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Effect of Extraction and Drying Methods on the Contents of Kava Pyrones and Phenolic Compounds in *Alpinia zerumbet* Leaves

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Abstract: *Alpinia zerumbet* (Pers.) Burtt and Smith (Zingiberaceae) is a perennial ginger growing in tropical and subtropical regions. It has a high valuable economic importance and it is considered as a multi-purpose plant. Previous studies have suggested that the biological activities of *A. zerumbet* leaves are due to its high contents of dihydro-5, 6-Dehydrokawain (DDK) along with phenolic compounds. In this study, three different extraction procedures (boiling water, autoclaving and ethanol) as well as two different drying methods (air-drying and oven-drying at 70°C) were used to obtain the extracts. The results showed that the significant high DDK content was recovered when *A. zerumbet* leaves were extracted with boiling water or oven-dried at 70°C. Although, autoclaving or boiling water extraction methods gave significantly higher amounts of phenolic compounds than ethanol, no significant difference was observed between both methods. Furthermore, air-drying and oven-drying at 70°C methods resulted into significant decrease in contents of phenolic compounds than not-dried *A. zerumbet* leaves. Thus, extraction and drying methods markedly affect the contents of DDK and phenolic compounds of *A. zerumbet* leaves.

Key words: *Alpinia zerumbet*, kava pyrones, DDK, phenolic compounds, extraction, drying

INTRODUCTION

Alpinia zerumbet (Pers.) B.L. Burtt. and R.M. Sm. (Zingiberaceae) is a perennial ginger growing in tropical and subtropical regions. It is economically important and considered as a multi-purpose plant. It produces essential oil, kava pyrones and phenolic compounds (Tawata *et al*., 1996; Zoghbi *et al*., 1999; Elzaawely *et al*., 2007b). It is used in folk medicine for the treatment of cardiovascular hypertension and as an antispasmodic agent (Bezerra *et al*., 2000). Furthermore, it exhibited anti-inflammatory, antibacterial, antifungal and antioxidant activities (Zoghbi *et al*., 1999; Elzaawely *et al*., 2007b). Kava pyrone (DDK) is a major compound in *A. zerumbet* and it has been detected in its leaves, rhizomes, flowers and seeds (Elzaawely *et al*., 2007a, b). It possessed several biological properties such as plant growth inhibition (Fujita *et al*., 1994), insecticidal activity and antifungal activity (Tawata *et al*., 1996). In addition it has antiplatelet, antiulcerogenic and antithrombotic effects (Mpalantinos *et al*., 1998). Some phenolic compounds have also been identified in *Alpinia* leaves (Mpalantinos *et al*., 1998; Elzaawely *et al*., 2007b).

Drying plant material is important to preserve their desirable qualities, reduce storage volume and to extend their shelf life (Lim and Murtijaya, 2007; Ashafa *et al*., 2008) however, it may lead to significant changes in their composition of phytochemicals (Capecka *et al*., 2005). Drying techniques such as ambient air-drying and low temperature processing are slow and might accelerate the oxidative and hydrolytic enzyme to destroy antioxidants in vegetables and fruits (Chiang *et al*., 2008) that may lead to quality loss of the plants e.g., color changes and loss in active ingredients (Harbourne *et al*., 2009). Chan *et al*. (2009) reported that microwave, oven and sun-drying *A. zerumbet* leaves resulted in a significant reduction in total phenolic content compared to those of fresh leaves by 50, 43 and 47%, respectively.

Previous studies have suggested that the biological activities of *A. zerumbet* leaves are due to its high contents of DDK along with phenolic compounds. However, information regarding the effect of drying methods on the contents of DDK and individual phenolics was not available. Thus, the objective of this study was to determine the optimal extraction and drying conditions for DDK and phenolic compounds from *A. zerumbet* leaves.

MATERIALS AND METHODS

Chemicals: Standard phenolic compounds (p-Hydroxybenzoic acid, syringic acid, vanillin,

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p-coumaric acid, ferulic acid and cinnamic acid) and all solvents used were of analytical grade and purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). DDK was isolated from *A. zerumbet* leaves by using the method described previously by Tawata *et al.* (1996).

Plant material: *A. zerumbet* leaves were collected from the farm of Faculty of Agriculture, University of the Ryukyus, Okinawa (Japan).

Sample preparation: *A. zerumbet* fresh leaves were cut into small pieces and 200 g were used in each experiment.

Methods of extraction: Three different extraction methods were carried out and 200 g of *A. zerumbet* fresh leaves were used in each method. In the first method, the leaves were boiled in distilled water for 20 min under normal pressure and the temperature did not exceed 100°C. In the second method, the leaves were autoclaved for 15 min under 1.5 atm. and 121°C. In the third method, the leaves were sonicated in ethanol for 20 min.

Methods of drying: *A. zerumbet* fresh leaves (200 g each) were subjected separately to two different drying methods including air-drying for one week in the laboratory at ambient temperature of 25-30°C and oven-drying at 70°C for 24 h, while fresh leaves (without drying) were used as a control. In each drying treatment, leaf pieces were boiled in distilled water for 20 min as described above.

Preparation of the extracts/fractions: Extracts of boiling water, autoclave or ethanol were separately filtered and fractionated with hexane (2×200 mL). Obtained hexane fractions were separately filtered and dried under vacuum at 40°C. The aqueous solutions remaining after extraction with hexane for each sample were separately dried and hydrolyzed with 200 mL NaOH 4 M at 50°C with stirring for 4 h to release phenolic acids from its bound form. After the pH was adjusted to 2.0 by HCl 6 N, the suspensions from each sample were separately filtered and fractionated with ethyl acetate (2×200 mL). The ethyl acetate fractions from each sample were separately filtered and dried under vacuum at 40°C.

GC-MS analysis: To confirm the identification of DDK and phenolic compounds, a 1 μL aliquot of acetone solution of hexane and ethyl acetate fractions from different samples of *A. zerumbet* leaves was injected into the GC-MS (QP-2010, Shimadzu Co., Kyoto, Japan). The DB-5MS column was 30 m in length, 0.25 mm id and 0.25l m in thickness (Agilent Technologies, J and W Scientific Products, Folsom, CA, USA). The carrier gas was helium. The GC oven temperature program was as follows: 50°C hold for 6 min, raised at 5°C min^{-1} to 280°C and hold for 5 min. The injector and detector temperatures were set at 250°C and 280°C, respectively. The mass range was scanned from 20 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 2.4.

Quantification by HPLC: DDK and phenolic compounds were measured at 280 nm using a Shimadzu HPLC (SCL-10A vp, Shimadzu Co., Kyoto, Japan) coupled with a UV-vis detector (SPD-20A, Shimadzu). Separations were achieved on a RP-18 ZORBAX ODS column (Agilent Technologies, USA) (25×0.46 cm i.d., 5 lm particle size). The mobile phase was water with 1% acetic acid (v/v) (solvent A) and methanol: Acetonitrile: Acetic acid (95:4:1, v/v/v) (solvent B) at a flow rate of 0.8 mL min^{-1}. 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. A 5 μL methanolic solution of hexane and ethyl acetate fractions of *A. zerumbet* leaves was used and the identification of the compounds was carried out by comparing their retention times to those of standards. The quantification of each compound was determined based on peak area measurements, which were reported to calibration curves of the corresponding standards.

Statistical analysis: Data were analyzed by SAS computer software version 6.12 using ANOVA with the Least Significant Difference (LSD) at the 0.05 probability level.

RESULTS AND DISCUSSION

Effect of different extraction methods: In order to evaluate the best extraction conditions for DDK and phenolic compounds from *A. zerumbet* leaves, three extraction methods were employed. Boiling water under normal pressure and autoclave under high pressure were used as extraction solvents for comparison with ethanol. Figure 1 shows the contents of DDK in hexane and ethyl acetate fractions of *A. zerumbet* leaves extracted by different methods. In both fractions, leaves extracted with boiling water under normal pressure retained significant amount of DDK (254.6 and 51.3 μg g^{-1}) than that extracted by autoclaving under high pressure (46.9 and 24.1 μg g^{-1}) or extracted by ethanol (21.3 and 1.9 μg g^{-1}) in hexane and ethyl acetate fractions, respectively.

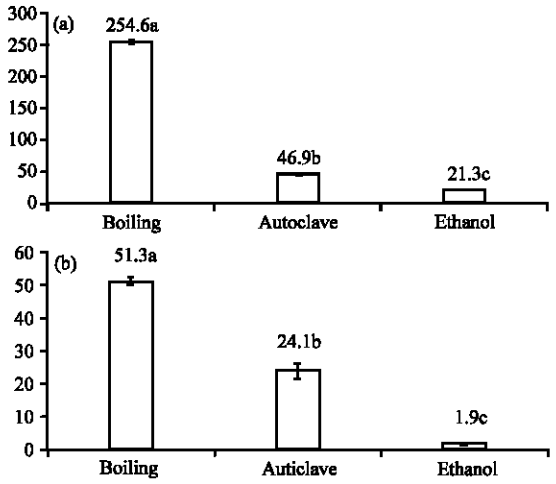

Fig. 1 (a, b): Contents of DDK (μg g^{-1} FW) in hexane fractions (a) and ethyl acetate fractions (b) of *A. zerumbet* leaves extracted with different methods

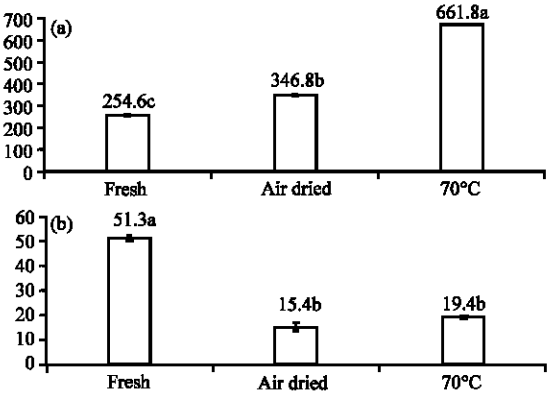

Fig. 2(a, b): Contents of DDK (μg g^{-1} FW) in hexane fractions (a) and ethyl acetate fractions (b) of *A. zerumbet* leaves dried with different methods

On the other hand, amounts of phenolic compounds detected in hexane and ethyl acetate fractions of *A. zerumbet* leaves extracted by different methods are presented in Table 1. Hexane fraction of the leaves extracted by boiling water under normal pressure contained significant higher vanillin than that in autoclaved leaves, while vanillin was not detected in the leaves extracted by ethanol (Table 1). Likewise, cinnamic acid was significantly higher in the boiled leaves than those in the leaves extracted by autoclave or ethanol (Table 1). In ethyl acetate fraction (Table 1), the results showed that leaves extracted with autoclave under high pressure significantly contained higher values of p-Hydroxybenzoic acid, vanillin and cinnamic acid than those in the leaves extracted by boiling water or ethanol. In contrast, p-coumaric acid was significantly higher in the leaves extracted by boiling water than those extracted by autoclave or ethanol. In addition, syringic acid was detected in the leaves extracted by boiling water only and not detected in the leaves extracted neither by autoclave nor ethanol. Although, autoclaving or boiling water extraction methods gave significantly higher amounts of ferulic acid than ethanol, no significant difference was observed between both methods. The results in Table 1 also indicated that the significant high amounts of total phenolics were recovered in the leaves extracted by autoclave or boiling water in comparison to that extracted by ethanol and no significant difference was observed in total phenolics between boiling water and autoclaving extraction methods (Table 1). Leaves extracted by ethanol significantly contained the lowest values of individual and total phenolics than leaves extracted by other methods. Trends in analytical extraction have been a movement toward less organic solvent consumption, faster extraction time and improved quantification (Teixeira *et al*., 2006). Boiling water extraction is important as it simulated the preparation of traditional medicine and the brewing of herbal tea (Lim and Murtijaya, 2007). As there was no significant difference in the amount of total phenolics extracted from fresh *A. zerumbet* leaves using autoclave and boiling water and taking in consideration the economic cost, boiling water could be the preferred solvent of choice to extract DDK and phenolic compounds from *A. zerumbet* leaves. Thus, boiling water at normal pressure was chosen as a solvent for the second experiment.

Effect of drying methods: Figure 2 shows the contents of DDK in hexane and ethyl acetate fractions of *A. zerumbet* leaves that dried by different methods. The two different drying methods resulted in a drastic increase in DDK content of dried leaves in comparison with fresh (not-dried leaves). The results in (Fig. 2a) showed that the significant high DDK content was recovered when *A. zerumbet* leaves were oven-dried at 70°C (661.8 μg g^{-1}), followed by air-dried at ambient temperature (346.8 μg g^{-1}) and fresh leaves (254.6 μg g^{-1}). Furthermore, DDK was higher in ethyl acetate fraction of fresh leaves than that of dried leaves (Fig. 2b). It was previously reported that very low solvent strength such as chloroform or hexane are the suitable to extract DDK from *A. zerumbet* (Tawata *et al*., 1996; Elzaawely *et al*., 2007a, b) and this may explain the reason that hexane fraction retained higher amounts of DDK than ethyl acetate.

Table 1: Contents of phenolic compounds in hexane fractions and ethyl acetate fractions of *A. zerumbet* leaves extracted with different methods

	Phenolic compounds (μg g^{-1} FW)						
Sample	PHBA	SA	V	PCA	FA	CA	
Hexane fraction							
Boiling	-	-	2.0±0.18a	-	-	1.0±0.00a	
Autoclave	-	-	0.6±0.06b	-	-	0.1±0.00b	
Ethanol	-	-	0.0c	-	-	0.1±0.00b	
LSD	-	-	0.37	-	-	0.012	
Ethyl acetate fraction	PHBA	SA	V	PCA	FA	CA	TPC
Boiling	62.1±0.04b	32.5±0.07a	7.3±0.00b	13.9±0.60a	261.5±10.8a	15.8±0.56b	393.2±9.7a
Autoclave	104.2±6.8a	0.0b	46.9±5.4a	9.7±1.0b	217.2±26.1a	48.5±7.8a	426.5±47.2a
Ethanol	4.3±0.02c	0.0b	1.4±0.03b	0.3±0.01c	3.0±0.29b	1.1±0.00b	10.1±0.36b
LSD	13.50	0.13	10.8	2.34	56.5	15.6	96.2

Values are means of three replications±S.E. Means with the same letter are not significantly different at $p \leq 0.05$. FW: Fresh Weight. -: Not detected. PHBA: p-hydroxybenzoic acid; SA: Syringic Acid; V: Vanillin; PCA: P-Coumaric Acid; FA: Ferulic Acid; CA: Cinnamic Acid and TPC: Total Phenolic Compounds

Table 2: Contents of phenolic compounds in hexane fractions and ethyl acetate fractions of *A. zerumbet* leaves dried with different methods

	Phenolic compounds (μg g^{-1} FW)						
Sample	PHBA	SA	V	PCA	FA	CA	
Hexane fraction							
Fresh	-	-	2.0±0.18a	-	-	1.0±0.00b	
Air dried	-	-	0.0b	-	-	0.0c	
70°C dried	-	-	2.4±0.26a	-	-	1.6±0.07a	
LSD	-	-	0.63	-	-	0.13	
Ethyl acelate fraction	PHBA	SA	V	PCA	FA	CA	TPC
Fresh	62.1±0.04a	32.5±0.07a	7.3±0.00b	13.9±0.60a	261.5±10.8a	15.9±0.56b	393.2±9.7a
Air dried	26.5±0.30c	0.0b	5.4±0.74c	2.3±0.01c	33.0±0.11c	10.3±0.01c	77.5±1.1c
70°C dried	51.9±0.44b	0.0b	17.1±0.27a	8.0±0.03b	14.9±0.44b	42.9±0.20a	260.8±0.9b
LSD	1.07	0.13	1.57	1.15	21.5	1.19	19.7

Values are means of three replications±S.E. Means with the same letter are not significantly different at $p \leq 0.05$. FW: Fresh Weight. -: Not detected. PHBA: p-HydroxyBenzoic Acid; SA: Syringic Acid; V: Vanillin; PCA: P-Coumaric Acid; FA: Ferulic Acid; CA: Cinnamic Acid and TPC: Total Phenolic Compounds

On the other hand, Table 2 represents the results of phenolic compounds detected in hexane and ethyl acetate fractions of fresh and dried *A. zerumbet* leaves. Different drying treatments affected both individual and total phenolics of *A. zerumbet*. Only vanillin and cinnamic acid were found in hexane fraction (Table 2). Although, hexane fraction of oven-dried leaves at 70°C significantly contained higher amounts of cinnamic acid than that in fresh leaves, there was no significant difference observed between the two samples in vanillin content. Contrarily, vanillin and cinnamic acid were not detected in hexane fraction of air-dried leaves (Table 2). In addition, drying resulted in a significant decline of individual and total phenolics in ethyl acetate fraction of *A. zerumbet* leaves, except for vanillin and cinnamic acid that were significantly higher in oven-dried leaves at 70°C (Table 2). Drying also caused a complete loss in syringic acid as it was only detected in fresh leaves. Of the two drying methods, air-drying at ambient temperature significantly caused 80.3% loss in total phenolics, followed by oven-drying at 70°C (33.7% loss) compared to fresh leaves (Table 2). The results also indicated that ferulic acid was the predominant phenolic that found in *A. zerumbet* leaves. Results obtained from this study also revealed that air-drying of *A. zerumbet* leaves at ambient temperature tend to lower the individual and total phenolics to their minimum values. Declines in phenolic contents resulting from air-drying could be due to enzymatic degradation as the process was carried out at room temperature and takes several days for samples to dry (Chan *et al*., 2009). It was previously reported that levels of flavonoids and phenolic compounds were decreased with drying in comparison with fresh or not-dried plant materials (Hassan *et al*., 2007a, b; Lim and Murtijaya, 2007; Julkunen-Tiitto and Sorsa, 2001; Chiang *et al*., 2008; Katsube *et al*., 2009; Chan *et al*., 2009; Sthishkumar *et al*., 2009; Sukrasno *et al*., 2011).

CONCLUSIONS

Based on these results, extraction and drying methods significantly affected the amounts of DDK and phenolic compounds in *A. zerumbet* leaves. Our study recommends that boiling water is the optimal method to extract active constituents from *A. zerumbet* leaves. While fresh leaves are a good source for phenolic compounds, oven-dried leaves at 70°C are an excellent supplier for DDK.

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