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Comparison of subcritical water and organic solvents for extracting kava lactones from kava root

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Abstract

Subcritical water extraction of lactones from a kava (*Piper methysticum*) root was compared to a Soxhlet extraction with water, to boiling in water, and to a sonication in acetone. For ground kava (250–500 μm), 2 h of subcritical water extraction were required for a complete extraction at 100°C, while at 175°C, 20 min were sufficient. For a complete extraction of the unground (shredded) kava, the time of extraction was extended to 40 min at 175°C. Boiling for 2 h and extraction with Soxhlet apparatus for 6 h, both of which employed water at atmospheric pressure, produced yields 40–60% lower than those obtained with subcritical water. With unground kava, 40 min of subcritical water extraction yielded essentially the same recoveries of lactones as 18 h of sonication with acetone, methylene chloride, or methanol.

Keywords: Subcritical water extraction; Extraction methods; Plant materials; Kava; *Piper methysticum*; Lactones

1. Introduction

Kava (*Piper methysticum*) root is used in the preparation of a ceremonial intoxicating beverage in the Pacific Islands and has a similar role in their society as coffee or tea [1]. Kava is also known for its beneficial effect on health and is used in phytomedicine. Pharmacological studies focused on purified compounds relate the kava biological activity to lactones, major constituents of kava resin [1–4]. Since 1850, a number of studies have been carried out on the isolation and identification of kava lactones [2–19], which can be classified as substituted α-pyrones and 5,6-dihydro-α-pyrones (Fig. 1).

A number of different techniques have been used to isolate and analyze kava lactones. Extracts have been analyzed using thin-layer chromatography [6,7], high-performance liquid chromatography [7–10], gas chromatography [11–16], and recently by micellar electrokinetic chromatography [17] and supercritical fluid chromatography [18]. In contrast to the broad range of techniques used to analyze kava extracts, little attention has been paid to the development of an efficient extraction method. Studies employing water extraction and hydrodistillation [12,15,19] and various organic solvents including chloroform [3,9,12], methylene chloride (CH_2Cl_2) [14], methanol [13,17], ethanol [10], and ethyl acetate [7,11,12] focused on identifying components and/or pharmacological effects, but do not present reasons for selecting solvents or other extraction conditions.

Although some studies reported concentrations of

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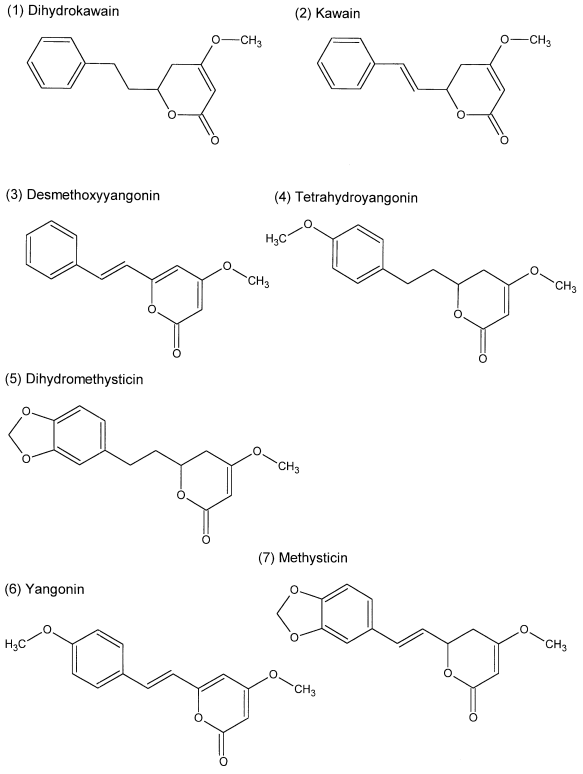

Fig. 1. Chemical structures of seven major kava lactones.

individual compounds extracted from kava, it is not possible to compare the extraction efficiencies because different kava samples were used (likely having different lactone concentrations), and different methods were used to analyze the extracts [4,6–9,11,16]. Only limited comparisons of extraction methods using the same kava samples have been reported. Lopez and Benedicto [16] compared supercritical fluid extraction (SFE) using carbon dioxide (CO_2) modified with 15% ethanol, to sonication with CH_2Cl_2, and to sonication with ethanol. Recoveries were comparable for the first two methods, and slightly lower for ethanol. Ashraf-Khorassani et al. [18] achieved similar recoveries with ethanol-modified CO_2 and CH_2Cl_2–MeOH (50:50), and slightly lower recoveries with pure CO_2.

The present study provides the first direct comparison of kava lactone extraction efficiencies using traditional water extraction and organic solvent extractions, as well as the new technique of subcritical water extraction.

2. Experimental

Air-dried kava root was obtained from Madis Botanicals (South Hackensack, NJ, USA) as received. The kava consisted of shredded material ca. 2–4 mm wide, 1 mm thick and several centimeters long. The development of the extraction method was performed on a ground, sieved kava root of 250–500 μm particle size fraction. Smaller particles were not suitable as their use resulted in plugging of the subcritical water extraction system. The most suitable subcritical water conditions obtained were then applied on the unground kava.

Sample sizes were 0.5 g for all extraction methods. After each extraction, the efficiency of each method was confirmed by extracting the plant tissue residues using 18 h of sonication in 15 mL of acetone.

Since individual standards of kava lactones were not available, capsules (standardized extract) from Nature's Resources Products (Mission Hills, CA, USA) with a defined content of kava lactones of 45 mg per capsule were used for quantitative calibration. An internal standard, *n*-heptadecane (Aldrich, Milwaukee, WI, USA), was employed.

2.1. Subcritical extraction with water

Subcritical water extraction was performed in a laboratory built apparatus previously described in detail [20,21]. The extraction system consisted of two ISCO model 100D syringe pumps (ISCO, Lincoln, NE, USA) delivering water and CH_2Cl_2 at a constant flow-rate to an HP 5890 gas chromatograph oven (Hewlett-Packard, Wilmington, DE, USA), where the extraction cell was mounted. The water (HPLC grade, Fisher Scientific, Pittsburgh, PA, USA), was purged for 2 h with nitrogen to remove dissolved oxygen prior to the extraction and supplied to the system with the pump at a constant flow of 1 mL/min. To preheat the extractant water to the required temperature, a 3-m preheating coil was installed in the oven before the extraction cell. A second pump delivered CH_2Cl_2 at a flow of 1 mL/min to a mixing ''tee'' installed inside the oven after the outlet of the extraction cell to collect the extracted compounds as the water cooled after exiting the extraction oven. Finally, a miniature

back-pressure regulator (Upchurch Scientific, Oak Harbor, WA, USA) was placed at the outlet of the extraction system (outside of the oven) to maintain the system pressure between 60 and 70 bar, thus ensuring that the water was in the liquid state at all temperatures tested.

It is important to note that CH_2Cl_2 was employed only to collect the kava lactones in a solvent suitable for gas chromatographic analysis and that the CH_2Cl_2 does not contact the kava sample (the organic solvent also aids in keeping the transfer tubing clean when solute concentrations are very high [21]). To show the ability of the system to extract and collect kava lactones without the collection solvent, the unground kava was also extracted without adding CH_2Cl_2 to the eluent stream.

All extractions were carried out in a 6.94 mL SFE cell (9.4 mm I.D., 100 mm long, Keystone Scientific, Bellefonte, PA, USA) equipped with a 0.5 μm frit at the inlet, and a 2 μm frit at the outlet. The larger pore frit was installed at the outlet to prevent plugging with plant material. In addition, four layers of glass microfiber filter (1 μm, Whatman, Maidstone, UK) followed by 7 g of 40 μm glass beads (3M Empore, Fisher Scientific, Pittsburgh, PA, USA) were placed inside the cell to protect the outlet frit. The extraction cell was filled with plant material (~0.5 g) and mounted vertically in the oven with water flowing from top to bottom.

The extraction procedure began by pressurizing the system with water to ~60 bar at a flow of 1 mL/min. At this time, the back-pressure regulator (set to 60 bar) opened, the CH_2Cl_2 flow was started, and collection of the eluent began. To prevent plugging, the extraction was started at ambient temperature followed by immediate heating to the required temperature. The start of the extraction (time=0) was set as the moment the oven reached the required temperature. For the temperature dependence experiment, each temperature was held for 10 min, the collection vial was replaced, and the system heated to the next higher temperature (requiring ca. 30 s). For the extraction rate experiments, the collection vial was replaced at frequent time intervals.

For initial temperature dependence and extraction rate experiments, the CH_2Cl_2 fractions were directly analyzed after addition of the internal standard. For quantitative determinations after addition of the internal standard, the CH_2Cl_2 fraction was collected and the water was acidified with 2 *M* HCl (to approx. pH 1) and rinsed twice more with additional 5 mL aliquots of CH_2Cl_2. The acidification and a centrifugation helped to break an emulsion. Combined fractions of the CH_2Cl_2 extracts were analyzed to determine extraction efficiencies. All experiments except the extraction rate experiments were performed in triplicate.

2.2. Boiling of kava

Replicate 0.5-g portions of ground kava (250–500 μm) were boiled under reflux for 2 h using 100 mL of water per extraction. After boiling, the cooled extract was filtered, then the water fraction was acidified and extracted using three 5 mL rinses of CH_2Cl_2. The residue from the filter was extracted using 18 h of sonication in 15 mL of acetone. All extractions were performed in triplicate.

2.3. Soxhlet extraction

Replicate 0.5-g portions of ground kava (250–500 μm) were extracted for 6 h using 150 mL of water per extraction. After the extraction, the water extract was acidified and extracted using three 5 mL rinses of CH_2Cl_2. The residue in the Soxhlet extraction thimble was extracted using 18 h of sonication in 15 mL of acetone. All extractions were performed in triplicate.

2.4. Solvent extraction

Extractions were performed with 0.5-g portions of ground kava (250–500 μm) by sonicating for 18 h in 15 ml of solvent, after the addition of the internal standard. Three different solvents, acetone, CH_2Cl_2, and methanol, were used to compare their extraction efficiencies. All extractions were performed in triplicate for each solvent.

2.5. Gas chromatographic analysis

Quantitative analyses were performed using gas chromatography with flame ionization detection (GC–FID) on a Hewlett-Packard Model 5890 Series

II gas chromatograph equipped with an autosampler. Chromatographic separations were accomplished with a 30 m×0.25 mm I.D. DB-5 column with a 0.25 μm film thickness (J&W Scientific, Rancho Cordova, CA, USA) with injections in the splitless mode for 0.1 min. The oven temperature was held at 130°C for 2 min followed by 8°C/min gradient to 320°C. Quantitations were based on the addition of *n*-heptadecane as the internal standard to each extract and on standard calibration curves generated from analyses of kava capsules containing seven major kava lactones. The identification of kava lactones was based on the literature data [2–15] and confirmed by GC–MS analyses. GC–MS analyses were performed using the same GC conditions and a Hewlett-Packard Model 6890 GC with a Hewlett-Packard Model 5973 MS system.

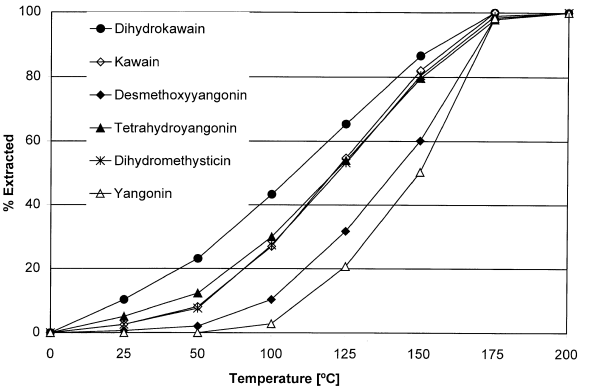

Fig. 2. Effect of temperature on the extraction efficiency of kava (*Piper methysticum*) lactones using subcritical water. Each temperature was held for 10 min. Percent removals are based on sonication of the residue after water extraction.

3. Results and discussion

The development of subcritical water extraction conditions consisted of four main steps. First, appropriate water temperatures were determined by sequentially extracting kava with step-wise increases in temperature. Second, the extract rate curves were determined at the selected temperatures. Third, the recoveries and selectivity of the subcritical water extractions were compared to other extraction techniques. Finally, the most suitable subcritical water conditions were applied to the unground kava.

3.1. Water temperature

The effect of extraction temperature on the recovery of kava lactones is shown in Fig. 2. Ground kava was sequentially extracted for 10 min at 25°C, followed by 50, 100, 125, 150, 175 and 200°C (each temperature for 10 min). Following this extraction sequence, the plant residues were further extracted by 18 h sonication in acetone to determine unextracted lactones and allow determination of overall extraction efficiencies (i.e., 100% extracted for Fig. 2 corresponds to the total mass of each compound extracted by the water and acetone residue extractions). As shown in Fig. 2, the extraction rate significantly increased with rise in temperature to 125°C, where about 20–30% of each compound was extracted in 10 min. At 175°C, two additional compounds were extracted which may or may not be degradation products of kava lactones in subcritical water, because they were not found in the residue. However, their total mass contribution was negligible (about 2% per compound relative to total concentration of kava lactones), and (as discussed later in the text) 175°C extractions did not result in lower recoveries of the major lactones compared to the other water (Soxhlet extraction and boiling) techniques.

3.2. Extraction rates

Based on the temperature behavior of kava shown in Fig. 2, three temperatures, 125, 150 and 175°C were selected to determine the effect of extraction time on the yields of kava lactones. The extraction rate at 100°C was also studied to allow direct comparisons of subcritical water extraction with boiling and Soxhlet extraction.

Fig. 3 confirms faster extraction at higher temperatures. At 175°C, all kava lactones were recovered in 20 min. At 100°C, 90% of most of the kava lactones were recovered after 90 min, but only 40 and 60% of desmethoxyyangonin and yangonin, respectively.

Surprisingly, the ability of CH_2Cl_2 extraction to recover kava lactones from the water extracts depended on the temperature at which the kava was extracted. As shown in Table 1, the concentration of

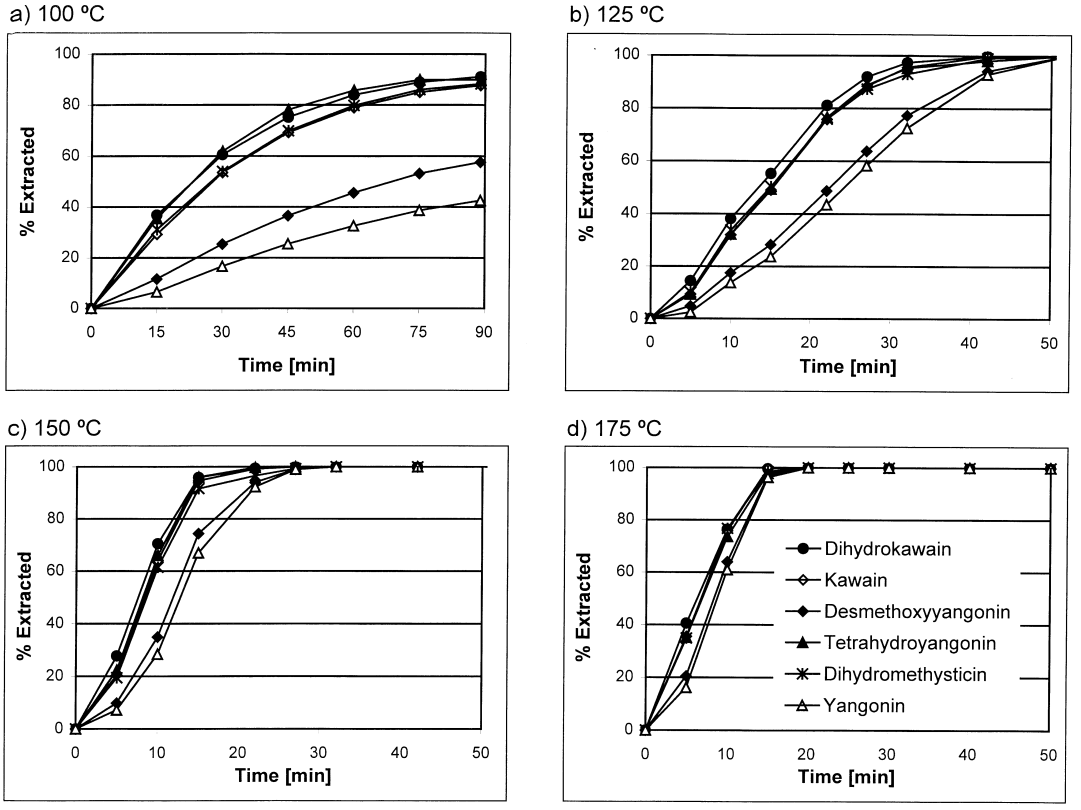

Fig. 3. Effect of extraction time on the extraction efficiency of kava (*Piper methysticum*) lactones using subcritical water at (a) 100°C, (b) 125°C, (c) 150°C and (d) 175°C. Percent removals are based on sonication of the residue after water extraction.

kava lactones obtained with a single, or with three consecutive rinses of CH_2Cl_2, from subcritical water extracts is related to the temperature at which the water extraction was performed. Water extracts obtained at lower temperatures (100°C) were more easily extracted by CH_2Cl_2 than those obtained at higher temperatures (175°C), apparently because of the formation of thicker emulsions at higher temperatures. However, if three rinses with CH_2Cl_2 are used, lactones are efficiently recovered from the water extracts regardless of extraction temperatures, demonstrated by the lack of any additional lactones in subsequent CH_2Cl_2 extracts.

Table 1
Total recovery efficiencies from kava water extracts obtained at different temperatures using single and triplicate rinses of CH_2Cl_2

Temperature of subcritical water extraction (°C)	Total concentration of seven kava lactones (mg/g)	
	Single CH_2Cl_2 rinse	3×CH_2Cl_2 rinses
100	46	51
125	78	93
150	85	111
175	76	121

3.3. Extraction efficiencies of subcritical water extraction versus Soxhlet extraction, boiling and sonication

Based on the extraction rate results, kava root was extracted with subcritical water at 100 and 175°C. As indicated by the extraction rate experiments (Fig. 3), two fractions were collected at each selected temperature; i.e., two 60 min fractions at 100°C, and two 10 min fractions at 175°C.

Chromatograms in Fig. 4 show a simple comparison among the extraction techniques. The methods employing water under atmospheric pressure,

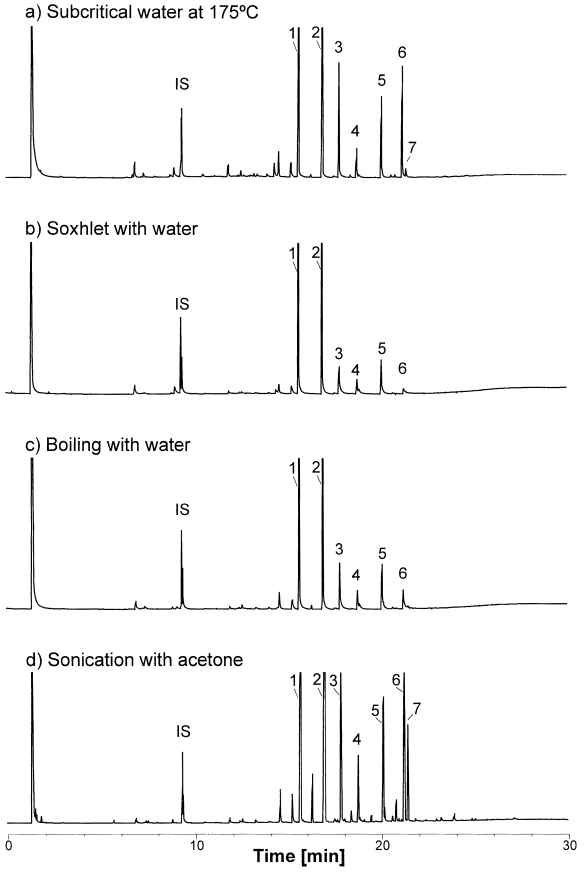

Fig. 4. GC–FID chromatograms of kava extracts obtained with different extraction techniques (for numbering of peaks, see Fig. 1).

Soxhlet extraction, and boiling water, exhibited ~50% lower recoveries than subcritical water (Table 2). For both techniques, lower recoveries were observed mainly for later eluting kava lactones: yangonin and desmethoxyyangonin (Fig. 4). (Note that methysticin was also found in all extracts, and displayed extraction curves similar to those in Fig. 4. However, since methysticin is known to degrade in GC injection ports [11], the quantitative data may not be reliable, and is, therefore, not included in this work.)

Subcritical water at 100°C required 120 min to extract 70% of kava lactones (compared to 18 h sonication in acetone). Higher recoveries of kava lactones (ca. 80%) were obtained in 20 min at 175°C (Table 2).

3.4. Comparison of subcritical water and organic solvent extractions for unground kava

Final comparisons of subcritical water extraction with organic solvent extractions (18 h with sonication) were performed with unground, shredded kava root. Subcritical water extractions were performed at 175°C using the standard technique where CH_2Cl_2 was added after the extraction cell to simplify collection of the kava lactones for GC injection. In order to investigate whether the extractions and collections could be efficiently performed without the addition of CH_2Cl_2, the extractions were per-

Table 2
Concentrations of kava lactones obtained with subcritical water extraction, Soxhlet extraction, boiling in water, and sonication in acetone

Kava lactones	Kava lactones concentrations (mg/g)±SD[a]						
	Extraction conditions						
	Subcritical water, 100°C		Subcritical water, 175°C		Soxhlet, water, 360 min	Boiling, water, 120 min	Sonication, acetone, 18 h
	60 min	120 min[b]	10 min	20 min[b]			
Dihydrokavain	28±1.6	30±0.9	24±3.4	30±2.3	21±1.5	21±4.6	33±1.9
Kavain	30±1.7	33±1.5	27±4.6	33±3.3	16±1.3	21±5.4	43±2.1
Desmethoxyyangonin	8±1.3	11±1.3	9±1.5	14±1.4	4.6±1.0	5.7±2.2	15±0.8
Tetrahydroangonin	2.4±0.2	2.4±0.2	2.6±0.4	3.2±0.3	1.5±0.2	1.7±0.6	4.0±0.2
Dihydromethysticin	6.8±0.4	6.8±0.4	6.1±1.2	7.5±0.8	4.2±0.7	4.1±0.9	10.5±0.4
Yangonin	5.8±0.8	7.5±2.1	10±2.0	15±2.0	1.8±0.9	3.5±1.9	20±1.0
Total of kava lactones	81±5	90±6	80±13	104±10	48±6	57±16	125±6

[a] Concentration and standard deviations (SD) were based on triplicate extractions performed on the same day.
[b] Includes the sum extracted in both time periods.

Table 3
Concentrations of kava lactones in unground kava obtained with subcritical water extraction and sonication in organic solvents

Kava lactones	Concentrations (mg/g)±SD[a]					
	Subcritical water, 175°C			Sonication in acetone, 18 h	Sonication in CH_2Cl_2, 18 h	Sonication in methanol, 18 h
	CH_2Cl_2 collection[b]		Without CH_2Cl_2[c]			
	20 min	40 min	40 min			
Dihydrokavain	23±1.1	32±2.8	31±1.6	33±1.3	37±0.5	35±3.3
Kavain	24±2.0	32±3.9	29±2.4	37±1.9	44±1.6	33±4.0
Desmethoxyyangonin	9.5±0.6	12±1.1	11±0.9	12±0.7	14±0.7	12±1.6
Tetrahydroangonin	2.4±0.1	3.5±0.3	3.1±0.3	3.7±0.2	4.1±0.1	3.6±0.4
Dihydromethysticin	5.6±0.2	9.3±0.7	8.3±0.5	9.7±0.6	11.1±0.3	8.7±1.2
Yangonin	10±0.3	14±1.6	11±1.8	14±1.1	16±0.6	11±2.2
Total of kava lactones	75±4	103±10	93±7	109±5	127±3	104±12

[a] Concentration and standard deviations (SD) were based on triplicate extractions performed on the same day.
[b] CH_2Cl_2 was added after the extraction cell to aid in preparing the extracted lactones for GC analysis.
[c] Extracted lactones were eluted from the extraction system without addition of CH_2Cl_2. The extractant water was then extracted with CH_2Cl_2 for GC analysis.

formed in an identical manner, but without the addition of CH_2Cl_2 to the water extraction effluent (see Experimental).

As shown in Table 3, somewhat longer subcritical water extraction times were required than for the ground kava (Table 2). However, 40 min of subcritical water extraction were sufficient to yield recoveries similar to those obtained with 18 h of sonication with acetone, CH_2Cl_2, or methanol for all lactones (Table 3). None of the organic solvents showed significantly higher recoveries than water, or other organic solvents.

Table 3 also demonstrates that the addition of CH_2Cl_2 after the extraction cell has no effect on the extraction efficiencies. No statistically significant change in extraction efficiencies occurs with or without CH_2Cl_2, demonstrating that only pure water is needed for quantitative recovery of kava lactones.

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