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SHORT COMMUNICATIONS

The Preliminary Detection of Cyanogenic Glycosides in Pra (*Elateriospermum tapos* Blume) by HPLC

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ABSTRACT

Seeds of Pra (*Elateriospermum tapos* Blume) are normally fermented and eaten by local villagers in Southern Thailand, especially in Nakhon Si Thammarat province. It is well known that fresh seeds can make the person who eats them even in small amounts feel dizzy. The amounts and types of cyanogenic glycosides in the seeds and the effect of heat and fermentation on the amount of the substances have never been reported before. Amygdalin, a type of cyanogenic glycoside, was detected in this preliminary study. It was found that fresh leaves contained 30 ppm of amygdalin, while fresh seeds contained much more amygdalin; 660 ppm, equivalent to a small amount of HCN, 0.005 ppm. Cooked and later fermented seeds had considerably less amygdalin than the fresh ones, 100 ppm and 25 ppm, respectively. It is clearly shown that heat (boiling) and fermentation can reduce the amount of amygdalin in the seeds.

Keywords: *Elateriospermum tapos*, cyanogenic glycoside, amygdalin, HCN

INTRODUCTION

Elateriospermum is a monotypic genus in the family Euphorbiaceae. *Elateriospermum tapos* Blume, known locally as Pra or Perah, and is distributed across the Malay Peninsula, including Southern Thailand [1]. The tree grows in lowland and hill forests up to 600 m. The most northerly latitude where a population has been found lies at ~8° 48' N in Khao Luang and Khao Nan national parks in Nakhon Si Thammarat province. The whole area of the population is estimated to be ~6,000 Rai or 9.6 km^2. The tree is deciduous and is also cultivated as an ornamental plant because of its reddish pink young leaves. Its flowers appear simultaneously with the new leaves each year at the beginning of summer (around March) while its fruits mature between August and September. The fruit is dehisced and scatters seeds, usually with 3 seeds per fruit. Seeds are safely edible after boiling and fermentation.

Because of the sheer numbers of seeds produced each year by the population at Khao Luang and Khao Nan national parks, the parks and local villages nearby annually arrange “a week of Pra collecting”. Local villagers and outside people come to the area and collect the seeds once a year. The fresh seeds are priced at ~25 - 35 baht per kilogram depending on the time of the season and the amount of seeds produced each year. Fermented seeds are sold at 35 - 40 baht per kilogram. It is estimated that the total value of the seeds in the market may exceed a million baht per year.

Seeds of Pra, especially the fresh ones, are well known to contain substances that make those who eat them feel dizzy. The substances in the seed are collectively known as prussic acid or cyanogenic glycosides [2]. The substances act as a natural defense for the plant deterring herbivores from eating its seeds [3]. Hydrogen cyanide (HCN) is released once hydrolyzed from endogenous cyanide containing compounds. The process is called cyanogenesis. Many cyanogenic plants release HCN in sufficient quantities to be toxic or distasteful, and as a result tend to be avoided by herbivores. HCN is an active compound in inhibiting the electron transport chain in cellular respiration [4].

The objectives of this preliminary study are firstly to determine the types of cyanogenic glycosides present in seeds and leaves of *Elateriospermum tapos*, secondly to compare the amounts of the cyanogenic glycosides found in the fresh seeds and leaves, and finally to investigate the effect of heat (boiling) and fermentation on the amount of cyanogenic glycosides left in the seeds.

MATERIALS AND METHODS

All samples, seeds and leaves, of *Elateriospermum tapos* used in this study were collected in September 2002. Fresh leaves and seeds were collected from Khao Nan national park (station: Huay Lake) and stored at −18 ºC before use. Fresh seeds were cooked in boiling water for 15 min. Fermented seeds (normally a week long with salty water) were available from the local market, and it is even possible to see them sold along the road (**Figure 1e**).

(a)

(b)

(c)

(d)

(e)

(f)

Figure 1 *Elateriospermum tapos* Blume: (a) young trees, (b) inflorescence and flowers, (c) fruits and seeds, (d) and (e) fermented seeds, (f) processed (cooked) seeds.

Chromatographic determination of cyanogenic glycosides, amygdalin and prunasin, was carried out on 0.2 g of each sample [5]. Each sample was extracted with 10 ml of methanol for 12 h at room temperature. Extractions of the samples were made in the presence of 0.1 g of polyvinylpolypyrrolidone (Sigma) to ensure that they were free from pigments (e.g. tannins, flavonoids and chlorophylls), which can interfere with the chromatographic determination. Chromatographic separation was performed isocratically using a 2690 Waters high-performance liquid chromatography (HPLC) system. The procedure was described under the following conditions: NOVA-Pax C_{18} column 150 mm × 3.9 mm, flow rate 0.8 ml/min, using acetonitrile:water 20:80 as an eluent, 20 µl of sample, and detection under UV at 218 nm.

The cyanogenic glycosides were identified by comparing their retention time with that of the authentic standards, amygdalin (D-mandelonitrile β-D-gentiobioside) (Sigma) and prunasin (D-mandelonitrile β-D-glucoside) (Sigma). The 2 substances were reported to be present in seeds, roots and leaves of many plant species [6], and were available from the chemical provider. Quantification was based on an external standard method where the calibration curve ranged from 2 to 15 mg/l of the reference compound, amygdalin.

RESULTS AND DISCUSSION

It was found that only the cyanogenic glycoside, amygdalin, was detected in the samples by HPLC. No signal for prunasin was detected in all samples. Under the chromatographic conditions, the amygdalin peak appeared at a retention time of 2.2 min (**Figure 2**) while that of prunasin appeared at 3 min. The highest amygdalin content was observed in the fresh seeds, 660 ppm, followed by the cooked seeds, 100 ppm (**Table 1**). The amount of amygdalin in the fresh leaves was 30 ppm, and similar to the amount found in the fermented seeds, 25 ppm.

Table 1 Amygdalin content found (ppm) in 0.2 g of seed and leaf samples determined by HPLC.

Sample	Seeds	Leaves
Fresh	660	30
Cooked	100	NA
Fermented	25	NA

NA = not applicable.

Only amygdalin was found in seed and leaf samples in this study. In a report by Ling and co-workers [2], 2 types of cyanogenic glycosides; linamarin and lotaustralin were found in leaves of *E. tapos*. There were other peaks in our HPLC results that still await identification with known standards. Perhaps one of those might be linamarin or lotaustralin as reported by Ling and co-workers [2].

The amount of amygdalin found in the fresh seeds (660 ppm) was equivalent to a very small calculated amount of HCN 0.005 ppm {660/(457.479 × 27 × 10)}. This level of HCN was well below the safety limit recommended by the Codex FAO/WHO for cassava flour (10 ppm) [7]. The amount of HCN was even lower in other seed samples studied. They all can be considered safe for consumption. For the leaves, it is assumed that in order to protect itself from being eaten, *E. tapos*, needs to produce small amounts of cyanogenic glycoside, such as amygdalin in the leaves as well.

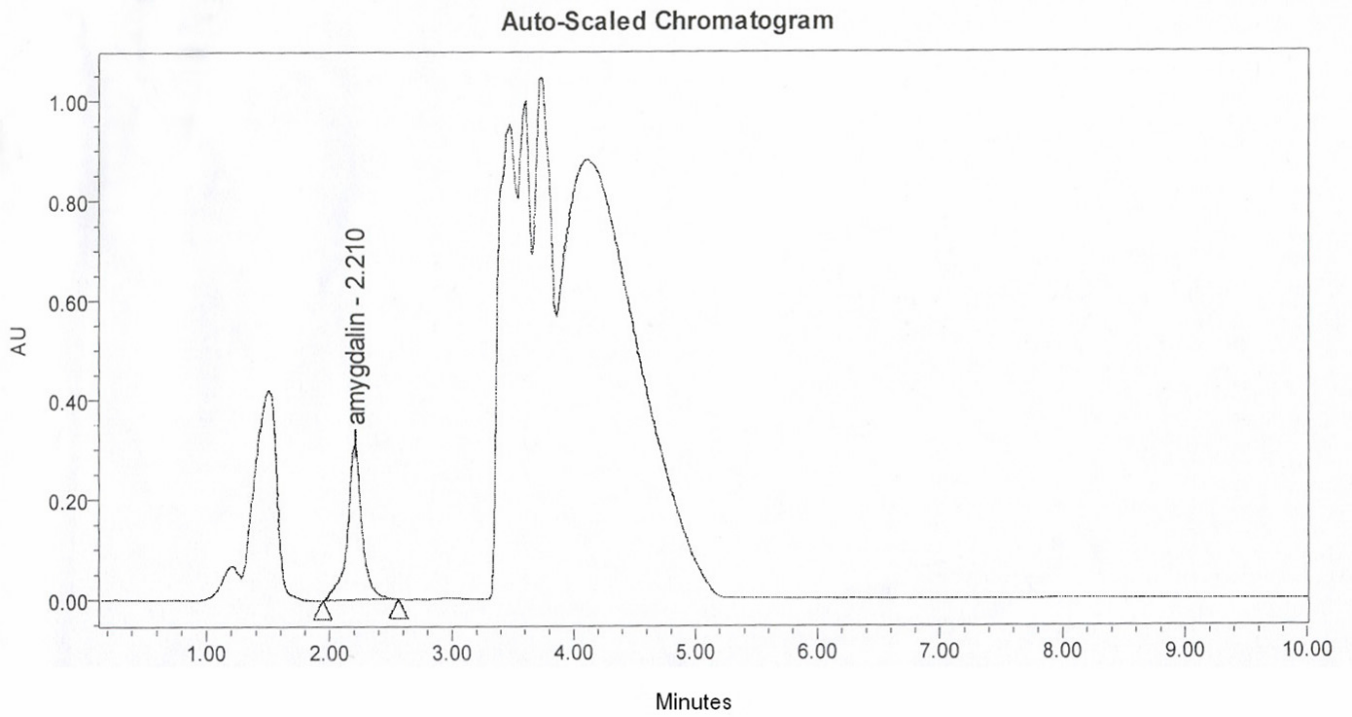

Figure 2 Chromatogram showing an amygdalin peak at retention time 2.2 min.

It is clearly shown in this study that heat (boiling) and fermentation can reduce the amount of amygdalin in *E. tapos* seeds. Amygdalin and other cyanogenic glycosides easily release the cyanide group upon hydrolysis [5]. It was observed that seed samples kept at room temperature for a lengthy period showed no signals of amygdalin (results not shown). Boiling can increase the breakdown of the cyanide group from the unstable amygdalin. Further fermentation, normally a week long with salty water, clearly

reduced the amount of amygdalin in the seeds. Folklore for processing Pra seeds appears to be an efficient method for reducing cyanogenic glycoside content by as much as 96.2 % (from ~660 ppm to 25 ppm), not just making the seeds taste better (less bitter).

CONCLUSIONS

It was found that a cyanogenic glycoside, amygdalin, was present in seeds and leaves of *Elateriospermum tapos*. The amount of amygdalin was much higher in fresh seeds (660 ppm) than cooked seeds (100 ppm) and fermented seeds (25 ppm). The amount of amygdalin found in the fresh leaves was also low (30 ppm). It is clearly shown that heat (boiling) and fermentation can reduce the amount of amygdalin in the edible seeds.

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บทคัดย่อ

ฉัตรชัย งามเรียบสกุล และ หทัยชนก คมเม่น

การตรวจสอบเบื้องต้นสารประกอบไกลโคโซด์ไซยาไนด์ในประ (*Elateriospermum tapos* Blume) ด้วยเทคนิค HPLC

เมล็ดประดองเป็นที่นิยมรับประทานโดยชาวบ้านหลายหมู่บ้านในภาคใต้ โดยเฉพาะในจังหวัดนครศรีธรรมราช และเป็นที่ทราบกันดีว่า การรับประทานเมล็ดประสดในระดับหนึ่งอาจทำให้เกิดอาการวิงเวียนศีรษะ ยังไม่เคยมีรายงานมาก่อนถึงชนิดและปริมาณของสารประกอบไกลโคโซด์ไซยาไนด์ในเมล็ดประ และผลของความร้อนและการดองต่อปริมาณสารประกอบไกลโคโซด์ไซยาไนด์ในเมล็ดประ จากการตรวจสอบเมล็ดและใบของประพบว่ามีสารประกอบไกลโคโซด์ไซยาไนด์ชนิดหนึ่งคือ Amygdalin โดยที่มีปริมาณของ Amygdalin ในใบสดเท่ากับ 30 ppm ขณะที่ในเมล็ดสดมีปริมาณ 660 ppm เมื่อคิดเป็นปริมาณของไฮโดรเจนไซยาไนด์ มีน้อยเท่ากับ 0.005 ppm เมล็ดที่ต้มสุกและเมล็ดต้มสุกแล้วดอง มีปริมาณ Amygdalin น้อยกว่าเมล็ดสดมาก โดยพบเท่ากับ 100 ppm และ 25 ppm ตามลำดับ จากการศึกษาครั้งนี้พบว่าความร้อนโดยการต้มและการดองสามารถลดปริมาณ Amygdalin ในเมล็ดประได้

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