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Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm

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Abstract

Alpinia zerumbet leaves and rhizomes have been extensively studied for their chemical compositions and biological activities. However, less attention has been given to its flowers and seeds. In our study, essential oil, total phenolics and antioxidant capacities assayed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and β-carotene bleaching methods were evaluated in flowers and seeds of *A. zerumbet*. In addition, their phenolic composition was determined by GC–MS and HPLC. 1,8-Cineol, camphor, methyl cinnamate and borneol were the major constituents in flower oils, whereas the main components in seeds oil were α-cadinol, T-muurolol, α-terpineol, δ-cadinene and terpinene-4-ol. The results showed that the hexane extract of flowers contained a significantly higher quantity of dihydro-5,6-dehydrokawain (DDK) than that of seeds. Total phenolic contents of flower and seed extracts were measured as 56.7 and 13.7 mg gallic acid equivalent per gram extract, respectively. The ethyl acetate extract of flowers and seeds possessed a high antiradical activity and prevented the bleaching of β-carotene. The HPLC analysis indicated that *p*-hydroxybenzoic acid, ferulic acid and syringic acid were the predominant phenolics in the ethyl acetate extract of flowers, whilst *p*-hydroxybenzoic acid, syringic acid and vanillin were the major phenolics in seeds.

Keywords: *Alpinia zerumbet*; Essential oil; Total phenolics; Dihydro-5,6-dehydrokawain; Antioxidant activity; Phenolic acids

1. Introduction

Phenolic compounds are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers (Proestos, Boziaris, Nychas, & Komaitis, 2006). Consumption of fruits and vegetables with high content of antioxidative phytochemicals such as phenolic compounds may reduce the risk of cancer, cardiovascular disease and many other diseases (Robbins & Bean, 2004 and Shui & Leong, 2006). Therefore, the interest in naturally occurring antioxidants has increased considerably in recent years for use in food and pharmaceutical products (Djeridane et al., 2006).

Alpinia zerumbet (Pers.) B.L. Burtt. & R.M. Sm. (Zingiberaceae) is a perennial plant growing widely in the subtropical and tropical regions. It is traditionally used for the treatment of cardiovascular hypertension and as an antispasmodic agent (Bezerra, Leal-Cardoso, Coelho-de-Souza, Criddle, & Fonteles, 2000). Cardamonin and alpinetin have been isolated from its seeds (Krishna & Chaganty, 1973). Furthermore, four sesquiterpenes including β–eudesmol, nerolidol, humulene epoxide II and 4α-hydroxydihydroagarofuran have been isolated from the seeds (Morita, Nakanishi, Morita, Mihashi, & Itokawa, 1996). Kava pyrones [dihydro-5,6-dehydrokawain (DDK) and 5,6-dehydrokawain (DK)] and some phenolics have been isolated from the leaves and rhizomes (Mpalantinos, de Moura, Parente, & Kuster, 1998 and Itokawa, Morita, & Mihashi, 1981 and Masuda, Mizuguchi, Tanaka, Iritani,

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& Takeda, 2000). However, DDK and phenolic compounds have not been previously reported from the flowers and seeds.

Regarding the lack of information about the antioxidant potential of *A. zerumbet* flowers and seeds, the aim of this study was to determine the contents of DDK and phenolic compounds as well as the antioxidant capacity of flowers and seeds of *A. zerumbet*. The chemical composition of their essential oils was also investigated.

2. Materials and methods

2.1. Standards

p-Hydroxybenzoic acid, syringic acid, vanillin, *p*-coumaric acid, ferulic acid and cinnamic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). DDK was isolated from *A. zerumbet* leaves by using the method described previously by Tawata, Taira, Kobamoto, Ishihara, and Toyama (1996).

2.2. Solvents and reagents

β-Carotene, linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), α-tocopherol, polyoxyethylene sorbitan monopalmitate (Tween-40) and all solvents used were of the highest purity needed for each application and purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.3. Plant material

The flowers and seeds were collected from the farm of Faculty of Agriculture, University of the Ryukyus, Okinawa (Japan).

2.4. Extraction of essential oil

Four hundred grams of fresh flowers or seeds were separately subjected to steam-distillation for 4 h. The distillates were separately extracted with diethyl ether (2 × 200 ml). The solvent was carefully removed under vacuum at 35 °C and essential oils were dissolved in diethyl ether at 1000 ppm and subjected to GC–MS analysis.

2.5. Preparation of extracts

Fresh flowers (300 g) and seeds (200 g) were separately boiled in water for 20 min. After cooling at room temperature, the water extracts from flowers or seeds were separately filtered and extracted with hexane (2 × 200 ml). Obtained hexane fractions were separately filtered and dried under vacuum at 40 °C to give 0.34 g (0.1%, w/w) and 0.07 g (0.04%, w/w) of dried hexane extract from fresh flowers or seeds, respectively. The aqueous solutions remaining after extraction of flowers or seeds with hexane were separately dried and hydrolyzed with 200 ml NaOH 4 M at 50 °C with stirring for 4 h. After the pH was adjusted to 2.0 by HCl 6 N, the suspensions from flowers or seeds were separately filtered and extracted with ethyl acetate (2 × 200 ml). The ethyl acetate extracts from flowers or seeds were separately filtered and dried under vacuum at 40 °C to give 1.6 g (0.53%, w/w) and 0.9 g (0.44%, w/w) of dried ethyl acetate extract from fresh flowers or seeds, respectively.

2.6. Total phenolic content

The amount of total phenolics was determined according to the Folin–Ciocalteu procedure (Kähkönen et al., 1999). Briefly, 1.0 ml Folin–Ciocalteu's reagent and 0.8 ml 7.5% (w/v) Na_2CO_3 were added to 0.2 ml of flowers or seeds extract dissolved in methanol. After shaking, the mixture was incubated at room temperature for 30 min. Absorption was measured at 765 nm using a Shimadzu UV-160A spectrometer, Kyoto (Japan). Total Phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram extract.

2.7. Antioxidant activity

2.7.1. DPPH assay

The radical-scavenging activity was evaluated as described previously (Abe, Murata, & Hirota, 1998). Two milliliters of the methanol solution of ethyl acetate extract from flowers or seeds were mixed with 1 ml of 0.5 mM DPPH methanol 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, and then the absorbance was measured at 517 nm using a Shimadzu UV-160A spectrometer, Kyoto (Japan). α-Tocopherol was used as positive reference while methanol was used as negative one. The EC_{50} value was determined as the concentration of each sample required to give 50% DPPH radical scavenging activity.

2.7.2. β-Carotene bleaching assay

Antioxidant activity was evaluated according to the β-carotene bleaching method (Siddhuraju & Becker, 2003). β-Carotene (2.0 mg) was dissolved in 10 ml chloroform. One milliliter of the chloroform solution was mixed with 20 μl linoleic acid and 200 mg Tween-40. The chloroform was evaporated under vacuum at 45 °C, then 50 ml oxygenated water was added, and the mixture was vigorously shaken. The emulsion obtained was freshly prepared before each experiment. An aliquot (250 μl) of the β-carotene-linoleic acid emulsion was distributed in each of the 96-wells of the microtitre plates. Methanolic solutions (30 μl) of the ethyl acetate extract from flowers or seeds of *A. zerumbet* and α-tocopherol at 1000 ppm were added. An equal amount of methanol was used as the control. The microtitre plates were incubated at 50 °C, and the absorbance was measured using a model MTP-32 microplate reader (Corona Electric, Ibaraki, Japan) at 492 nm. Readings of all

samples were performed immediately at zero time and every 15 min up to 180 min.

2.8. GC–MS analysis

A 1 μl aliquot of 500 ppm acetone solution of hexane and ethyl acetate extracts from flowers or seeds 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 0.25 μm in thickness (Agilent Technologies, J&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 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.

For essential oil analysis, an aliquot of 1 μl oil dissolved in diethyl ether was injected into the GC–MS using the same column described above. The carrier gas was helium and the GC oven temperature program was as follows: 40 °C hold for 5 min, raised at 6 °C/ min to 280 °C, and hold for 5 min. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. The essential oil components were identified by comparing their retention times and mass fragmentation pattern with those of standards and MS library. Quantitative determinations of essential oil components were carried out based on peak area measurements.

2.9. 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 μm 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. 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 extracts from flowers or seeds at 10,000 ppm 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.

2.10. Statistical analysis

All experiments were repeated three times. Data were analyzed by SAS computer software version 6.12 using ANOVA with the least significant difference (LSD) at the 0.05 probability level.

3. Results and discussion

3.1. Chemical composition of the essential oils

The results obtained by GC–MS analysis of the essential oils from flowers or seeds of *A. zerumbet* are presented in Table 1. Essential oils from fresh flowers or seeds of *Alpinia* were obtained as yellow oils with aromatic-spicy odour. The yield of oil extracted from flowers (0.07%, w/w) was higher than that from seeds (0.04%, w/w). The main components determined in flower oil were 1,8-cineol (16.63%), camphor (14.1%), methyl cinnamate (12.81%) and borneol (6.41%), whereas seed oil mainly contained α-cadinol (13.46%), T-muurolol (10.79%), α-terpineol (10.67%), δ-cadinene (6.19%) and terpinene-4-ol (6.18%). Zoghbi, Andrade, and Maia (1999) studied the chemical composition of essential oil from flowers of *A. zerumbet* growing in Brazil. The main constituents identified were 1,8-cineol, terpinene-4-ol and sabinene. However, there are no reports on chemical compositions of the essential oils obtained from flowers or seeds of *A. zerumbet* growing in Okinawa (Japan). It has been stated that camphor, borneol, terpinene-4-ol and 1,8-cineol were the main antimicrobial constituents of the oils of *Achillea setacea* and *A. teretifolia* (Ünlü et al., 2002), whereas α-cadinol and T-muurolol showed a strong antimite activity (Chang, Chen, Wang, & Wu, 2001). Moreover, α-cadinol showed selective toxicity against the human colon adenocarcinoma cell line HT-29 (He et al., 1997). The chemical composition of the flower essential oil suggests the possibility of using this oil to flavour food products as well as in cosmetics e.g., shower gels, soaps, shampoos and bath products (Jirovetz, Buchbauer, Shafi, & Leela, 2003). On the other hand, the seed essential oil may have anticancer and pesticidal activity as it contains α-cadinol and T-muurolol as major components.

3.2. Total phenolic content

The amount of total phenolics in the water extracts from flowers or seeds of *A. zerumbet* was determined using the Folin–Ciocalteu method and the results were expressed as gallic acid equivalents. Flowers contained a significantly higher amount of total phenolics than seeds (56.7 ± 0.2 and 13.7 ± 0.4 mg GAE/g extract, respectively). The Folin–Ciocalteu procedure is a widely used method and provides a rapid and useful estimation of the phenolic content of plant extracts (Luximon-Ramma, Bahorun, & Crozier, 2003). It is well known that phenolic compounds contribute directly to the antioxidant activity of plant

Table 1
Essential oil components of fresh flowers and seeds of *A. zerumbet*

Compound	R.I.	Peak area (%)	
		Flowers	Seeds
2-Methyl-1-butyl acetate	876	–	0.19
Camphene	949	0.03	–
Benzaldehyde	961	0.14	–
B-pinene	976	–	0.82
Methyl 2-hydroxy-3-methylpentanoate	992	–	0.33
α-Phellandrin	1006	0.22	0.47
α-Terpinene	1018	0.07	0.07
p-Cymene	1026	0.10	–
1,8-Cineol	1035	16.63	2.33
γ-Terpinene	1060	0.01	–
Linalool	1102	4.16	0.50
Phenylethyl alcohol	1114	0.17	–
4-Isopropyl-1-methyl-2-cyclohexen-1-ol	1129	0.28	1.23
Camphor	1152	14.1	3.55
Camphene hydrate	1160	0.71	0.23
Pinocarvon	1167	0.10	0.04
Borneol	1178	6.41	1.32
Pinocamphone	1180	–	0.71
Terpinene-4-ol	1186	3.85	6.18
Cryptone	1193	4.68	1.72
α-Terpineol	1199	4.25	10.67
Sabinyl acetate	1208	0.36	0.15
trans-p-Menth-1-en-3-ol	1213	0.18	0.35
trans-p-Mentha-6,8-dien-2-ol	1223	0.12	–
Benzylacetone	1247	0.92	–
Cuminaldehyde	1248	0.21	–
p-Menth-1-en-3-one	1259	0.37	0.09
2-Isopropyl-4-methylhex-2-enal	1269	0.28	0.27
Phellandral	1284	0.36	0.12
Thymol	1291	0.10	–
Cuminalcohol	1294	0.50	–
Isothymol	1300	0.94	–
trans-Methyl cinnamate	1302	0.31	–
Benzalacetone	1322	0.04	–
Methyl cinnamate	1332	12.81	–
β-Elemene	1333	–	0.74
4-Isopentyl-1,3-cyclopentanedione	1343	0.10	–
β-Caryophyllene	1345	0.09	0.86
α-Caryophyllene	1358	0.34	2.63
Calarene	1357	0.06	1.67
δ-Cadinene	1376	0.06	6.19
Epizonarene	1378	0.01	0.54
α-Muurolene	1382	–	0.17
Elemol	1386	0.36	5.20
Caryophyllene oxide	1399	0.17	–
γ-Eudesmol	1443	1.03	1.13
T-muurolol	1454	–	10.79
α-Cadinol	1467	–	13.46
α-Eudesmol	1469	0.95	–
DDK	1935	3.84	0.42
Heneicosane	2102	0.40	0.53
DK	2159	0.07	–

R.I., retention index relative to *n*-alkanes on the DB-5 column.

extracts and therefore, it is quite important to evaluate the total phenolic content in tested extracts.

3.3. DDK content

DDK and DK are major compounds in *A. zerumbet* and they have been detected in its leaves, stems and rhizomes (Tawata et al., 1996). However, this is the first report describes the DDK content in flowers or seeds of *A. zerumbet*. Hexane extract of flower contained significantly higher amount of DDK (339.6 ± 1.3 μg/g of fresh weight) than that of seeds, which contained very small quantity of DDK (3.1 ± 0.3 μg/g of fresh weight). DDK and DK are medically-important natural substances as they showed antiplatelet activity in rabbits (Teng et al., 1990). Furthermore, they exhibited anti-ulcerogenic and anti-thrombotic effects (Mpalantinos et al., 1998). Our result is interesting as it shows a very big difference in DDK content between flowers and seeds. It is known that flowers convert to seeds through different stages of maturity and the explanation for this big decline in DDK content remains unknown and still needs more studies to understand and clarify the biosynthetic pathway and the physiological role of DDK within the plant.

3.4. DPPH free radical-scavenging activity

The DPPH radical scavenging is a commonly used method to evaluate the ability of plant extracts to scavenge free radicals generated from DPPH reagent (Chung, Chien, Teng, & Chou, 2006). Hexane extracts from flowers or seeds showed very weak DPPH radical-scavenging activity; therefore, only ethyl acetate extracts that were active and exhibited 50% inhibition of DPPH radicals were included in this test. A high antiradical activity was observed in the ethyl acetate extract from both flowers and seeds. However, no significant differences were observed between their DPPH EC_{50} values (0.08 mg/ml for each). It has been reported that free radical-scavenging activity is greatly influenced by the phenolic composition of the sample (Cheung, Cheung, & Ooi, 2003). Thus, the antioxidant activity of the ethyl acetate extracts from flowers or seeds may be attributed to their phenolics content. Although the seeds extract contained lower amount of total phenolics than that of flowers, it showed a high DPPH radical-scavenging efficiency similar to that of flower extract and this activity may have also been partly contributed to some constituents other than phenolics.

3.5. Antioxidant activity measured by β-carotene bleaching method

Fig. 1 shows the antioxidant activity of the ethyl acetate extracts from flowers and seeds of *A. zerumbet* as measured by β-carotene bleaching method. Both extracts inhibited the oxidation of β-carotene to different degrees. β-Carotene in this model system undergoes rapid discoloration in the absence of an antioxidant and it may be oxidized and subsequently, the system loses its orange colour. The presence of samples with antioxidant activity can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical formed in the system (Jayaprakasha, Singh, & Sakariah, 2001). In the present study, the ethyl acetate extract of flowers showed higher ability to prevent the

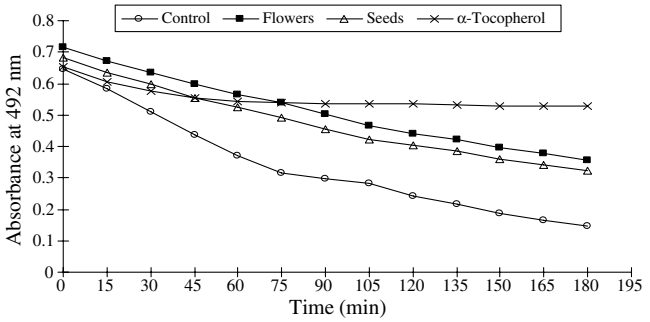

Fig. 1. Antioxidant activity of ethyl acetate extracts from fresh flowers and seeds of *A. zerumbet* measured by β-carotene bleaching method.

bleaching of β-carotene than that of seeds and this antioxidant capacity may be due to its high levels of phenolic compounds.

3.6. Phenolic compounds content

Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids, which often occur in plants as esters, glycosides and bound complexes and are rarely present in free forms (Germanò et al., 2006). In our study, a 4 h hydrolysis with 4 M NaOH at 50 °C was used to release conjugated phenolics. HPLC successfully identified six phenolic compounds in the ethyl acetate extract of flowers or seeds including *p*-hydroxybenzoic acid, syringic acid, vanillin, *p*-coumaric acid, ferulic acid and cinnamic acid with total amounts of 91.0 and 62.0 μg/g of fresh weight for flowers or seeds, respectively (Table 2). *p*-Hydroxybenzoic acid, ferulic acid and syringic acid were the predominant phenolic compounds in the flowers contributing about 81.9% to the total amount. On the other hand, *p*-hydroxybenzoic acid, syringic acid and vanillin were the major phenolics in seeds contributing about 73.9% to the total amount. Furthermore, GC–MS was used to confirm the phenolic profile of flower or seed extracts and a total of 17 compounds including benzoic acid, pyrocatechin, 2-methyl benzoic acid, benzyl acetone, hydrocinnamic acid methyl ester, benzenepropanoic acid, *p*-chlorobenzoic acid, *p*-hydroxybenzaldehyde, vanillin, cinnamic acid, *p*-hydroxybenzoic acid, vanillic acid, hydrocinnamic acid-*p*-hydroxy-methyl ester, syringic acid, ferulic acid, DDK and DK were tentatively identified on the basis of spectral data and standard chemicals (Table 3). Flower or seed extracts, which exhibited strong antioxidant activities, were found to contain high amounts of total and individual phenolics that may contribute to this activity. Phenolic compounds are commonly found in plants and they have been reported to have a strong antioxidant activity (Elzaawely, Xuan, & Tawata, 2005 and Mansouri, Embarek, Kokkalou, & Kefalas, 2005). The antioxidant potential of phenolic compounds is dependent on the number and arrangement of the hydroxyl groups as well as the presence of electron-donating substituents in the ring structure (Lapornik, Prošek, & Wondra, 2005).

Table 2
Phenolic compounds content in ethyl acetate extracts of *A. zerumbet* flowers and seeds

Compound	Phenolic compounds of fresh weight (μg/g)		
	Flowers	Seeds	LSD
p-Hydroxybenzoic acid	28.8 ± 0.11^{a}	23.4 ± 0.38^{b}	1.1
Syringic acid	20.1 ± 0.01^{a}	12.8 ± 0.90^{b}	2.5
Vanillin	5.5 ± 0.18^{b}	9.6 ± 0.58^{a}	1.7
p-Coumaric acid	4.9 ± 0.13^{a}	3.9 ± 0.93^{a}	2.6
Ferulic acid	25.6 ± 2.39^{a}	8.7 ± 0.72^{b}	6.9
Cinnamic acid	6.1 ± 0.43^{a}	3.6 ± 0.30^{b}	1.4
Total phenolics	91.0 ± 2.63^{a}	62.0 ± 0.95^{b}	7.7

Values are means of three replications ±S.E.
Means with the same letter are not significantly different at $P \leqslant 0.05$.

Table 3
Compounds detected by GC–MS in hexane and ethyl acetate extracts of *A. zerumbet* flowers and seeds

Compound	Flowers		Seeds	
	Hexane	Ethyl acetate	Hexane	Ethyl acetate
Benzoic acid	–	+	–	+
Pyrocatechin	–	+	–	–
2-Methyl benzoic acid	–	–	–	+
Benzyl acetone	–	+	–	–
Hydrocinnamic acid methyl ester	–	+	–	+
Benzenepropanoic acid	–	+	–	+
p-Chlorobenzoic acid	–	–	–	+
p-Hydroxybenzaldehyde	–	+	–	+
Vanillin	–	+	–	+
Cinnamic acid	–	+	–	+
p-Hydroxybenzoic acid	–	+	–	+
Vanillic acid	–	+	–	–
Hydrocinnamic acid, *p*-hydroxy-methyl ester	–	+	–	+
Syringic acid	–	+	–	–
Ferulic acid	–	+	–	+
DDK	+	+	+	+
DK	+	–	+	–

(–), Not detected.
(+), Detected.

4. Conclusions

The present study investigates the contents of essential oil, DDK and phenolic compounds in flowers and seeds of *A. zerumbet* for the first time. The results verify that flower extracts contained higher amounts of total phenolics, DDK and individual phenolics than seeds. Results also reveal that flowers and seeds exhibited antioxidant actions in terms of free radical scavenging and β-carotene bleaching activities. Flowers had stronger activity than seeds in β-carotene bleaching method; however, no differences were found between flowers and seeds in DPPH radical-scavenging activity. Six phenolic compounds were identified and quantified in the ethyl acetate extract of flowers or seeds and it is observed that their antioxidant activity may be associated with these phenolics. Our results indicate that

the flowers and seeds of *A. zerumbet* may be considered as a good source of health-promoting phenolic substances to be used in tea preparations or food products. There is also a high possibility to use the flowers as an interesting source to produce DDK for medical use. Further studies are needed to evaluate the biological activities of essential oils from flowers and seeds of *A. zerumbet* and to identify and characterize the compounds other than phenolics in seed extract.

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References

Abe, N., Murata, T., & Hirota, A. (1998). Novel DPPH radical scavengers, bisorbicillinol and demethyltrichodimerol, from a fungus. *Bioscience Biotechnology and Biochemistry, 62*, 661–666.

Bezerra, M. A. C., Leal-Cardoso, J. H., Coelho-de-Souza, A. N., Criddle, D. N., & Fonteles, M. C. (2000). Myorelaxant and antispasmodic effects of the essential oil of *Alpinia speciosa* on rat ileum. *Phytotherapy Research, 14*, 549–551.

Chang, S. T., Chen, P. F., Wang, S. Y., & Wu, H. H. (2001). Antimite activity of essential oils and their constituents from *Taiwania cryptomerioides*. *Journal of Medical Entomology, 38*, 455–457.

Cheung, L. M., Cheung, P. C. K., & Ooi, V. E. C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry, 81*, 249–255.

Chung, Y., Chien, C., Teng, K., & Chou, S. (2006). Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides Sieb & zucc*. *Food Chemistry, 97*, 418–425.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry, 97*, 654–660.

Elzaawely, A. A., Xuan, T. D., & Tawata, S. (2005). Antioxidant and antibacterial activities of *Rumex japonicus* Houtt. aerial parts. *Biological and Pharmaceutical Bulletin, 28*, 2225–2230.

Germanò, M. P., D'Angelo, V., Biasini, T., Sanogo, R., De Pasquale, R., & Catania, S. (2006). Evaluation of the antioxidant properties and bioavailability of free and bound phenolic acids from *Trichilia emetica* Vahl. *Journal of Ethnopharmacology, 105*, 368–373.

He, K., Zeng, L., Shi, G., Zhao, G., Kozlowski, J. F., & McLaughlin, J. L. (1997). Bioactive compounds from *Taiwania cryptomerioides*. *Journal of Natural Products, 60*, 38–40.

Itokawa, H., Morita, M., & Mihashi, S. (1981). Phenolic compounds from the rhizomes of *Alpinia speciosa*. *Phytochemistry, 20*, 2503–2506.

Jayaprakasha, G. K., Singh, R. P., & Sakariah, K. K. (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chemistry, 73*, 285–290.

Jirovetz, L., Buchbauer, G., Shafi, M. P., & Leela, N. K. (2003). Analysis of the essential oils of the leaves, stems, rhizomes and roots of the medicinal plant *Alpinia galanga* from southern India. *Acta Pharmaceutica, 53*, 73–81.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J., Pihlaja, K., Kujala, T. S., et al. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry, 47*, 3954–3962.

Krishna, B. M., & Chaganty, R. B. (1973). Cardamonin and alpinetin from the seeds of *Alpinia speciosa*. *Phytochemistry, 12*, 238.

Lapornik, B., Prošek, M., & Wondra, A. G. (2005). Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering, 71*, 214–222.

Luximon-Ramma, A., Bahorun, T., & Crozier, A. (2003). Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. *Journal of the Science of Food and Agriculture, 83*, 496–502.

Mansouri, A., Embarek, G., Kokkalou, E., & Kefalas, P. (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). *Food Chemistry, 89*, 411–420.

Masuda, T., Mizuguchi, S., Tanaka, T., Iritani, K., & Takeda, Y. (2000). Isolation and structure determination of new antioxidative ferulic acid glucoside esters from the rhizome of *Alpinia speciosa*, a zingiberaceae plant used in Okinawan food culture. *Journal of Agricultural and Food Chemistry, 48*, 1479–1484.

Morita, M., Nakanishi, H., Morita, H., Mihashi, S., & Itokawa, H. (1996). Structure and spasmolytic activities of derivatives from sesquiterpenes of *Alpinia speciosa* and *Alpinia japonica*. *Chemical and Pharmaceutical Bulletin, 44*, 1603–1606.

Mpalantinos, M. A., de Moura, R. S., Parente, J. P., & Kuster, R. M. (1998). Biologically active flavonoids and kava pyrones from the aqueous extract of *Alpinia zerumbet*. *Phytotherapy Research, 12*, 442–444.

Proestos, C., Boziaris, I. S., Nychas, G.-J. E., & Komaitis, M. (2006). Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. *Food Chemistry, 95*, 664–671.

Robbins, R. J., & Bean, S. R. (2004). Development of a quantitative high-performance liquid chromatography–photodiode array detection measurement system for phenolic acids. *Journal of Chromatography A, 1038*, 97–105.

Shui, G., & Leong, L. P. (2006). Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. *Food Chemistry, 97*, 277–284.

Siddhuraju, P., & Becker, K. (2003). Studies on antioxidant activities of mucuna seed (*Mucuna pruriens* var *utilis*) extract and various non-protein amino/imino acids through in vitro models. *Journal of the Science of Food and Agriculture, 83*, 1517–1524.

Tawata, S., Taira, S., Kobamoto, N., Ishihara, M., & Toyama, S. (1996). Syntheses and biological activities of dihydro-5,6-dehydrokawain derivatives. *Bioscience, Biotechnology and Biochemistry, 60*, 1643–1645.

Teng, C. M., Hsu, S. Y., Lin, C. H., Yu, S. M., Wang, K. J., Lin, M. H., et al. (1990). Antiplatelet action of dehydrokawain derivatives isolated from *Alpinia speciosa* rhizoma. *The Chinese Journal of Physiology, 33*, 41–48.

Ünlü, M., Daferera, D., Dönmez, E., Polissiou, M., Tepe, B., & Sökmen, A. (2002). Compositions and the in vitro antimicrobial activities of the essential oils of *Achillea setacea* and *Achillea teretifolia* (Compositae). *Journal of Ethnopharmacology, 83*, 117–121.

Zoghbi, M. G. B., Andrade, E. H. A., & Maia, J. G. S. (1999). Volatile constituents from leaves and flowers of *Alpinia speciosa* K. Schum. and *A. purpurata* (Viell.) Schum. *Schum. Flavour and Fragrance Journal, 14*, 411–414.