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NOTE

Efficacy of extracting solvents to chemical components of kava (*Piper methysticum*) roots

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Abstract The chemical composition of kava (*Piper methysticum*) lactones and various phytochemicals obtained following the sonication of ground kava roots extracted in the solvents hexane, chloroform, acetone, ethanol, methanol and water, respectively, was analyzed. Eighteen kava lactones, cinnamic acid bornyl ester and 5,7-dimethoxy-flavanone, known to be present in kava roots, were identified, and seven compounds, including 2,5,8-trimethyl-1-naphthol, 5-methyl-1-phenylhexen-3-yn-5-ol, 8,11-octadecadienoic acid-methyl ester, 5,7-$(OH)_2$-4′-one-6,8-dimethylflavanone, pinostrobin chalcone and 7-dimethoxyflavanone-5-hydroxy-4′, were identified for the first time. Glutathione (26.3 mg/g) was found in the water extract. Dihydro-5,6-dehydrokavain (DDK) was present at a higher level than methysticin and desmethoxyyagonin, indicating that DDK is also a major constituent of kava roots. Acetone was the most effective solvent in terms of maximum yield and types of kava lactones isolated, followed by water and chloroform, whereas hexane, methanol, and ethanol were less effective as solvents. Total phenolic and antioxidant activity varied among the extracting solvents, with acetone and chloroform producing the highest effects, followed by water, while methanol, ethanol and hexane were less effective.

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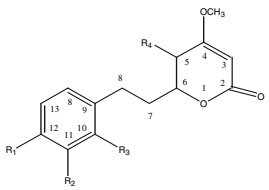
Keywords Antioxidant · Extracting solvent · Kava lactone · Kava root · *Piper methysticum* · Total phenolics

Introduction

A water infusion of kava (*Piper methysticum*) roots has been used as a traditional beverage in the Pacific Islands since ancient times for its soporific and narcotic effects. Several standardized extracts of the biologically active kava lactones are marketed both as herbal medicinal products for anxiety disorders and as dietary supplements to improve stress disorders, nervous tension and restlessness [1]. Kava is a traditional herbal medicine for treating gonorrhea, menstrual pain, tuberculosis, respiratory tract infections, and chronic pain related to gout and arthritic conditions [2, 3]. Pacific Islanders routinely apply kava root extracts as an analgesic and as a mouthwash for toothache and canker sores [4, 5]. In Europe, it was used for the treatment of chronic inflammations of the urinary tract at the beginning of the twentieth century [6, 7]. However, the consumption of kava has recently been reported to cause serious side effects such as hepatitis and acute liver failure [8–11, 13] and, consequently, European and Canadian drug control authorities have withdrawn the registration of all herbal medicinal products containing kava preparations. The U.S. FDA advises hepatitis patients to get their doctor's advice before consuming kava [9, 12]. Whitton et al. [9] noted that the consumption of high doses of kava lactones resulted in hepatotoxic side effects due to glutathione depletion in the liver. Other biological activities of kava lactones have been reported, including herbicidal and antifungal activities [14] and cyclooxygenase (I and II) enzyme inhibition [15].

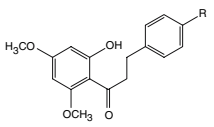
To date, the phytochemicals identified in kava roots include 18 kava lactones and three chalcones, bornyl ester of cinnamic acid derivatives and several flavanones [15] (Fig. 1). The pharmaceutical and biological effects of kava roots may result from all a combined effect of all compounds present in kava. However, kavain, 7,8-dihydrokavain, dihydromethysticin, desmethoxyyagonin, methysticin and yagonin and the three dihydrochalcones have been the focus of most of the research carried out on kava, and little attention has been paid to other kava lactones and constituents in kava roots.

The aim of this study was to (1) determine the efficacies of extracting solvents having different polarities on the chemical profile of kava roots, including lactones and other phytochemicals, and (2) to investigate the effect of using extracting solvents on the total phenolic content and on the antioxidant activities of kava roots.

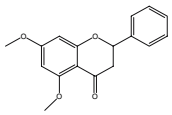


Kava lactones	R1	R2	R3	R4	C5-C6	C7-C8
11-Hydroxy-12-methoxydihydrokavain	OCH_3	OH				
7,8-Dihydro-5-hydroxykavain				β-OH		
11,12-Dimethyxydihydrokavain	OCH_3	OCH_3				
Methysticin		OCH_2O				=
Dihydromethysticin		OCH_2O				
Dihydro-5,6-dehydrokavain (DDK)		OCH_3			=	
Kavain						=
7,8-Dihydrokavain						
Dihydro-5,6-dehydrokavain					=	
5,6-Dehydromethysticin		OCH_2O			=	=
Desmethoxyyagonin					=	=
Yagonin	OCH_3				=	=
5,6,7,8-Tetrahydroyagonin		OCH_3				
5,6-Dihydroyagonin	OCH_3					=
7,8-Dihydroyagonin	OCH_3				=	
10-Methoxyyagonin	OCH_3		OCH_3		=	=
11-Methoxyyagonin	OCH_3	OCH_3			=	=
11-Hydroxyyagonin	OCH_3	OH			=	=
Hydroxykavain				OH		=
11-Methoxy-12-hydroxydehydrokavain	OH	OCH_3			=	=

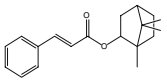
Dihydrochalcones	R
Flavokavain A	OCH_3
Flavokavain B	H
Flavokavain C	OH



Other compounds



5,7-Dimethoxyflavanone



Cinnamic acid bornyl ester

Fig. 1 Common kavalactones, dihydrochalcones and other constituents identified in kava roots (Bilia et al. [23]) with some modifications

Materials and methods

Reference substances

Kavain, 7,8-dihydrokavain, dihydromethysticin, desmethoxyyagonin, methysticin and yagonin were purchased from PhytoLab GmbH & Co. KG, Germany, and glutathione was obtained from the Wako Company (Osaka City, Japan). Dihydro-5,6-dehydrokavain (DDK) was isolated from Alpinia (*Alpinia zerumbet*) leaves using the method described by Tawata et al. [16].

Solvents and reagents

Acetone, acetic acid, acetonitrile, chloroform, methanol, ethanol and hexane were of analytical grade and purchased from Wako Pure Chemical Industries.

Plant materials

Dried kava roots were imported from Ronnies Nakamal, Port Vila, Vanuatu (specimen number: Ka04-31), ground into powder and stored in the freezer at –20°C before use.

Preparation of extracts

Extractions were performed by sonicating 5 g ground kava root for 30 min in 200 ml of solvent, followed by filtration; this procedure was repeated three times. Six different solvents – hexane, chloroform, acetone, ethanol, methanol, and distilled water – were compared their extraction efficiency. The filtered extracts obtained from extraction with any one solvent were combined, and dried under vacuum at 25°C. The kava lactones and glutathione were dissolved in acetone and methanol, filtered through a 0.25-μm membrane and analyzed directly by gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC), respectively.

HPLC analysis

Glutathione were measured at 280 nm using a Shimadzu HPLC (model SCL-10 A vp; Shimadzu Co, Kyoto, Japan) coupled with a UV-Vis detector (model SPD-20A; Shimadzu). Separations were achieved on a RP-18 ZORBAX ODS column (Agilent Technologies, J&W Scientific Products, Folsom, CA) (internal diameter 25 × 0.46 cm; particle size 5 μm). The mobile phase

consisted of water with 1% acetic acid (v/v) (solvent A) and methanol:acetonitrile:acetic acid (95:4:1, v/v/v) (solvent B). The gradient elution was performed as follows: 0–2 min, 5% B isocratic; 2–10 min, linear gradient 5–25% B; 10–20 min, linear gradient 25–40% B; 20–30 min, linear gradient 40–50% B; 30–40 min, linear gradient 50–100% B; 40–45 min, 100% B isocratic and 45–55 min, linear gradient 100–5% B. The flow rate was 1.0 ml/min and the injection volume was 5 µl. The quantification of glutathione was determined based on peak area measurements that were compared to calibration curves of the corresponding reference compound.

GC–MS analysis

An aliquot of 1 µl aliquot of each kava sample was injected into the GC–MS (model QP-2010; Shimadzu). The data were obtained on a DB-5MS column (length 30 m, internal diameter 0.25 mm, thickness 0.25 µm) (Agilent Technologies, J&W Scientific Products). The carrier gas was helium, and the GC oven temperature program consisted of: a 50°C hold for 6 min, increases in temperature at a rate of 5°C/min to 280°C, followed by a hold for 5 min. The injector and detector temperatures were set at 250 and 280°C, respectively. The mass range was scanned from 15 to 900 amu. The control of the GC–MS system and the data peak processing were carried out using Shimadzu's GC–MS Solution software ver. 2.4.

Identification and quantification of phytochemicals in kava

Chemicals in kava roots were identified by comparing the retention times and peak areas between the standard chemicals and the samples and by mass spectra using Shimadzu's GC–MS Solution software ver. 2.4 and from literature. Kavain, 7,8-dihydrokavain, dihydromethysticin, desmethoxyyagonin, methysticin, yagonin and DDK were quantified by GC using a program similar program in the GC–MS, whereas glutathione was identified and quantified by HPLC. Four dilutions of 50, 100, 500 and 1000 ppm were used to obtain standard curves of the reference chemicals ($r > 0.99$). These compounds were identified by HPLC by comparing their retention times to those of the standards. However, with the exception of kavain, 7,8-dihydrokavain, dihydromethysticin, desmethoxyyagonin, methysticin, yagonin, DDK and glutathione, all other constituents were determined by GC as the percentage of peak area using a software program similar to that used in the GC–MS analysis, as these latter constituents could neither be purchased nor successfully purified in our laboratory. The content of the quantified compounds was expressed in miligrams per gram of extract.

Measurement of total phenolics

The amount of total phenolics in the water, chloroform, acetone, methanol, ethanol and hexane extracts of kava roots was determined according to the Folin–Ciocalteu procedure described by Kähkönen et al. [17]. Two hundred microliters of each extract (500 ppm) was introduced into a test tube to which 1.0 ml of Folin–Ciocalteu's reagent and 0.8 ml of sodium carbonate (7.5%) was added. The solutions were well mixed and allowed to stand for 30 min. Absorption was measured at 765 nm using a Shimadzu UV-160 A spectrometer. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram extract.

Antioxidant activity using the DPPH radical scavenging system

The radical scavenging activity was evaluated as described previously [18]. A 2-ml aliquot of the ethanol solution of the root extracts was mixed with 1 ml of 0.5 m*M* 1,1-diphyenyl-2-picrylhydrazyl (DPPH) ethanol solution and 2 ml of 0.1 *M* sodium acetate buffer (pH 5.5). After shaking, the mixture was incubated at room temperature in the dark for 30 min, following which the absorbance was measured at 517 nm using a Shimadzu UV-160A spectrometer. Ethanol was used as negative reference. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula of Son and Lewis [19] as follows:

$$\%\,\text{radical scavenging activity} = [(A_{\text{control}} - A_{\text{test}})/A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the control (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution plus antioxidant).

Statistical analysis

All treatments were arranged in a completely randomized design with at least three replications. Data were subjected to analysis of variance (ANOVA) with significant differences between means identified by GLM procedures using SAS ver. 6.12 (SAS Institute, Raleigh, NC) with $P < 0.05$ adopted as the criterion of significance.

Results and discussion

Compounds detected for the first time from kava roots

Based on the GC–MS analysis, we identified a total of 18 kava lactones, three chalcones (flavokavains A, B, and C), cinnamic acid bornyl ester and 5,7-dimethoxyflavanone (Fig. 1). Seven compounds were detected in kava roots for the first time, including 2,5,8-trimethyl-1-naphthol (1), 5-methyl-1-phenylhexen-3-yn-5-ol (2), 8,11-octadecadienoic acid-methyl ester (3), 5,7-$(OH)_2$-4′-one-6,8-dim ethylflavanone (4), pinostrobin chalcone (5), and 7-dimethoxyflavanone-5-hydroxy-4′ (6). Compounds (1–6) were tentatively identified based on mass spectra obtained by GC–MS and from the literature. The chemical structure, retention time and molecular weight of these compounds are shown in Figs. 1 and 2 and Table 1. In addition to the long chain fatty acid (3) and flavanones (4, 6), another type of chalcone (5) was found. This study reconfirmed the existence of DDK in kava roots. Hocart et al. [20] reported that kava roots possess DDK; however, they did not quantify it, despite that fact that several pharmaceutical properties of DDK had been reported [16, 21].

The six newly detected compounds (1–6) were identified as phenolics, flavanones and fatty acids, as well as pinostrobin chalcone (6). Compound (3) was found in all extracting solvents (Table 1), and compound (5) was present in all solvents with the exception of hexane. Conversely, substances (1) and (2) were detected only in hexane, and compound (4) was identified only in water, acetone and hexane. Extraction with chloroform and hexane did not yield compound (6). These compounds need to be purified and their identity confirmed by analytical instruments such as nuclear magnetic resonance, GC–MS and infrared spectroscopy. The biological activities of these constituents should also be examined for their involvement in kava roots.

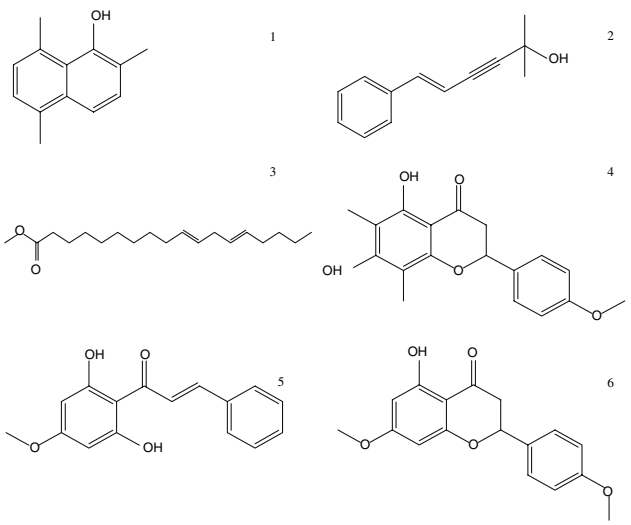


Fig. 2 Compounds (1–6) were detected and identified for the first time from kava roots. *1* 2,5,8-Trimethyl-1-naphthol, *2* 5-Methyl-1-phenylhexen-3-yn-5-ol, *3* 8,11-Octadecadienoic acid-methyl ester, *4* 5,7-$(OH)_2$-4′-one-6,8-dimethylflavanone, *5* Pinostrobin chalcone, *6* 7-Dimethoxyflavanone-5-hydroxy-4′

Content of kava lactones and other compounds in different solvents

By comparing the percentage of peak area, we determined that acetone was the most effective solvent in terms of maxium yield of kava lactones (89.5%), followed by water (78.5%) and chloroform (71.9%). Hexane (68.6%) was more efficacious than both methanol (64.3%) and ethanol (52.3%). A similar order of effectiveness and efficacy was obtained when total percentage of peak areas of compounds other than kava lactones was used. The quantities of each compound based on percentage of peak area (18 lactones, three dihydrochalcones, cinnamic acid bornyl ester, 5,7-dimethoxyflavanone and the other six substances detected for the first time from kava roots) are shown in Table 1. Glutathione was detected only in the water extract (26.3 mg/g); the HPLC analysis did not reveal the presence of glutathione in any of the other extracting solvents.

We quantified the seven kava lactones (kavain, 7,8-dihydrokavain, dihydromethysticin, desmethoxyyagonin, methysticin, yagonin, and DDK) and glutathione, as shown in Table 2. Similar to the results shown in Table 1, the highest levels of these lactones were detected in acetone (286.2 mg/g), with water and chloroform yielding approximately similar amounts of kava lactones (108.6 and 106.2 mg/g, respectively) and methanol (45.6 mg/g) being more effective than ethanol and hexane (22.5 and 26.3 mg/g, respectively). The amount of each individual kava lactone varied among extracting solvents (Table 2). There was more DDK (1.9–27.1 mg/g) than methysticin (0–14.4 mg/g) in kava roots in all solvents, and more DDK than desmethoxyyagonin (2.1–21.0 mg/g) in a number of solvents, particularly water and acetone (Table 2). Our analyses therefore that DDK is also a major lactone in kava roots.

Whitton et al. [9] reported that high consumption of lactones will lead to rapid depletion in glutathione levels, resulting in free lactone exposure of the hepatocytes and consequent damage [22]. As observed in Table 2, the amount of total lactones in the water extract (108.6 mg/g) was almost similar to that in chloroform extract (106.2 mg/g) and much higher than that found in the methanol, ethanol and hexane extracts (45.6, 22.5, and 26.3 mg/g, respectively).

Table 1 Composition of kava root constituents by different extracting solvents

Compounds	Retention time (min)	Molecular weight	Peak area (%)					
			Water	Acetone	Chloroform	Methanol	Ethanol	Hexane
Kava lactones								
10-Methoxyyagonin	26.5	288	0.1	–	–	–	–	–
Hydroxykavain	35.8	246	–	–	–	0.3	–	0.2
Dihydro-5,6-dehydrokavain	43.6	230	2.9	5.2	0.8	1.4	1.1	1.1
7,8-Dihydrokavain	44.5	232	28.9	24.2	21.4	33.2	27.5	32.4
7,8-Dihydroyagonin	45.9	260	–	0.9	0.1	0.1	–	–
Kavain	46.6	230	17.6	13.2	10.9	9.6	7.5	12.1
7,8-Dihydro-5-hydroxykavain	47.2	248	–	0.2	0.1	–	–	–
5,6-Dihydroyagonin	47.4	260	–	0.6	0.9	–	–	–
11-Hydroxy-12-methoxydihydrokavain	47.5	278	–	5.7	5.1	–	–	–
11-Methoxyyagonin	47.6	288	–	1.6	0.2	–	–	–
Desmethoxyyagonin	47.9	228	3.9	5.7	5.1	5.5	4.3	6.3
5,6,7,8-Tetrahydroyagonin	49.3	262	5.9	3.9	-	3.0	2.2	3.2
Methysticin	50.5	274	–	0.5	–	–	–	0.9
Dihydromethysticin	51.5	276	15.5	15.2	13.6	7.4	7.1	9.1
11,12-Dimethoxydihydrokavain	51.6	292	–	–	1.8	–	–	–
Yagonin	53.1	258	1.9	14.6	8.1	3.8	2.6	2.5
11-Methoxy-12-hydroxydehydrokavain	53.2	274	–	–	3.1	–	–	0.1
11-Hydroxyyagonin	53.6	274	1.8	4.0	0.2	–	–	–
5,6-Dehydromethysticin	55.4	272	–	0.2	0.5	–	–	0.7
Dihydrochalcones								
Flavokavain B	49.7	286	0.1	0.5	0.8	–	–	0.4
Flavokavain C	49.9	286	–	–	0.2	–	–	0.1
Flavokavain A	56.1	316	–	–	0.2	–	–	–
Compounds other than kava lactones								
Cinnamic acid bornyl ester	33.1	284	0.2	0.1	0.2	0.6	0.4	0.3
5,7- Dimethoxyflavanone	40.2	284	–	0.3	0.4	0.3	0.3	0.2
Compounds detected first time from kava roots								
2,5,8-Trimethyl-1-naphthol	15.2	186	–	–	–	–	–	0.1
5-Methyl-1-phenylhexen-3-yn-5-ol	18.0	186	–	–	–	–	–	0.1
8,11-Octadecadienoic acid-methyl ester	30.8	294	0.1	0.1	0.1	0.2	0.2	0.1
Pinostrobin chalcone	36.8	270	0.1	0.1	0.1	0.1	0.1	–
7-Dimethoxyflavanone-5-hydroxy-4′	42.7	300	0.6	3.5	–	0.1	0.1	–
5,7-$(OH)_2$-4′-one-6,8-dimethylflavanone	47.7	314	1.2	0.1	–	–	–	0.1
Total lactones			78.5	89.5	71.9	64.3	52.3	68.6
Total all compounds			83.7	99.4	74.7	67.0	54.5	71.1

–, Not detected

Total phenolics

The content of total phenolics varied among the extracting solvents (Fig. 3). The chloroform extract provided maximum phenolic content (63.1 mg GAE/g extract), followed by the acetone extract (43.0 mg). The amount of total phenolics in the water extract (6.1 mg) was markedly reduced relative with that of the acetone extract. However, methanol, ethanol and hexane extracts yielded a minimum of total phenolics (2.9, 2.9, and 2.2 mg, respectively). The quantity of total phenolics in terms of maxium yield by a particular solvent was chloroform > acetone > water > methanol, and ethanol > hexane extracts.

Antioxidant activity

The acetone extract showed the highest DPPH scavenging activity, followed by the chloroform and water extracts (Fig. 4). The antioxidant activities of the methanol and

Table 2 Quantity of seven major kava lactones and glutathione in kava roots (mg/g extract)

Chemicals	Water	Acetone	Chloroform	Methanol	Ethanol	Hexane
Methysticin	0.0	5.5	14.4	0.0	0.0	1.2
Dihydromethysticin	31.5	51.9	18.9	5.4	3.2	3.6
Kavain	36.9	41.5	14.7	6.9	3.3	4.7
7,8-Dihydrokavain	3.8	55.1	23.0	18.6	9.4	10.1
Dihydro-5,6-dehydrokavain (DDK)	22.9	27.1	4.7	4.7	2.1	1.9
Desmethoxyyagonin	6.7	21.0	7.6	4.3	2.1	2.7
Yagonin	6.8	84.1	22.9	5.7	2.4	2.1
Total lactones	108.6	286.2	106.2	45.6	22.5	26.3
Glutathione	26.3	0.0	0.0	0.0	0.0	0.0

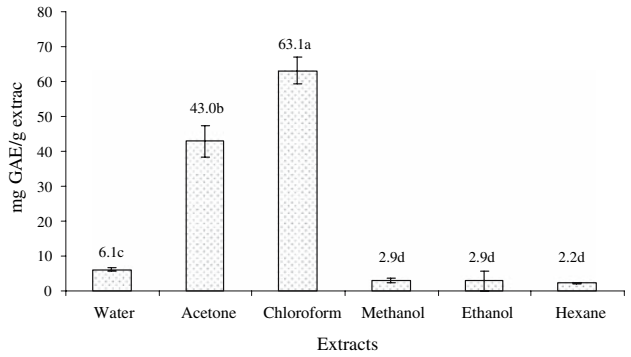


Fig. 3 Total phenolic content in extracting solvents of kava roots. Each value represents the mean of three replicates ± standard errors. Means with the same letter are not significantly different at $P < 0.05$

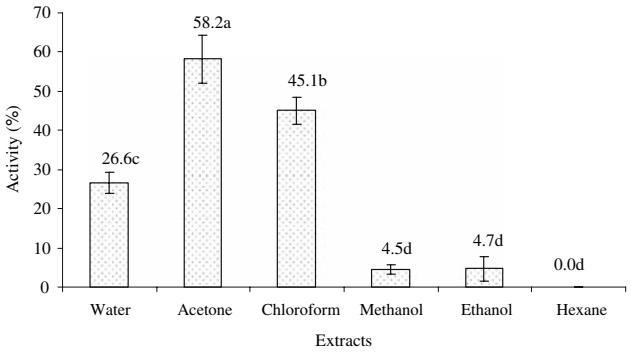


Fig. 4 Antioxidant activity (%) in kava root extracting solvents. Each value represents the mean of three replicates ± standard errors. Means with the same letter are not significantly different at $P < 0.05$

ethanol extracts were significantly lower than that of the water extract, while the extract of hexane showed no activity. In terms of antioxidant activity of the solvent, acetone > chloroform > water > methanol > ethanol > hexane extracts.

Our study revealed that acetone was the most effective in extracting kava lactones and other constituents from kava roots. Water and chloroform were useful, but were less effective than acetone. Hexane, methanol and ethanol were not ideal solvents for extracting phytochemicals in kava roots. The results of this study may be useful in developing protocols for the effective use of the pharmaceutical and other biological properties of kava roots.

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References

1. Bilia AR, Gallori S, Vincieri FF (2002) Kava–kava and anxiety: growing knowledge about the efficacy and safety. Life Sci 70:2581–2597
2. Singh YN (1992) Kava: an overview. J Ethnopharm 37:13–45
3. Singh YN, Blumenthal M (1997) Kava, an overview. HerbalGram 39:34–55
4. Chevallier A (1996) The encyclopedia of medicinal plants. DK Publ, New York
5. Leung AY, Foster S (1996) Encyclopedia of common natural ingredients. Wiley, New York
6. Mors WB, Magalhaes MT, Gottlieb OR (1962) Naturally occurring aromatic derivatives of monocyclic α-pyrones. In: Par R (ed) Progress in the chemistry of organic natural products, vol 20. Vienna New York Springer, pp 131–161
7. Sahelian R (1998) Clinical uses of kava. In kava, the miracle antianxiety herb. St Martin's Press, New York, pp 85–88
8. Bilia AR, Bergonzi MC, Lazari D, Vinceiri FF (2002) Characterization of commercial kava–kava herbal drug and herbal drug preparations by means of nuclear magnetic resonance spectroscopy. J Agric Food Chem 50:5016–5025
9. Whitton PA, Lau A, Salisbury A, Whitehouse J, Evans CS (2003) Kava lactones and the kava–kava controversy. Phytochemistry 64:673–679
10. Stickel F, Baumuller HM, Seitz K, Vasilakis D, Seitz G, Seitz HK, Schuppan D (2002) Hepatitis induced by kava (*Piper methysticum rhizoma*). J Hetatol 39:62–67
11. Zou L, Henderson GL, Harkey MR, Sakai Y, Li A (2004) Effects of kava (kava–kava, 'Awa', Yaqona, *Piper methysticum*) on c-DNA-expressed cytochrome P450 enzymes and human cryopreserved hepatocytes. Phytomedicine 11:285–294
12. Clouatre DL (2004) Kava kava: examining new reports of toxicity. Toxicol Lett 150:85–96
13. Meissner O, Haberlein H (2005) HPLC analysis of flavokavins and kavapyrones from *Piper methysticum* Forst. J Chromatogr B 826:46–49

14. Xuan TD, Elzaawely AA, Fukuta M, Tawata S (2006) Herbicidal and fungicidal activities of lactones in kava (*Piper methysticum*). J Agric Food Chem 54:720–725
15. Wu D, Nair MG, Dewitt DL (2002) Novel compounds from *Piper methysticum* Forst (kava kava) roots and their effect on cyclooxygenase enzyme. J Agric Food Chem 50:701–705
16. Dragull K, Yoshida WY, Tang CS (2003) Piperidine alkaloids from *Piper methysticum*. Phytochemistry 63:193–198
17. Tawata S, Taira S, Kobamoto N, Ishihara M, Toyama S (1996) Syntheses and biological activities of dihydro-5,6-dehydrokawain derivatives. Biosci Biotech Biochem 60:1643–1645
18. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha J, Pihlaja K, Kujala TS, Heinonen M (1999) Antioxidant activity of plant extract containing phenolic compounds. J Agric Food Chem 47:3954–3962
19. Abe N, Murata T, Hirota A (1998) Novel DPPH radical scavengers, bisorbicillinol and demethltrichdimerol, from a fungus. Biosci Biotech Biochem 62:661–666
20. Son S, Lewis BA (2002) Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: structure–activity relationship. J Agric Food Chem 50:468–472
21. Hocart HC, Fankhauser B, Buckle DW (1993) Chemical archaeology of kava, a potent brew. Rapid Commun Mass Spectrometry 7:219–224
22. Zhu J, Majikina M, Tawata S (2001) Syntheses and biological activities of pyranyl-substituted cinnamates. Biosci Biotech Biochem 65:161–163
23. Bilia AR, Scalise L, Bergonzi MC, Vincieri FF (2004) Analysis of kavalactones from *Piper methysticum* (kava-kava). J Chromatogr B 812:203–214