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A traditional method of *Cinnamomum carolinense* preparation eliminates safrole from a therapeutic Pohnpean tea

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Abstract

Cinnamomum carolinense, locally known as *madeu*, is a tree endemic to the volcanic mountains of the Island of Pohnpei in the Eastern Carolines of the South Pacific. The bark is harvested from trees and brewed to make a medicinal tea and hot beverage that is regularly consumed. Many species of *Cinnamomum* contain the known hepatocarcinogen safrole, sparking concern regarding habitual consumption of this beverage. HPLC-PDA analysis confirmed the presence of the carcinogen in alcoholic extracts of *Cinnamomum carolinense* bark shavings (0.435%, w/w), but safrole was not detected in the tea. The limit of detection and limit of quantitation of safrole were determined to be 1.25 and 3.75 μg/mL, respectively. The traditional preparation method, which boils the bark shavings, degrades the safrole.

Keywords: *Cinnamomum carolinense*; *Madeu*; Pohnpei; Carcinogen; Safrole; Traditional medicine; Ethnopharmacology

1. Introduction

We have been involved in a collaborative, multidisciplinary study of plant resources and traditional use on the Island of Pohnpei, Federated States of Micronesia since 1998. We are working with local people who gather plant resources on the island for use as medicine, food, construction material, fuel, in personal care, and for other purposes. Many of these plant resources are now less abundant than the past, when they were more important to the traditional economy. One of these resources is *madeu* [*Cinnamomum carolinense* Koidz. (Lauraceae)]. A large tree endemic to Pohnpei, it is found in the volcanic montane areas of the upper altitude forests, at ca. 800 feet. We worked with a family that still harvests the bark of this tree in limited quantities for use in their home and for sale in the local market.

When the tree was plentiful, and regeneration through seedling growth occurred, the method of harvesting *madeu* involved stripping the bark from the tree, chopping it into small pieces, and sun-drying it (Fig. 1A). The result was that the girdled trees died. Today, however, there are only remnant populations of the trees extant on Pohnpei, and the Pelep family, wishing to maintain the resource, has developed a sustainable method of harvesting the bark. This method involves scraping a few millimeters of the outer bark with a machete held at a 90° angle to the trunk of the tree until a light reddish-white layer of wood is reached (Fig. 1B). This layer is then gently scraped onto a folded palm leaf (Fig. 1C), and the scrapings are carefully dried in an electric oven and packaged for use in a sealed bag or jar to avoid humidity. About a teaspoon of the dried scrapings is boiled in several cups of water and consumed warm as a therapeutic tea. It is often

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Fig. 1. (A) Yosio Pelep demonstrates the traditional destructive method of *madeu* harvest by stripping bark from the tree; (B) Y. Pelep demonstrates the sustainable method of *madeu* harvest by scraping the inner bark with a machete; (C) Elter and Yosio Pelep holding a palm leaf with *madeu* bark shavings harvested from a *Cinnamomum carolinense* tree.

drunk sweetened, in the morning or with meals. When used medicinally as a treatment for backache and joint pain, the tea is consumed unsweetened. Traditionally, the leaves, roots, and bark were all used for the treatment of backache, but the bark was considered the most potent.

Many ethnomedical uses have been reported for this plant. Raynor (1994) noted the use of *madeu* as a medicinal tea. Riesenberg (1948) reported several conditions that used *madeu* as part of a therapeutic mixture, including excessive menstruation and a disease specific to Pohnpei known as "mangrove sickness." Additional ethnobotanical and botanical observers have confirmed its widespread use as a tea on the island (Glassman, 1950, 1952; Kanehira, 1915a,b, 1916; Merlin et al., 1992). Our fieldwork (Balick et al. 4131-NY) has identified many other traditional uses for the plant: to treat syphilis; as a tonic to boost the immune system; to enlarge a urine stream that has contracted perhaps as the result of a prostate problem; and to treat something locally known as "men's sickness" that involves swelling and pain in the groin area that is said to come from overwork.

In his monograph of the genus *Cinnamomum*, Kostermans (1986) lumped *Cinnamomum carolinense* Koidz. under *C. pedatinervium*, but as he was lacking complete voucher material, and as these species appear different, we consider the Pohnpean plant under study to be *Cinnamomum carolinense*. Kostermans (1986) noted that safrole was present in the bark based on an unspecified Gildmeister reference to safrole in the Pohnpean *Cinnamomum*. Many species of *Cinnamomum* are known to contain safrole (bin Jantan and Goh, 1992), and concern that this beverage contains a known carcinogen led to this study.

Safrole (Fig. 2) is a hepatocarcinogen (Daimon et al., 1998; Ioannides et al., 1981; Liu et al., 1999), and its use in food is restricted by the U.S. Food and Drug Administration (Title 21, Part 172.510, 2004; Title 21, Part 189.90, 1997). It has historically been used in cosmetics and as a topical anesthetic, and is a component of oil of sassafras and many culinary spices. Insoluble in water, safrole is very soluble in alcohol (Budavari, 1999). When ingested, safrole is metabolized to form many by-products. The metabolite

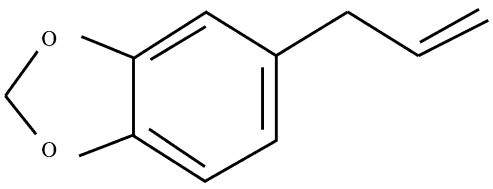

Fig. 2. Chemical structure of safrole.

1′-hydroxysafrole is known to be partially responsible for the carcinogenicity of safrole, and is more carcinogenic and mutagenic than the parent compound (Ioannides et al., 1981).

2. Materials and methods

2.1. Plant material

Bark shavings of *Cinnamomum carolinense* were collected according to the method described above. Voucher specimens (Balick et al. 4131) were prepared and deposited in The New York Botanical Garden Herbarium (NY).

2.2. Chemicals and supplies

Reagent-grade methanol (MeOH) from EM Science (NJ, USA) was used for extractions. HPLC-grade acetonitrile (ACN) and MeOH from J.T. Baker (NJ, USA) were used for HPLC analysis. Safrole was purchased from Chem Service (PA, USA).

2.3. Extraction and preparation

Cinnamomum carolinense bark shavings were extracted in both MeOH and water (Fig. 3). The MeOH extract was prepared by homogenizing the plant material (2.01 g) in a blender with MeOH (200 mL) and extracting in a sonicator at room temperature for 1 h. Another portion (5.01 g) was extracted sequentially in boiling water (300 mL) for 20 min followed by MeOH (200 mL) as above. A third portion was spiked with 50 μg safrole standard and boiled as above. A Sentron (Roden, The Netherlands) 2001 pH meter was used to determine the pH of the boiling water extract. All extracts were filtered, concentrated under vacuum, and lyophilized.

2.4. Quantitative and qualitative analysis

Samples were resuspended in MeOH and filtered through 0.45 μm syringe filters prior to HPLC analyses. HPLC conditions and quantitative analysis were adapted from previously published methods (Carlson and Thompson, 1997; Curro et al., 1987). Four dilutions (from 2.5 to 50 μg/mL) were used to create a standard curve and quantify the amount of safrole present in the extracts. Triplicate chromatographic runs were used and standard deviations calculated for each sample. The limit of detection (LOD) at a signal-to-noise (S/N) ratio of 3:1 and lower limit of quantitation (LOQ) at a S/N ratio of 10:1 were established.

Analysis was performed on a Waters (NJ, USA) 2695 Separations module with a Waters 996 PDA scanning from 190 to 320 nm using a Phenomenex (CA, USA) Aqua 250 mm × 4.6 mm i.d. 5 μm reversed-phase C_{18} column. Results were monitored at 235 nm. The solvent system for quantitative analysis was optimized to clearly separate safrole and was a linear gradient of water (A) and ACN (B) from 55% A to 50% A in 10 min, then a linear gradient to 10% A over 20 min, followed by 4 min of 100% MeOH at a flow rate of 1 mL/min. The solvent system for qualitative analysis was optimized to better separate the polar constituents with a linear gradient from 15% B to 100% B over 35 min.

2.5. Safrole degradation analysis

Three samples of neat safrole standard (0.5 mL each) were boiled in water for 20 min in: (1) neutral pH; (2) pH 2 adjusted with triflouroacetic acid; and (3) neutral pH under reflux. The samples were then either partitioned with chloroform or dried under vacuum and resuspended in MeOH for HPLC analysis and CD_3OD for NMR analysis. Samples were analyzed by HPLC-PDA (qualitative conditions described above) and by ^1H NMR (Bruker Avance 300 MHz; MA, USA).

3. Results

In the quantitative HPLC analysis, standard safrole eluted at a retention time of approximately 20 min, with UV λ_{max} at 235 and 286 nm. Safrole was positively identified by UV

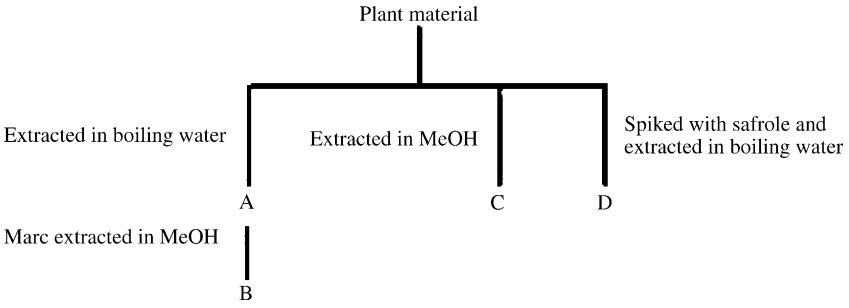

Fig. 3. Extraction and analysis scheme.

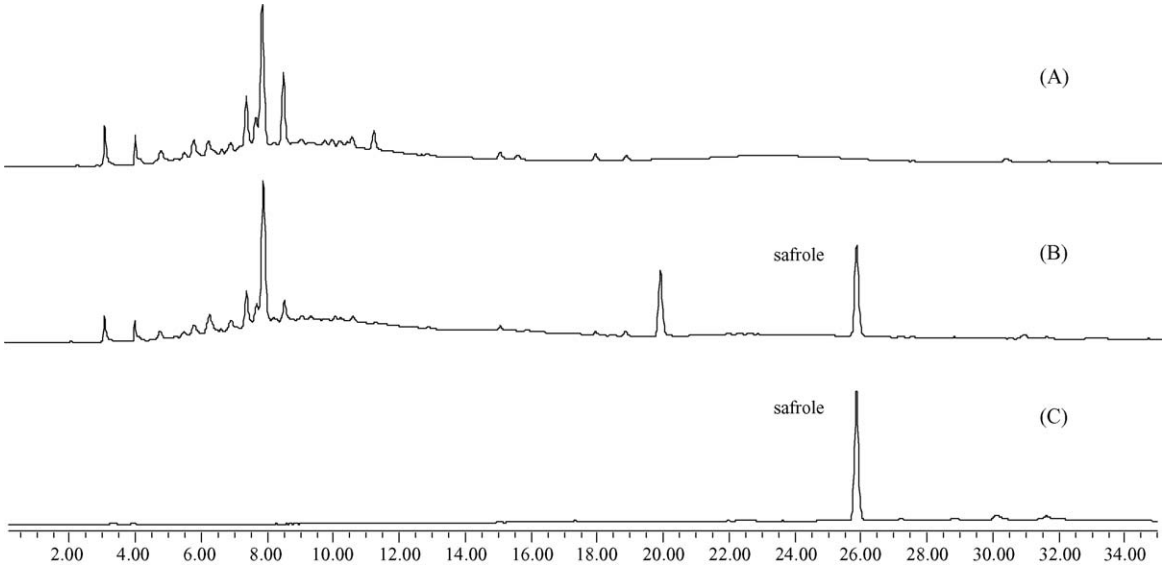

Fig. 4. HPLC-PDA chromatograms at 235 nm showing (A) boiling water extraction of *madeu*; (B) methanolic extract of *madeu*; and (C) safrole standard.

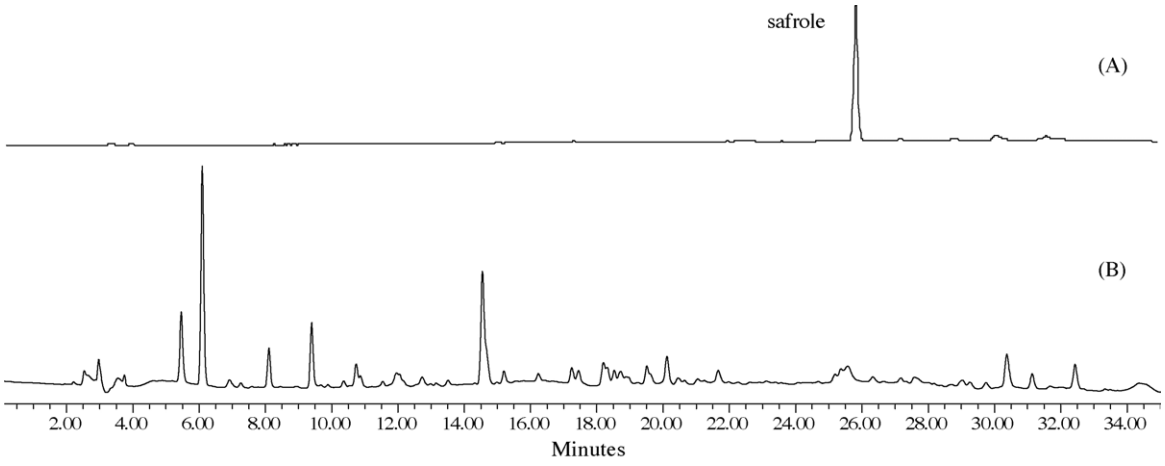

Fig. 5. HPLC-PDA chromatogram at 235 nm of safrole standard (A) before and (B) after boiling in water (using the qualitative HPLC conditions described in the text).

spectra and retention time in the methanolic extracts. Using the regression curve generated ($R^2 = 0.9999$), the amount of safrole in the methanolic extract of the plant material was determined to be 0.435% (w/w) ($\pm 5 \times 10^{-6}$). The methanolic extract of the plant material that remained following the boiling water extraction contained only 0.0001% (w/w) ($\pm 5 \times 10^{-7}$). Safrole was not detected in any boiling water extractions, including a sample that had been fortified with safrole standard. The LOD and LOQ were determined to be 1.25 and 3.75 μg/mL, respectively. Qualitative chromatograms of extracts are presented in Fig. 4. The water extracts were slightly acidic (pH 4.4). An aliquot of standard safrole that was boiled in water maintained an oily appearance and remained water immiscible, but showed evidence of degradation when analyzed by HPLC-PDA (Fig. 5) and by NMR (Fig. 6).

4. Discussion and conclusions

Initial methanolic extracts of *Cinnamomum carolinense* bark shavings indicated that the plant material used to make the traditional tea does contain safrole. However, it could not be detected in the water extractions that were prepared according to traditional Pohnpean methods. The importance of reproducing the traditional preparation of an herbal product for evaluation and wider use is reminiscent of another contemporary controversy involving a Pacific island plant. The well known series of adverse event reports involving the non-traditional use of preparations of kava (*Piper methysticum*) as a dietary supplement for the treatment of anxiety have been ascribed by some authors to the commercial extraction with organic solvents as the cause of potential toxicity (Singh and Devkota, 2003; Whitton et al., 2003). The same

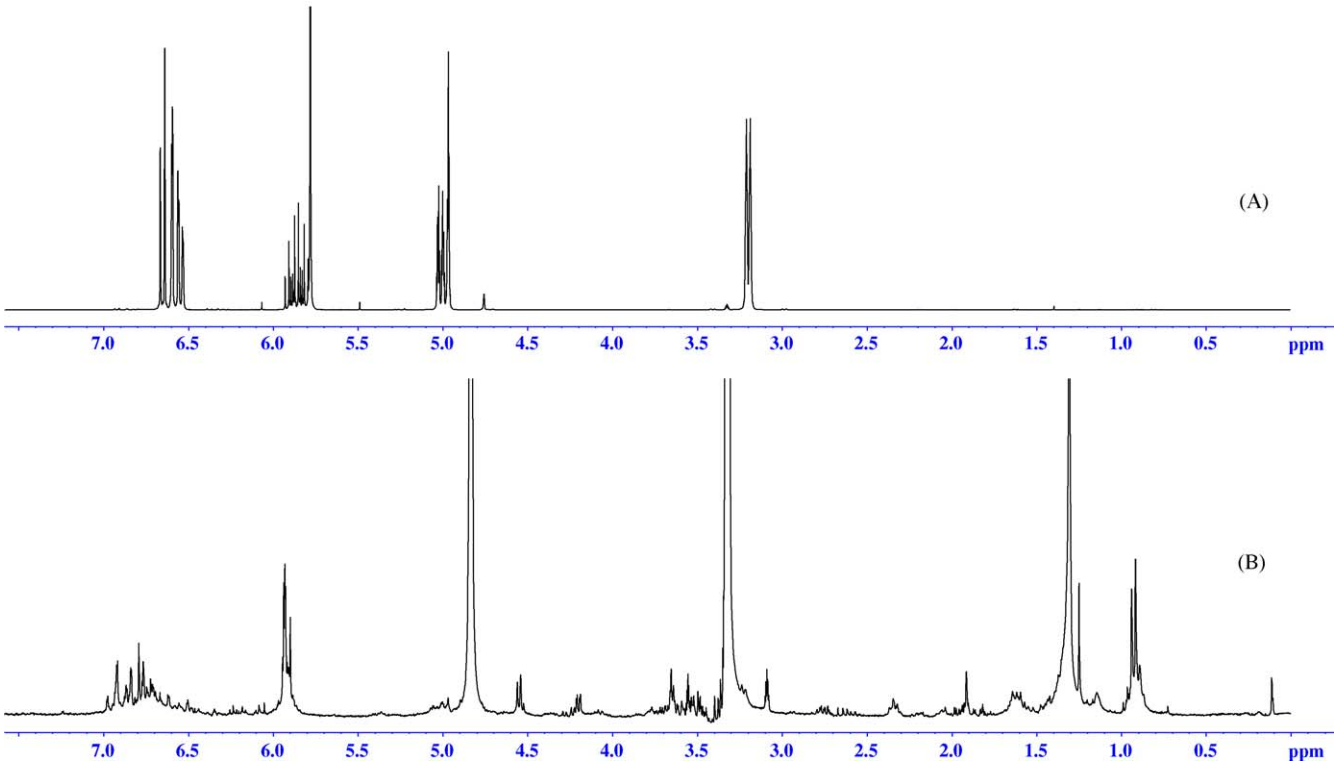

Fig. 6. ^{1}H NMR spectra (300 MHz) of safrole standard in CD_3OD (A) before and (B) after boiling in water.

adverse events do not seem to be observed with traditional use (extraction of the root with tepid water) in Samoa (Tavana et al., 2003) or Pohnpei (Roberta Lee, personal observation, 2004). In this current project involving *Cinnamomum carolinense*, we analyzed the traditional preparation to ascertain the fate of the carcinogen safrole. Safrole is insoluble in water, but our analysis shows that the traditional method of preparation results in the degradation of safrole prior to consumption of the tea. Only a minor amount of safrole (0.0001%, w/w) remained in the plant material after it had been extracted in the boiling water, indicating that it is not simply a matter of insolubility. The methanolic extract of the bark contained nearly 300 times more safrole than the methanolic extracts of the plant material that had been extracted with boiling water. Furthermore, safrole was not detected in a boiling water extraction that was spiked with safrole prior to extraction. It has been noted by other authors that boiling spices that contain safrole for 1–5 min greatly reduces the safrole content (Farag and Abo-Zeid, 1996). The water extract of *madeu* is slightly acidic (pH 4.4), which may contribute to safrole degradation by hydrolyzing the dioxolane ring (Fig. 2). In our experiments with the safrole standard, boiling in either normal or acidic conditions created a large number of degradation by-products, indicating that the safrole was not simply volatilized. The HPLC chromatograms of the boiled standard show many peaks, and very little safrole (Fig. 5). The NMR spectrum supports these results, as the proton signals for safrole have nearly disappeared and many additional signals appear after the standard is boiled (Fig. 6).

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References

Budavari, S., 1999. The Merck Index. Merck & Co., Rahway, NJ, p. 8286.

Carlson, M., Thompson, R.D., 1997. Liquid chromatographic determination of safrole in sassafras-derived herbal products. Journal of AOAC International 80, 1023–1028.

Code of Federal Regulations, 2004. Title 21, Part 172.510. Washington, DC, U.S. Government Printing Office.

Code of Federal Regulations, 1997. Title 21, Part 189.90. Washington, DC, U.S. Government Printing Office.

Curro, P., Micali, G., Lanuzza, F., 1987. Determination of β-asarone, safrole, isosafrole and anethone in alcoholic drinks by high-performance liquid chromatography. Journal of Chromatography 404, 273–278.

Daimon, H., Sawada, S., Asakura, S., Sagami, F., 1998. In vivo genotoxicity and DNA adduct levels in the liver of rats treated with safrole. Carcinogenesis 19, 141–146.

Farag, S.E.A., Abo-Zeid, M., 1996. Mutagenicity and degradation of natural carcinogenic compound safrole in spices under different processing methods. Al-Azhar Journal of Pharmaceutical Sciences 17, 149–158.

Glassman, S.F., 1950. Ponape's "national" beverage. Research Reviews, July 1950, pp. 16–18.

Glassman, S.F., 1952. The flora of Ponape. Museum Bulletin 209, Bernice P. Bishop Museum, Honolulu, HI.

Ioannides, C., Delaforge, M., Parke, D.V., 1981. Safrole: its metabolism, carcinogenicity and interactions with cytochrome *P*-450. Food and Cosmetics Toxicology 19, 657–666.

bin Jantan, I., Goh, S.I., 1992. Essential oils of *Cinnamomum* species from peninsular Malaysia. Journal of Essential Oil Research 4, 161–171.

Kanehira, R., 1915a. Forests and plants of the south seas islands. Daimippon Sanrin Kaiho, Part I, no. 394, 18–32.

Kanehira, R., 1915b. Forests and plants of the south seas islands. Daimippon Sanrin Kaiho, Part II, no. 395, 1–17.

Kanehira, R., 1916. Forests and plants of the south seas islands. Daimippon Sanrin Kaiho, Part III, no. 401, 45–61.

Kostermans, A.J.G.H., 1986. A monograph of the genus *Cinnamomum* Schaeff. Part I (Lauraceae). Academia Scientific Book Inc., Tokyo.

Liu, T.Y., Chem, C.C., Chen, C.L., Chi, C.W., 1999. Safrole-induced oxidative damage in the liver of Sprague-Dawley rats. Food and Chemical Toxicology 37, 697–702.

Merlin, M., Jano, D., Raynor, W., Keene, T., Juvik, J., Sebastian, B., 1992. Tuhke en Pohnpei. Plants of Pohnpei. John D. and Catherine T. MacArthur Foundation, Chicago, IL.

Raynor, B., 1994. Resource management in upland forests of Pohnpei: past practices and future possibilities. ISLA: A Journal of Micronesian Studies 2, 47–66.

Riesenberg, S.H., 1948. Magic and medicine in Ponape. Southwestern Journal of Anthropology 4, 406–429.

Singh, Y.N., Devkota, A., 2003. Aqueous kava extracts do not affect liver function tests in rats. Planta Medica 69, 496–499.

Tavana, G., Stewart, P., Snyder, S., Ragone, D., Fredrickson, K., Cox, P.A., Borel, J., 2003. Lack of evidence of kava-related hepatotoxicity in native populations in Savaii, Samoa. Herbalgram 59, 28–32.

Whitton, P.A., Lau, A., Sailisbury, A., Whitehouse, J., Evans, C.S. Kava lactones and the kava-kava controversy. Phytochemistry, 64, 673–679.