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ORIGINAL ARTICLE

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A The South Pacific islanders have consumed kava beverage for thousands of years. The quality of kava and kava beverage is evaluated through determination of the content of six major kavalactones including methysticin, dihydromethysticin, kavain, dihydrokavain, yangonin and desmethoxyyangonin. In this study, we determined contents of kavalactones in and chemotype of kava beverages prepared from roots and rhizomes of Isa and Mahakea varieties and extraction efficiency of five different solvents including hexane, acetone, methanol, ethanol and ethyl acetate. The six major kavalactones were detected in all kava beverages with these five solvents. Different solvents had different extraction efficiencies for kavalactones from the lyophilized kava preparations. The contents of kavalactones in the extracts with acetone, ethanol, and methanol did not differ significantly. Ethanol had the highest extraction efficiency for the six major kavalactones whereas hexane gave the lowest extraction efficiency.

K *Piper methysticum* · Kava · Kavalactone · Chemotype · Extraction efficiency

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Kava is known as the common name of both a shrub plant, *Piper methysticum* and the beverage prepared from the plant materials (BHMA 1996). The South Pacific islanders have consumed kava beverages for thousands of years (Singh 1992). The kava beverage is traditionally prepared from macerated roots and/or stump with water or coconut water (BHMA 1996; Kilham 1996). It is reported that beneficial effects of the kava beverage include relaxation, euphoria, anti-convulsion, neuroprotection, analgesia and attenuation of menopausal symptoms (Lebot et al. 1992; Bilia et al. 2002; Lebot and Lévesque 1989; Baum et al. 1998; Whitton et al. 2003; Schulz et al. 2001). The safety of kava consumption has been a topic of debate in recent years (Anke and Ramzan 2004; Russmann et al. 2001; Anon 2001; Campo et al. 2002; Gow et al. 2003; Brauer et al. 2003). In general, the kava beverage and products made from rhizomes and roots have been considered being safe (Teschke and Lebot 2011).

Six major kavalactones are considered to be the main psychoactive components of kava. Some reports suggested

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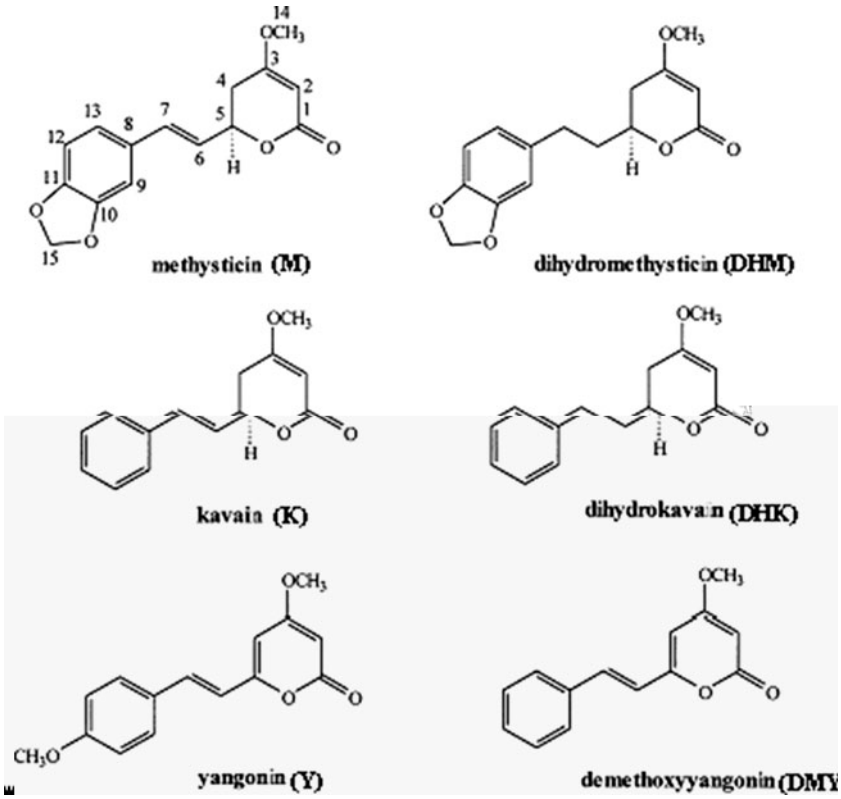

F . 1 Molecular structures of the six major kavalactones

that these kavalactones exert advantageous physiological functions on humans such as diuretic, soporific, antiepileptic, spasmolytic, analgesic, local anaesthetic, bacteriocidal and antimycotic functions (Whitton et al. 2003; Lebot and Lévesque 1996a). Lebot and Lévesque divided the active ingredients of kava into two main groups — major kavalactones and minor kavalactones, and numbered and used only the six major kavalactones (1 = desmethoxyyangonin, DMY; 2 = dihydrokavain, DHK; 3 = yangonin, Y; 4 = kavain, K; 5 = dihydromethysticin, DHM; and 6 = methysticin, M (Fig. 1) (Lebot and Lévesque 1989; Wang et al. 2010)). It was reported that no liver enzymes were elevated in rats with a daily dose of 200 or 500 mg kavalactones/kg for 2 weeks (Singh and Devkota 2003). Dragull et al. (2003) suggested that an alkaloid, piperrmethystine, in kava may be toxic to liver cells.

It was reported that contents and chemotypes of kavalactones vary among parts, kava varieties and plant ages (Siméoni and Lebot 2002). The contents and chemotypes of kavalactones are the main criteria of kava beverage quality. The traditional method for preparing kava beverage made the content of kavalactones decline in the kava beverage. However, no studies were reported about extraction efficiency of different solvents for kavalactones in kava material and kava beverage. In this study, the aim was to study chemotypes and contents of kavalactones from rhizomes and roots of two kava varieties in five different solvents and extraction efficiency of different solvents for kavalactones from kava materials.

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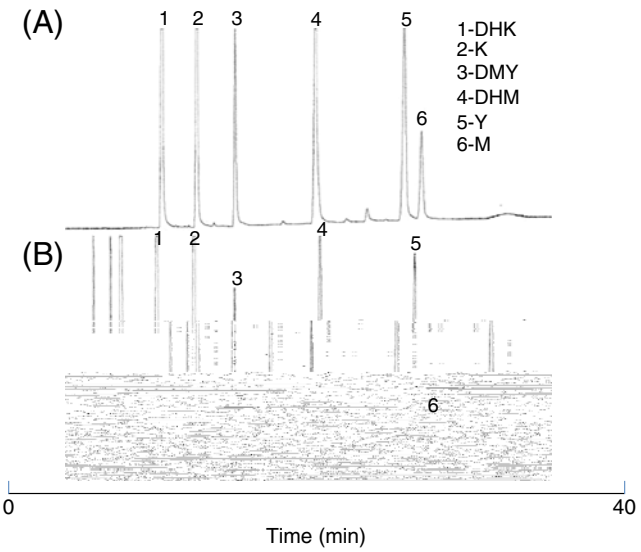
Raw materials

All solvents were of high-performance liquid chromatography (HPLC) grade from Fisher Scientific (Fairlawn,



F . 2 GC chromatograms of six kavalactone standards (**A**) and a typical kava extract (**B**)

NJ). Kavalactone standards including methysticin (M), dihydromethysticin (DHM), kavain (K), dihydrokavain (DHK), yangonin (Y) and desmethoxyyangonin (DMY) (Fig. 1) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Other reagents were purchased from Sigma (St. Louis, MO) too otherwise stated. Kava materials were Kava roots and rhizomes of Isa variety and Mahakea variety at 3.4 years of age grown in the Waimanalo Research Farm on Oahu, University of Hawaii.

Preparation of kava materials and beverages

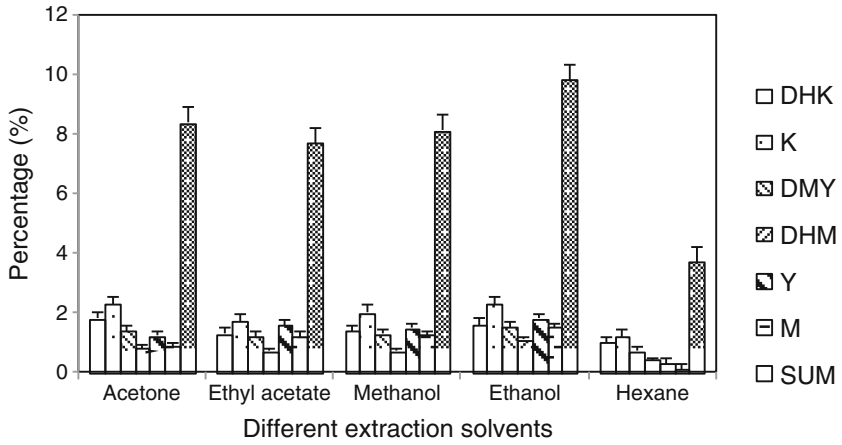
The fresh kava root or rhizome materials mixed with warm water (45 °C) at a ratio of was 1:3 (W/V) in blender, shredded (0.1–3 mm diameter) for 1 min and agitated, and then filtered and squeezed manually. The extractions were repeated three times and the water extracts (beverage) were combined. An aliquot (40 mL) of the beverage was frozen dried. The dried kava powders were extracted with 10 mL of ethyl acetate, methanol, ethanol, acetone or hexane under sonication for 10 min. After a brief centrifugation at a low speed (2,000 rpm), the supernatant (solvent layer) was decanted into a test tube. This extraction process was repeated three more times. The extracts were combined followed by evaporation of the solvent under a gentle stream of nitrogen gas to an appropriate volume (1 mL).

Analysis of kavalactones with gas chromatography-flame ionization detector (GC-FID)

The six major kavalactones were analyzed on a Hewlett Packard model 5890 series II gas chromatograph equipped with an FID, an autosampler, and a DB-5 capillary column (30 m×0.25 mm, 0.25 μm; J&W Scientific, Rancho Cordova, CA). The carrier gas was set at a flow rate of 1 mL/min of helium. The injector and detector temperatures were 250 and 300 °C, respectively. The oven temperature started at 120 °C for 1 min, increased to 300 °C at a rate of 10 °C/min and then held at 300 °C for 5 min. The injection was at splitless mode and the injection volume was 2 μL. Octadecane was used as an internal standard to calculate the concentrations of the six major kavalactones. Figure 2 shows GC chromatograms of six kavalactone standards and a typical kava extract.

Quality control and quality assurance (QA/QC)

Sample preparations and analytical procedures were performed according to quality assurance and quality control measures. Work standard solutions of kavalactones were run at the beginning of sample analysis to evaluate peak resolution and possible contamination. The limits of detection (LOD) were determined as signals three times of the background signals. Peaks that were smaller than three times of



the signal-to-noise ratio were considered undetected. Each sample was analyzed in triplicate otherwise stated. The LOD for six kavalactones ranged from 0.5 ppm to 1 ppm.

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Comparison of extraction efficiency of different solvents for kavalactones

Extraction efficiency of kavalactones from kava beverages varied with extracting solvents (Table 1). Among all solvents tested, ethanol yielded the highest total content of kavalactones from Mahakea and Isa root beverages and Mahakea rhizome beverages. The total kavalactone content of acetone extract of Isa rhizome beverages was higher than that of ethanol. Ethanol had the strongest extraction efficiency, whereas hexane had the lowest extraction efficiency among the five solvents. Solvent polarity is apparently important for good extraction efficiencies for kavalactones as kavalactones have moderate polarity.

Comparison of kavalactone content in kava beverages prepared from root and rhizomes of Isa and Mahakea varieties as extracted with different solvents

The total kavalactone content in roots was higher than that in rhizome of the same kava variety, as reported by others (Siméoni and Lebot 2002). The total kavalactone content in Isa root beverages was approximately twice as much as in Mahakea root beverages, which the kava plants were grown in the same field and harvested at the same age (Table 1). The total kavalactone content in Isa and Mahakea rhizome beverages was approximately equal (Table 1). The results suggest that kavalactone content depends up on kava varieties and plant part.

Figure 3 shows the profiles of six major kavalactones and their total content in the beverages prepared from Mahakea rhizome and extracted with different solvents. In general, the concentrations of dihydrokavain (DHK) and kavain (K) were higher than the other four kavalactones. Figure 4 shows percentages of six major kavalactones and their total content in the beverages prepared from Mahakea root and extracted with different solvents. Except hexane

extracts, the concentrations of the six major kavalactones were approximately similar. Figure 5 shows patterns of six major kavalactones and their total content in the beverages prepared from Isa rhizome and extracted with different solvents. DHK had the highest concentrations in the beverages followed by dihydromethysticin (DHM) and then K. Figure 6 shows the profiles of six major kavalactones and their total content in the beverages prepared from Isa rhizome and extracted with different solvents, which the profiles are similar to those of Isa rhizome beverages.

It is well documented that the content of kavalactones in kava plant or kava beverage depend on kava plant varieties, part and age and geographic locations of the plants, and even orientation of kava plant and time of harvest (Siméoni and Lebot 2002; Lebot and Lévesque 1989; Lebot et al. 1999). Smith (1983) and Smith et al. (1984) found that the content of the kavain, demethoxyyangonin and yangonin were higher in the roots than in the stems and leaves, whereas dihydrokavain, methysticin, and dihydromethysticin were higher in the roots than in the stems and the leaves. Content of kavalactone in kava beverages also depend on temperature of water, ratio of water and kava material, and size of kava material and times of extraction.

Chemotype in kava beverages prepared from roots and rhizomes of Isa and Mahakea varieties as extracted with different solvents

The results of the present study indicate that there are some variations in chemotype in kava beverages prepared from Mahakea roots and rhizomes, as extracted with different solvents, whereas the chemotypes of kava beverages prepared from Isa roots and rhizomes appear to be stable (Table 1). The chemotypes of kava beverages prepared from Isa roots and rhizomes as extracted with the five solvents were the same

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