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Herbicidal and Fungicidal Activities of Lactones in Kava (*Piper methysticum*)

T. D. XUAN, A. A. ELZAAWELY, M. FUKUTA, AND S. TAWATA*

Department of Bioscicence and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Okinawa 903-0123, Japan

This is the first report showing that kava lactones are plant and plant fungus growth inhibitors. Aqueous extract of kava roots showed high allelopathic potential and strongly suppressed germination and growth of lettuce, radish, barnyardgrass, and monochoria. Nine kava lactones were detected using GC-MS including desmethoxyyagonin, kavain, 7,8-dihydrokavain, hydroxykavain, yagonin, 5,6,7,8-tetrahydroxyyagonin, methysticin, dihydromethysticin, and 11-hydroxy-12-methoxydihydrokavain. Quantities of desmethoxyyagonin, kavain, 7,8-dihydrokavain, yagonin, methysticin, and dihydromethysticin detected were 4.3, 6.9, 18.6, 5.7, 1.4, and 5.4 mg/g of dry weight, respectively. These six major lactones in kava roots showed great herbicidal and antifungal activities. Growth of lettuce and barnyardgrass were significantly inhibited at 1−10 ppm, and four plant fungi including *Colletotrichum gloeosporides*, *Fusarium solani*, *Fusarium oxysporum*, and *Trichoderma viride* were significantly inhibited at 10−50 ppm. The biological activities of kava lactones were characterized by different double-bond linkage patterns in positions 5,6 and 7,8. The findings of this study suggest that kava lactones may be useful for the development of bioactive herbicides and fungicides.

KEYWORDS: Fungicide; herbicide; inhibition; kava roots; kava lactones; plant fungi; weeds

INTRODUCTION

Kava is a perennial pepper plant found in the Oceanic Islands of the South Pacific. Kava root is the source of perhaps the most important traditional beverage for many South Pacific Island people (*1*). Aqueous extracts of kava roots have been consumed over the past 2000 years without serious effects on health (*2*). Historically, kava has been a popular remedy due to its anxiolytic properties (*3*). Many pharmaceutical products prepared from the lipophilic extracts have been widely available as nonpresciptive botanical dietary supplements (*4*). However, kava extracts were banned in the entire European Union and Canada in January 2003 and have been subjected to alerts and advisories by the U.S. FDA as a result of 11 cases of hepatic failure leading to liver transplants, including four deaths (*5*). Kava lactones, which are mainly found in the roots, are considered to be the active constituents responsible for the pharmacological activity in humans and animals (*6*). Three possible mechanisms for kava lactone hepatotoxicity are known, namely, inhibition of cytochrome P450, reduction in liver glutathione content, and inhibition of cyclooxygenase enzyme activity (*5*). The pharmaceutical properties of kava lactones are postulated to include blockade of voltage-gated sodium ion channels, enhanced ligand binding to γ-aminobutyric acid (GABA) type A receptors, diminished excitatory neurotransmitter release due to calcium ion channel blockade, reduced neuronal re-uptake of noradrenaline (norepinephrine), reversible inhibition of monoamine oxidase B, and suppression of the synthesis of the eicosanoid thromboxane A^2, which antagonizes GABA (A) receptor function (*7*−*10*).

In our previous research we observed that incorporation of kava roots into soils reduced spontaneous emergence of paddy weeds by 80% and increased rice tillering and root growth (*11*, *12*). The aqueous extract of kava also significantly inhibited the growth of five fungi including *Fusarium solani*, *Pyricularia griesa*, *Rhizopus stolonifer*, *Taphrina deformans*, and *Thanatephorus cucumeris* (*12*). Xuan et al. (*13*) identified eight phenolic compounds in kava roots including gallic, protocatechuic, *p*-hydroxybenzoic, *p*-coumaric, ferulic, salicylic, *trans*-*o*-coumaric, and *trans*-cinnamic acids. However, several substances present in high quantities with greater inhibitory activity than that shown by the phenolic acids remained unidentified. This study was conducted to clarify what substances are actually responsible for the strong inhibitory activities of kava roots and to determine their effects on plant and plant fungus growth.

MATERIALS AND METHODS

Kava Roots. Commercial dried kava roots were imported from Vanuatu, ground into powder, and stored in the freezer at −20 °C before use.

Tested Plants. Commercial seeds of lettuce (*Lactuca sativa* L. cv. Great Lakes 366) and radish (*Raphanus sativus* L. cv. Gensuke) and two noxious paddy weeds, barnyardgrass (*Echinochloa crus-galli* Vasing.) and monochoria (*Monochoria vaginalis* Presl var. *plantaginea* Solms-Laub.), were used for bioassays in this experiment. Empty and undeveloped seeds were discarded after they floated in tap water. The

* Corresponding author (telephone/fax 81-98-895-8734; e-mail g056003@agr.u-ryukyu.ac.jp).

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remaining seeds were then air-dried and hermetically stored at −20 °C. These seeds were sterilized with 1% sodium hypochlorite for 30 min and rinsed many times with distilled water before use. The germination percentage of each of these plants was randomly checked, and all were >80%.

Preparation of Extracts. Five grams of kava root powder was extracted with 100 mL of 70% MeOH at 40 °C in a water bath for 4 h. The solvent was then filtered and the supernatant evaporated in a rotary evaporator at 40 °C until dryness. The dry extract was dissolved in distilled water, and its influence on germination and growth of lettuce, radish, barnyardgrass, and monochoria was tested.

Bioassays. Twenty seeds of each indicator plant were sown evenly in a Petri dish (9 cm in diameter) lined with filter paper and moistened with 8 mL of each concentration of 50, 100, and 500 ppm of kava. Five replicates of all treatments were placed in an incubator (25 °C, 4000 lx, with an 8/16 h day/night cycle, humidity = 75%) using a completely random design. Treatments with distilled water only were the controls. After 7 days, the number of germinated seeds was counted and the lengths of shoots and roots were measured.

TLC Experiment. After it had been confirmed that the aqueous extract of kava root exhibited strong inhibition on the emergence of the indicator plants, the precipitate of kava root extracts was dissolved in MeOH for the TLC experiment. The combination of benzene/ethyl acetate (24:1) gave the best separation and was therefore selected. The TLC plates were coated with a 500 μm layer of silica gel (Merck). The prepared kava extract was applied to the TLC plate (16 × 20 cm). R_f values of the colored spots detected under the UV light were recorded, and the plate area was scraped and eluted with methanol. Total solids were determined for each fraction to refer all of the results to the same concentration and reported as percent of total. The effects of these spots on germination and growth of barnyardgrass were examined using the bioassay method described above. Treatments were replicated three times. Spots that showed inhibition on barnyardgrass emergence were collected, dissolved in acetone, and used for GC-MS analysis.

GC-MS Analysis. A 1 μL aliquot of each spot/acetone solution was injected (splitless) into the GC-MS (QP-2010, Shimadzu Co., Kyoto, Japan). The data were obtained on a DB-5MS column, 30 m length, 0.25 mm i.d., and 0.25 μm thickness (Agilent Technologies, J&W Scientific Products, Folsom, CA). The carrier gas was helium, and the GC oven temperature program was as follows: 50 °C hold for 5 min, raised at 5 °C/min to 280 °C, and 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 by means of Shimadzu's GC-MS solution software, version 2.1.

Quantification of Kava Lactones in Kava Roots. Six lactones including 7,8-dihydrokavain, dihydromethysticin, desmethoxyyagonin (5,6-dehydrokavain), kavain, methysticin, and yagonin were purchased from PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany. They were dissolved in acetone and injected into the GC-MS using GC conditions similar to those described above to quantify kava lactones in kava roots. Kava lactones were identified and quantified by comparing the mass spectra, retention times, and peak areas between the standard chemicals and the samples. However, some of the identified constituents could not be quantified as they could neither be purchased nor purified in our laboratory.

Herbicidal Activity. Dilutions of six pure reference kava lactones at 1 and 10 ppm were prepared and examined for their influence on the emergence of barnyardgrass and lettuce using the reported bioassay method.

Antifungal Activity. *Colletotrichum gloeosporides*, *Fusarium solani*, *Fusarium oxysporum*, and *Trichoderma viride* are noxious plant fungi. *C. gloeosporides* causes anthracnose, a serious foliar disease of mango in South and Southeast Asia, South Africa, southern and middle America, France, and Hawaii (*14*). *F. solani* is a soilborne fungus attacking a wide range of host plants, which includes 66 plant families, and has a worldwide distribution. The fungus is ubiquitous in soil under citrus cultivation. *F. solani* was also implicated as a possible causal agent of citrus blight, an important decline of citrus in many countries (*15*). *F. oxysporum* has been characterized as causing the following symptoms: vascular wilt, yellows, corm rot, root rot, and damping-off. Hosts of this harmful fungus include potato, sugarcane, garden bean, cowpea, prickly pear, cultivated zinnia, pansy, Assam rattlebox, and *Musa* sp. (*16*). *T. viride* can be ranked as one of the most widely distributed of all soil fungi. There are many references to its existence in a wide range of forests, grasslands, and cultivated soils. This fungus can cause problems in the industrial cultivation of mushrooms, and also tulip bulbs may frequently be infected (*17*).

The fungal strains of *C. gloeosporides*, *F. solani*, *F. oxysporum*, and *T. viride* were used in this test. Antifungal activity was determined by using the method described by Masika and Afolayan (*18*). Fungal cultures were maintained on potato dextrose agar (PDA) and were recovered for testing by subculturing on fresh medium for 3 days. The prepared PDA plates containing 7,8-dihydrokavain, dihydromethysticin, desmethoxyyagonin (5,6-dehydrokavain), kavain, methysticin, and yagonin dissolved in water at concentrations of 10 and 50 ppm were inoculated in plugs obtained from the actively growing margin of the fungus plates and incubated at 25 °C. Controls contained distilled water only. After 4 days, the antifungal activities of these kava lactones were determined.

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 version 6.12 (SAS Institute, 1997) with $P \leq 0.05$ adopted as the criterion of significance.

Table 1. Effects of Kava Root Extracts on Germination and Growth of Tested Plants (Percent of Control)[a]

	inhibition (% of control)		
treatment (ppm)	germination	shoot length	root length
	Lettuce		
50.0	71.3 a	52.9 a	78.8 a
100.0	94.1 b	82.2 b	94.5 a
500.0	100.0 c	100.0 b	100.0 a
	Radish		
50.0	61.4 a	56.8 a	68.4 a
100.0	89.7 b	61.5 a	86.2 ab
500.0	100.0 b	100.0 b	100.0 b
	Barnyardgrass		
50.0	26.8 a	27.2 a	30.0 a
100.0	33.3 a	48.6 b	84.3 b
500.0	43.5 a	62.3 b	95.9 b
	Monochoria		
50.0	43.3 a	21.9 a	46.1 a
100.0	71.2 b	70.1 b	86.4 b
500.0	82.3 b	90.5 b	98.1 b

[a] Values in a column with the same letter are not significantly different at $P \leq 0.05$.

RESULTS

Herbicidal Activity. The aqueous extracts of kava roots strongly stunted germination and growth of lettuce, radish, barnyardgrass, and monochoria (**Table 1**). However, the level of inhibition varied among these plants. At 50−100 ppm, germination and growth of all plants were strongly inhibited, but barnyardgrass and monochoria were less influenced than lettuce and radish. At 500 ppm, emergence of both lettuce and radish was completely inhibited, germination and growth of monochoria were reduced by 80−90%, and barnyardgrass was least inhibited. The aqueous extracts of kava roots exhibited strong inhibition on the emergence of the four plants, indicating that kava roots may contain water-soluble allelochemicals which are responsible for the strong influence of kava root extracts on plant emergence.

Eight spots were observed on the TLC plate under UV light and examined for their activities on the germination and growth

Table 2. Effects of Spots Isolated from TLC on Emergence of Barnyardgrass at 50 ppm[a]

	R_f value	rel content (% of total)	inhibition (% of control) germination	shoot	radicle
crude extract			14.8 b	31.8 a	21.8 a
spot 1	0.15	13.4	–3.7 a	5.4 a	5.1 a
spot 2	0.38	3.7	–3.7 a	–1.3 a	7.5 a
spot 3	0.45	10.6	–7.4 a	0.5 a	1.3 a
spot 4	0.49	20.7	–3.7 a	6.2 a	13.8 a
spot 5	0.61	11.6	14.8 b	42.9 b	44.4 b
spot 6	0.67	9.9	18.6 b	63.1 c	60.7 b
spot 7	0.78	4.9	25.9 b	74.1 c	96.3 c
spot 8	0.89	21.7	3.7 ab	8.9 a	7.0 a

[a] Values in a column with the same letter are not significantly different at $P \leq 0.05$. Means with (–) indicate percent stimulation over control.

of barnyardgrass. Of these, only three spots significantly suppressed the emergence of barnyardgrass, with R_f values of 0.61, 0.67, and 0.78. Yields of individual spots are presented in **Table 2**. Spots 5, 6, and 7 yielded 11.6, 9.9, and 4.9% of the total extract and were white, yellow, and deep yellow in color, respectively. These spots were selected for analysis of their chemical components. The compounds in spots 5 and 6 reduced barnyardgrass germination and growth by 14−18 and 40−60%, respectively. However, the constituents of spot 7 exerted the greatest suppression on germination and shoot and root lengths of the weed by 25.9, 74.1, and 96.3%, respectively. The substances contained in other spots did not significantly influence weed growth. Although kava crude extract gave significant reduction on the weed germination and shoot elongation, its inhibitory magnitude was lower than those of spots 5, 6, and 7. It appears that these spots may contain phytotoxins released by kava roots.

Effects of individual kava lactones on the germination and growth of lettuce and barnyardgrass are presented in **Table 3**. The level of inhibition varied among kava lactones. Dihydromethysticin was the most inhibitory of all tested compounds, reducing lettuce germination by 90%. The inhibitory effect of the kava lactone mixture was higher than that of each individual kava lactone. In general, barnyardgrass showed greater resistance to kava lactones than lettuce (**Table 3**). Methysticin was maximally phytotoxic toward root elongation of barnyardgrass (80% inhibition). However, the mixture of kava lactones was less phytotoxic than each individual kava lactone.

Fungicidal Activity. Kava lactones inhibited the growth of *F. solani* and *F. oxysporum*, whereas they were less effective on the emergence of *C. gloeosporides* and *T. viride* (**Table 4**). For *T. viride*, only 7,8-dihydrokavain was fungitoxic at 50 ppm, whereas the other compounds did not show any activity. None of these kava lactones was fungitoxic to *C. gloeosporides* at 10 ppm; however, when the dose increased to 50 ppm, 7,8-dihydrokavain, desmethoxyyagonin, and dihydromethysticin strongly inhibited its growth. The difference in antifungal activities among dihydromethysticin, yagonin, desmethoxyyagonin, and methysticin was negligible. The kava lactone mixture did not affect the growth of *T. viride* and *C. gloeosporides* at 50 ppm, but it was fungitoxic against *F. solani* and *F. oxysporum* at this concentration (**Table 4**).

GC-MS Results. Nine kava lactones were detected in the three spots collected by TLC. Their chemical structures are described in **Figure 1**. Spot 5 consists of desmethoxyyagonin and yagonin. Spot 6 contains kavain, hydroxykavain, methysticin, and 11-hydroxy-12-methoxydihydrokavain. Three kava lactones were detected in spot 7, including 7,8-dihydroxykavain,

Table 3. Effects of Some Kava Lactones on Emergence of Lettuce and Barnyardgrass[a]

kava lactone	concn (ppm)	inhibition (% of control) germination	shoot length	root length	av[b]
		Lettuce			
methysticin	1.0	48.1 bc	71.1 ab	61.2 de	60.1
	10.0	55.6 cd	73.3 ab	53.1 cd	60.7
7,8-dihydrokavain	1.0	–[c]	–	–	–
	10.0	25.9 a	74.3b	15.6 ab	38.6
desmethoxyyagonin	1.0	48.1 bc	74.9 b	50.3 cd	57.8
	10.0	59.2 d	82.4 ab	63.3 de	68.3
kavain	1.0	51.9 bcd	75.9 ab	59.9 de	62.6
	10.0	59.2 d	86.1 b	62.6 de	69.3
yagonin	1.0	44.4 b	63.1 ab	36.1 bc	47.9
	10.0	59.2 d	78.6 ab	68.0 de	68.6
dihydromethysticin	1.0	–	–	–	–
	10.0	89.9 e	76.5 ab	74.8 e	80.4
mixture	1.0	51.6 bcd	78.6 ab	54.4 cd	61.5
	10.0	59.4 d	83.4 ab	75.5 e	72.8
		Barnyardgrass			
methysticin	1.0	50.0 cd	55.0 bc	75.4 e	60.1
	10.0	60.0 e	56.0 bc	83.9 e	66.6
7,8-dihydrokavain	1.0	46.7 c	56.6 bc	53.1 cd	52.1
	10.0	50.0 c	61.2 bc	58.9 d	56.7
desmethoxyyagonin	1.0	43. 3c	31.7 a	46.4 bcd	40.5
	10.0	60.0 e	59.9 c	51.3 bcd	57.1
kavain	1.0	46.7 c	–	39.7 ab	41.6
	10.0	46.7 c	47.6 b	46.9 bcd	47.1
yagonin	1.0	56.7 de	53.1 bc	42.9 abc	50.9
	10.0	56.7 de	58.2 c	43.8 abc	52.9
dihydromethysticin	1.0	50.0 cd	48.6 b	32.1 a	43.6
	10.0	56.7 de	55.6 bc	48.2 bcd	53.5
mixture	1.0	0.0 a	38.3 a	35.3 ab	24.5
	10.0	20.0 b	50.7 b	38.4 ab	36.4

[a] Values in a column with the same letter are not significantly different at $P \leq 0.05$. [b] The inhibition is averaged from suppressive magnitudes of germination percentage and shoot and root length of the plants. [c] Experiments were not conducted.

Table 4. Antifungal Activity of Some Kava Lactones[a]

kava lactone	concn (ppm)	inhibition (% of control) *C. gloeosporides*	*F. solani*	*F. oxysporum*	*T. viride*	av[b]
methysticin	10.0	0.0 a	37.1 cde	11.6 b	0.0 a	12.2
	50.0	0.0 a	33.9 bcd	36.2 ghi	0.0 a	17.5
7,8 dihydrokavain	10.0	0.0 a	0.0 a	10.1 bc	0.0 a	2.5
	50.0	33.3 c	38.7 de	21.7 de	16.2 b	27.5
desmethoxyyagonin	10.0	0.0 a	27.4 b	11.6 bc	0.0 a	9.8
	50.0	22.2 b	33.9 cde	24.6 ef	0.0 a	20.2
kavain	10.0	0.0 a	29.0 bc	0.0 a	0.0 a	7.3
	50.0	0.0 a	29.0 bc	17.4 cd	0.0 a	11.6
yagonin	10.0	–[c]	–	–	–	–
	50.0	0.0 a	41.9 def	39.1 j	0.0 a	20.3
dihydromethysticin	10.0	0.0 a	40.3 def	18.8 de	0.0 a	14.8
	50.0	27.8 bc	48.4 f	31.9 gh	0.0 a	27.0
mixture	10.0	0.0 a	29.0 bc	30.4 gh	0.0 a	14.9
	50.0	5.6 a	40.3 def	37.7 hij	0.0 a	20.9

[a] Values in a column with the same letter are not significantly different at $P \leq 0.05$. "0" indicates no inhibition zone was detected. [b] The inhibition is averaged from suppressive magnitudes of individual fungal species. [c] Experiments were not conducted.

5,6,7,8-tetrahydroxyyagonin, and dihydromethysticin (**Table 5**). This is the first report that kava lactones are involved in the allelopathic activities of kava roots. Only six of the nine detected kava lactones were quantified as the remaining chemicals could not be purchased or successfully purified in our laboratory. The compound detected in the greatest amount was 7,8-dihy-

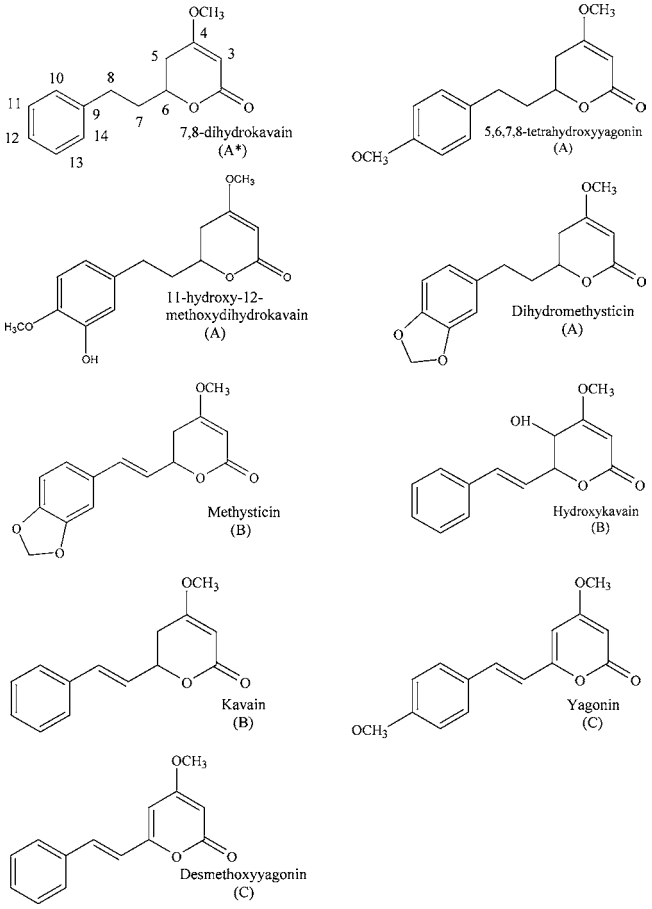

Figure 1. Chemical structures of kava lactones involved in inhibitory activities of kava roots. *, Structure of kava lactones divided by A–C moieties.

Table 5. Kava Lactones Identified from Spots That Showed Strong Inhibition on Emergence of Barnyardgrass and Their Amounts in Kava Roots ($n = 5$)

compound	spot	retention time[a] (min)	mol wt	amount in kava roots[a] (mg g^{-1} of dry wt)
desmethoxyyagonin	5	41.6 ± 0.02	228	4.3 ± 0.07
kavain	6	43.6 ± 0.01	230	6.9 ± 0.05
7,8-dihydrokavain	7	44.2 ± 0.04	232	18.6 ± 0.1
hydroxykavain	6	44.5 ± 0.04	246	–[b]
yagonin	5	49.1 ± 0.03	258	5.7 ± 0.07
5,6,7,8-tetrahydroxyyagonin	7	49.3 ± 0.02	262	–
methysticin	6	50.5 ± 0.03	274	1.4 ± 0.06
dihydromethysticin	7	51.3 ± 0.01	276	5.4 ± 0.04
11-hydroxy-12-methoxy-dihydrokavain	6	52.1 ± 0.05	278	–

[a] ± standard errors. [b] Measurement was not conducted.

drokavain (18.6 mg/g), whereas methysticin was the lowest (1.4 mg/g). Quantities of the other four kava lactones ranged between 4 and 7 mg/g.

DISCUSSION

Kava lactones are major constituents in kava roots, and they can be easily extracted by common solvents such as acetone, methanol, and ethanol, which can yield high amounts of crude kava lactones (*19*). Recent papers have revealed that high doses of kava lactones cause hepatotoxic side effects (*5*, *20*, *21*). Lactones are usually metabolized in the liver by the cytochrome P450 system and in the serum by lactone hydrolases (*20*, *21*). The use of kava lactones has been banned throughout the European Union and Canada since January 2003 (*5*). Major work on kava lactones conducted thus far has focused on its pharmaceutical properties and effects on human health. However, no information on the allelopathic activities of kava lactones has been reported, except for some of our previous research which suggests that kava lactones may be responsible for the strong allelopathic potential of kava roots (*11*−*13*). To date, much research has been conducted on lactones, mainly on sesquiterpene lactones, to examine their biological activities. Several sequiterpenes lactones have been described to have potent pharmacological (*22*, *23*), allelopathic (*24*, *25*), and plant growth regulatory activities (*26*), as well as being potent germination inhibitors (*24*, *25*, *27*−*31*). Insecticidal (*32*−*34*) and herbicidal (*35*) activities have also been described. These compounds have potential for the development of herbicides possessing novel structures. However, very little is known about their structure−activity relationships or their possible modes of actions in plants. Sequiterpene lactones are reported to react with the sulfide group of glutathione in a reversible pH-dependent reaction (*36*). There are two potentially reactive groups in sesquiterpenes for reaction with glutathione: the lactone ring and the α-methylene substituent. For kava lactones, Whitton (*19*) proposed that glutathione binds irreversibly with kava lactones by a Michael-type reaction, due to opening of the lactone ring because no α-methylene groups are present in kava lactones. This may explain the consumption of high doses of kava lactones resulting in hepatotoxic side effects due to glutathione depletion in liver (*37*). Excluding glutathione, it is still not known whether sesquiterpene lactones and kava lactones can react with other enzymes belonging to an SH group.

Among the kava lactones that have been detected so far, the six kava lactones quantified in this study (methysticin, 7,8-dihydrokavain, desmethoxyyagonin, kavain, yagonin, and dihydromethysticin) are major constituents in kava roots (*37*−*42*). Structurally, the nine lactones detected in kava roots in this study can be characterized by different double-bond linkage patterns in positions 5,6 and 7,8. The pyrone moieties of these kava lactones are divided by structures A−C. System A is characterized by the absence of double-bond linkages in both positions 5,6 and 7,8. System B has an unsaturated bond at position 7,8, and system C is a completely unsaturated (**Figure 1**). On the other hand, it seems that the substitution patterns of aromatic rings of kava lactones do not influence their activities (*43*).

Interestingly, the separation of kava lactones on the TLC plate was dependent on their pyrone moieties. All kava lactones belonging to structures A and C were moved to spots 7 and 5, respectively, whereas spot 6 contained structure B lactones and one compound of system A (11-hydroxy-12-methoxydihydrokavain) (**Table 5**). In the TLC bioassay, the inhibitory effect of A−C structures against barnyardgrass germination can be ranked as follows: A > B> C (**Table 2**). To compare the influence among individual kava lactones against the growth of plants and fungi, the average inhibition was used. The average was calculated from the levels of inhibition on germination and shoot and root lengths of test plants and on each of the individual fungal strains (**Tables 3** and **4**). The lactones belonging to system B showed the greatest suppression against both lettuce and barnyardgrass, whereas the effect of compounds with structures A and C was lower (**Table 3**). The fungal activity of the kava lactones in this study can be ranked as follows: A > C > B (**Table 4**). However, two lactones of system A (5,6,7,8-tetrahydroxyyagonin and 11-hydroxy-12-methoxydihydrokavain)

and one compound of system B (hydroxykavain) were not examined in these bioassays, as they could be neither purchased nor successfully purified in our laboratory. Therefore, the TLC trial may predict more exactly the phytotoxic action of the kava lactones (A > B > C) than the bioassays. Both hydroxykavain and 11-hydroxy-12-methoxydihydrokavain have a hydroxyl group at positions 5 and 13, respectively, which differs from other detected kava lactones (**Figure 1**). Allelopathic activities of individual compounds are dependent on the number and arrangement of the hydroxyl groups and the extent of structural conjugation (*44*). Therefore, these two substances may have stronger allelopathic activities than the other detected kava lactones and may cause greater reduction of spots 6 and 7 than spot 5.

The herbicidal and antifungal activities among kava lactones in each individual structure group (A, B, and C) were similar (**Tables 3** and **4**), suggesting that the phytotoxic actions of kava lactones are dependent on the double-bond linkage patterns in positions 5,6 and 7,8. The absence of double-bond linkages in both positions 5,6 and 7,8 (structure A kava lactones) gave the greatest herbicidal and antifungal activities. Quantitatively, the structure A kava lactones account for the greatest amount (24.0 mg/g), followed by the structure C lactones (10.0 mg/g), whereas the structure B lactones are present in the lowest amount (8.3 mg/g) (**Table 5**). This evidence suggests that kava lactones belonging to system A play a major role in the phytotoxic activities of kava lactones. Kava lactones of systems B and C may have a role, but their quantities and activities were lower than those of the system A compounds. However, the biological activities of kava roots may be the results of interactions between all lactones present.

The findings of this research suggest that kava lactones may be used as potent bioactive herbicides and fungicides. This is the first paper demonstrating that kava lactones are plant and plant fungus growth inhibitors at low concentrations. Furthermore, the method used in this research to extract lactones from kava roots efficaciously and to synthesize kava lactones may be useful to enhance the herbicidal and fungicidal potential of kava lactones as well as for pharmaceutical purposes.

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NOTE ADDED AFTER ASAP PUBLICATION

Table 3 in the original posting of January 13, 2006, was incomplete and has been corrected with the revised ASAP posting of January 19, 2006.

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