrol. Since no standard miroestrol was available in the market, we isolated this compound in purified form according to the method previously described (Jones and Pope, 1961). This purified compound was identified by NMR, IR, spectrophotometry indicating the maximum absorption at 254 nm and one single peak by HPLC at 254 nm (data not shown). This purified miroestrol was used as the reference standard to determine the amount in the crude extract samples. Contents of miroestrol were calculated in mg/kg of the dried root.

## RESULTS AND DISCUSSION

The maximum percentage yield of crude extract was 25.72% of White Kwao Krua found in the sample from Chiang Mai province. Among the three bioactive isoflavonoid compounds in all samples, puerarin was the highest (Table 1). Genistein was the lowest and not found in some root samples. This may be due to the instability of this compound. White Kwao Krua at 6 y old collected from Chiang Mai province Site No. 4 and Site No. 2.4 gave the highest amount of puerarin 29 mg/kg and daidzein 8.9 mg/kg, respectively, while at the age of 5.5 y old at Site No. 3 gave the highest amount of genistein of 1.6 mg/kg. Isoflavonoid contents in tuberous roots of White Kwao Krua collected from Chiang Mai (Site No. 2) at different ages were determined by HPLC fingerprint chromatogram and compared to reference standards. No genistein was found in roots of all ages collected at this site (Table 1). It appeared that the amounts of isoflavonoids (puerarin and daidzein) depended on the root ages. The highest amount puerarin (15.2 mg/kg) was obtained at 5 y old in Site No. 2.2 whereas the highest amounts of daidzein of 8.9 and 7.3 mg/kg were found at 6 y old in Site No. 2.4 and 14 y old in Site No. 2.6, respectively. However, only minute amounts of these three isoflavonoid compounds were found in Red Kwao Krua collected from Kanjanaburi province. But, Red Kwao Krua roots from Chiang Mai province had isoflavonoid contents, especially daidzein, more than that from Kanjanaburi province. Red Kwao Krua from Chiang Mai province appeared to contain less puerarin, but more daidzein and genistein than White Kwao Krua collected from the same province.

The amounts of miroestrol ranged from 2.4 to 45.0 mg/kg of the dried root of White Kwao Krua. No miroestrol was found in the two Red Kwao Krua samples collected from Chiang Mai and Kanjanaburi. The amounts of 29.8 to 45.0 mg of miroestrol per kg of the dried root in White Kwao Krua collected from Chiang Mai province (at Site Nos.

2.1 to 2.5) at the ages of 4.5 to 6.5 y were close to that previously reported (Jones and Pope, 1961). Interestingly, at the age of 5.5 y of White Kwao Krua root collected from Chiang Mai Site No.2.3, the amount of miroestrol found in the crude extract was the greatest at 45 mg/kg. The miroestrol contents appeared to depend on sites of cultivation more than the ages, since roots at the age of 6 y but from different sites of Chiang Mai (Site No.4) and Chiang Rai had lower amounts of miroestrol of only 5.80 and 13.40 mg/kg of dried root, respectively compared to 29.8 mg/kg of the dried root from Chiang Mai (Site No.2.4).

This study indicated that isoflavonoid and miroestrol contents in White Kwao Krua depended on locations of cultivation and ages of its tuberous roots. Puerarin and miroestrol seemed to be the main bioactive compounds in White Kwao Krua while daidzein and genistein were isoflavonoids that were found more in Red Kwao Krua.

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## Tables

Table 1. Comparison of isoflavonoid contents in tuberous roots of *Pueraria mirifica* and *Butea superba* at different ages collected from various locations in Thailand.

| Plant   | Age (years) | Location (site)  | % yield of crude extract of the dried root (g/100 g) | Isoflavonoid contents (% of the dried root) |           |          | Miroestrol contents |                         |
|---|-------------|------------------|--|---|-----------|----------|---------------------|-------------------------|
|   |             |                  |  | puerarin                                    | genistein | daidzein | % of the dried root | mg/kg of the dried root |
| <i>Pueraria mirifica</i><br>(White Kwao Krua) | 3.5         | Chiang Mai (1)   | 7.82   | 0.0065                                      | 0.0003    | 0.0005   | 0.00024             | 2.40                    |
|   | 4.5         | Chiang Mai (2.1) | 15.20  | 0.0068                                      | ND        | 0.0067   | 0.00440             | 44.00                   |
|   | 5.0         | Chiang Mai (2.2) | 16.60  | 0.0152                                      | ND        | 0.0028   | 0.00369             | 36.90                   |
|   | 5.5         | Chiang Mai (2.3) | 16.20  | 0.0121                                      | ND        | 0.0031   | 0.00450             | 45.00                   |
|   | 5.5         | Chiang Mai (3)   | 23.30  | 0.0124                                      | 0.0016    | 0.0032   | 0.00151             | 15.10                   |
|   | 6.0         | Chiang Mai (2.4) | 25.72  | 0.0111                                      | ND        | 0.0089   | 0.00298             | 29.80                   |
|   | 6.0         | Chiang Mai (4)   | 13.10  | 0.0290                                      | 0.0009    | 0.0020   | 0.00058             | 5.80                    |
|   | 6.0         | Chiang Rai       | 8.67   | 0.0090                                      | ND        | 0.0043   | 0.00134             | 13.40                   |
|   | 6.5         | Chiang Mai (2.5) | 11.10  | 0.0086                                      | ND        | 0.0026   | 0.00346             | 34.60                   |
|   | 14.0        | Chiang Mai (2.6) | 20.00  | 0.0026                                      | ND        | 0.0073   | 0.00161             | 16.10                   |
|   | NK          | Kanjanaburi      | 15.70  | 0.0218                                      | ND        | 0.0042   | 0.00014             | 1.40                    |
| <i>Butea superba</i><br>(Red Kwao Krua)       | NK          | Kanjanaburi      | 18.77  | 0.0003                                      | ND        | 0.0001   | ND                  | ND                      |
|   | NK          | Chiang Mai (5)   | 6.90   | 0.0019                                      | 0.0045    | 0.0372   | ND                  | ND                      |

Note : ND = Not detectable

NK = Not known