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Essential oils, kava pyrones and phenolic compounds from leaves and rhizomes of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. and their antioxidant activity

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Abstract

During essential oil production from *Alpinia zerumbet*, large volumes of water and solid wastes are produced and subsequently discarded. An extraction protocol to obtain essential oil, dihydro-5,6-dehydrokawain (DDK) and enriched antioxidant phenolic extracts from fresh leaves or rhizomes of *A. zerumbet* and their wastes was developed. The main components determined in leaf oil were 1,8-cineol, camphor and methyl cinnamate, whereas rhizome oil mainly contained DDK and methyl cinnamate. The highest DDK content was found in the hexane extract of fresh rhizomes. Ethyl acetate extracts from leaves showed higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities than those from rhizomes. Ethyl acetate extract from wastewater of leaves possessed the strongest inhibition to β-carotene oxidation. Ferulic and *p*-hydroxybenzoic acids were the major phenolics present in these extracts. The results indicate that disposed wastes produced during essential oil production from *A. zerumbet* leaves or rhizomes may be utilized in foodstuffs as a cheap source of natural antioxidants.

Keywords: *Alpinia zerumbet*; Essential oil; Antioxidant activity; Phenolics; DDK; Disposed wastes

1. Introduction

Reactive oxygen species (ROS) are major contributors to various serious diseases such as aging, cancer, atherosclerosis, inflammation, cardiovascular disease, cataracts, immune system decline and brain dysfunction (Atoui, Mansouri, Boskou, & Kefalas, 2005; Parejo et al., 2003). Antioxidants are used to preserve foods by retarding discoloration, rancidity or deterioration (Yen, Duh, & Chuang, 2000). However, currently used synthetic antioxidants such as *tert*-butyl hydroxyanisole (BHA) and *tert*-butyl hydroxytoluene (BHT) have been suspected to cause or promote toxic and carcinogenic effects (Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002; Tepe, Sokmen, Sokmen, Daferera, & Polissiou, 2005). Therefore, the interest for cheap, renewable and abundant sources of natural antioxidants has grown due to safety concerns, contradictory toxicological data about synthetic antioxidants

Abbreviations: DDK, dihydro-5,6-dehydrokawain; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ROS, reactive oxygen species; BHA, *tert*-butyl hydroxyanisole; BHT, *tert*-butyl hydroxytoluene; LDL, low density lipoprotein; DK, 5,6-dehydrokawain; SA, syringic acid; PHA, *p*-hydroxybenzoic acid; V, vanillin; PCA, *p*-coumaric acid; FA, ferulic acid; CA, cinnamic acid; FL, fresh leaves; FR, fresh rhizomes; LSW, leaves solid waste; RSW, rhizomes solid waste; FLh, hexane extract of fresh leaves; FRh, hexane extract of fresh rhizomes; LSWh, hexane extract of leaves solid waste; RSWh, hexane extract of rhizomes solid waste; FLea, ethyl acetate extract of fresh leaves; FRea, ethyl acetate extract of fresh rhizomes; LSWea, ethyl acetate extract of leaves solid waste; RSWea, ethyl acetate extract of rhizomes solid waste; LWW, leaves wastewater; RWW, rhizomes wastewater; LWWh, hexane extract of leaves wastewater; RWWh, hexane extract of rhizomes wastewater; LWWea, ethyl acetate extract of leaves wastewater; RWWea, ethyl acetate extract of rhizomes wastewater; EC_{50}, effective concentration required to give 50% DPPH radical scavenging activity; UV–vis, ultraviolet–visible; LSD, least significant difference; RI, retention index.

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and consumer preferences for natural additives (Garrote, Cruz, Moure, Dominguez, & Parajo, 2004).

Phenolic compounds comprise a major group of plant secondary metabolites. They are biochemically synthesized via the shikimate pathway, which produces the group of phenolics called phenylpropanoids (Singer, Crowley, & Thompson, 2003). They can act as antioxidants by donating hydrogen to highly reactive radicals, thereby preventing further radical formation (Lapornik, Prosek, & Wondra, 2005). It has been reported that a high consumption of fruits and vegetables containing phenolic antioxidants inhibits the oxidation of low density lipoprotein (LDL), and thus slows the process of atherosclerosis and also reduces the risk of cancer and many other diseases (Mohd Zin, Abdul Hamid, Osman, & Saari, 2006).

Alpinia [*Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. (Family Zingiberaceae)] is a perennial ginger growing widely in the subtropics and tropics. It is used in folk medicine for its anti-inflammatory, bacteriostatic and fungistatic properties (Zoghbi, Andrade, & Maia, 1999). The essential oil extracted from its leaves possessed both relaxant and antispasmodic actions in rate ileum (Bezerra, Leal-Cardoso, Coelho-de-Souza, Criddle, & Fonteles, 2000). Kava pyrone, dihydro-5,6-dehydrokawain (DDK), is a major compound in Alpinia leaves and it has shown plant growth inhibition against lettuce seeds (Fujita, Nishimura, Kaburagi, & Mizutani, 1994), insecticidal activity against *Coptotermes formosanus* and antifungal activity against *Pythium* sp. and *Corticium rolfsii* (Tawata, Taira, Kobamoto, Ishihara, & Toyama, 1996). Methyl *trans*-cinnamate, DDK, flavokawin B, dihydroflavokawin B, 5,6-dehydrokawain (DK), cardamonin and alpinetin have been detected in the rhizomes (Itokawa, Morita, & Mihashi, 1981). Furthermore, DDK, DK and some flavonoids have also been identified in the leaves (Mpalantinos, de Moura, Parente, & Kuster, 1998). However, the contents of phenolic acids have not been previously reported from Alpinia leaves or rhizomes.

Wastes of agricultural industries are recognized as a major source of environmental pollution, therefore, efforts are needed to use or recycle these wastes (Ulloa, van Weerd, Huisman, & Verreth, 2004). It has been reported that vegetable byproducts are an interesting and cheap source of health-promoting antioxidant polyphenols (Llorach, Tomas-Barberan, & Ferreres, 2004). In Okinawa (Japan), there are several companies producing essential oils from Alpinia leaves. During the extraction of essential oil from Alpinia by steam distillation, large volumes of wastewater in addition to the solid waste (deodorized leaves) are produced and subsequently discarded. During this process, the leaves are subjected to high temperatures which may release DDK and many phenolics into these wastes. The aim of this work was to evaluate Alpinia wastes as a source of DDK and natural antioxidants for utilization in food and pharmaceutical products. For this reason, contents of DDK and phenolic compounds were studied in Alpinia wastes as well as their antioxidant capacity. The composition of phenolic acids in fresh leaves or rhizomes of Alpinia was reported for the first time.

2. Materials and methods

2.1. Standards

Syringic acid (SA), *p*-hydroxybenzoic acid (PHA), vanillin (V), *p*-coumaric acid (PCA), ferulic acid (FA) and cinnamic acid (CA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). DDK was isolated from Alpinia leaves by using the method described by Tawata et al. (1996).

2.2. Solvents and reagents

β-Carotene, linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), *tert*-butyl hydroxytoluene (BHT), polyoxyethylene sorbitan monopalmitate (Tween-40), diethyl ether (99.5%), acetone (99.8%), methanol (99.7%), ethyl acetate (99.8%), hexane (96.0%), acetonitrile (99.8%) and chloroform (99.0%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.3. Plant material

Leaves and rhizomes of Alpinia 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 leaves or rhizomes of Alpinia 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

Extracts of fresh leaves (FL) or rhizomes (FR) of Alpinia and their wastes (water or solid residues of leaves or rhizomes after extraction of oil) were prepared according to the extraction protocol described in Fig. 1. Two hundred grams of fresh leaves (FL), 200 g of fresh rhizomes (FR), 260 g of leaves solid waste (LSW) and 260 g of rhizomes solid waste (RSW) were separately boiled in 500 ml distilled water for 20 min. After cooling at room temperature, the water extracts from FL, FR, LSW and RSW were separately filtered and extracted with hexane (2×200 ml). Obtained hexane extracts were separately filtered and dried under vacuum at 40 °C to give hexane extracts of fresh leaves (FLh), fresh rhizomes (FRh), leaves solid waste (LSWh) and rhizomes solid waste (RSWh). The aqueous solutions remaining from previous samples after extraction with hexane were separately dried and hydrolyzed with 150 ml NaOH

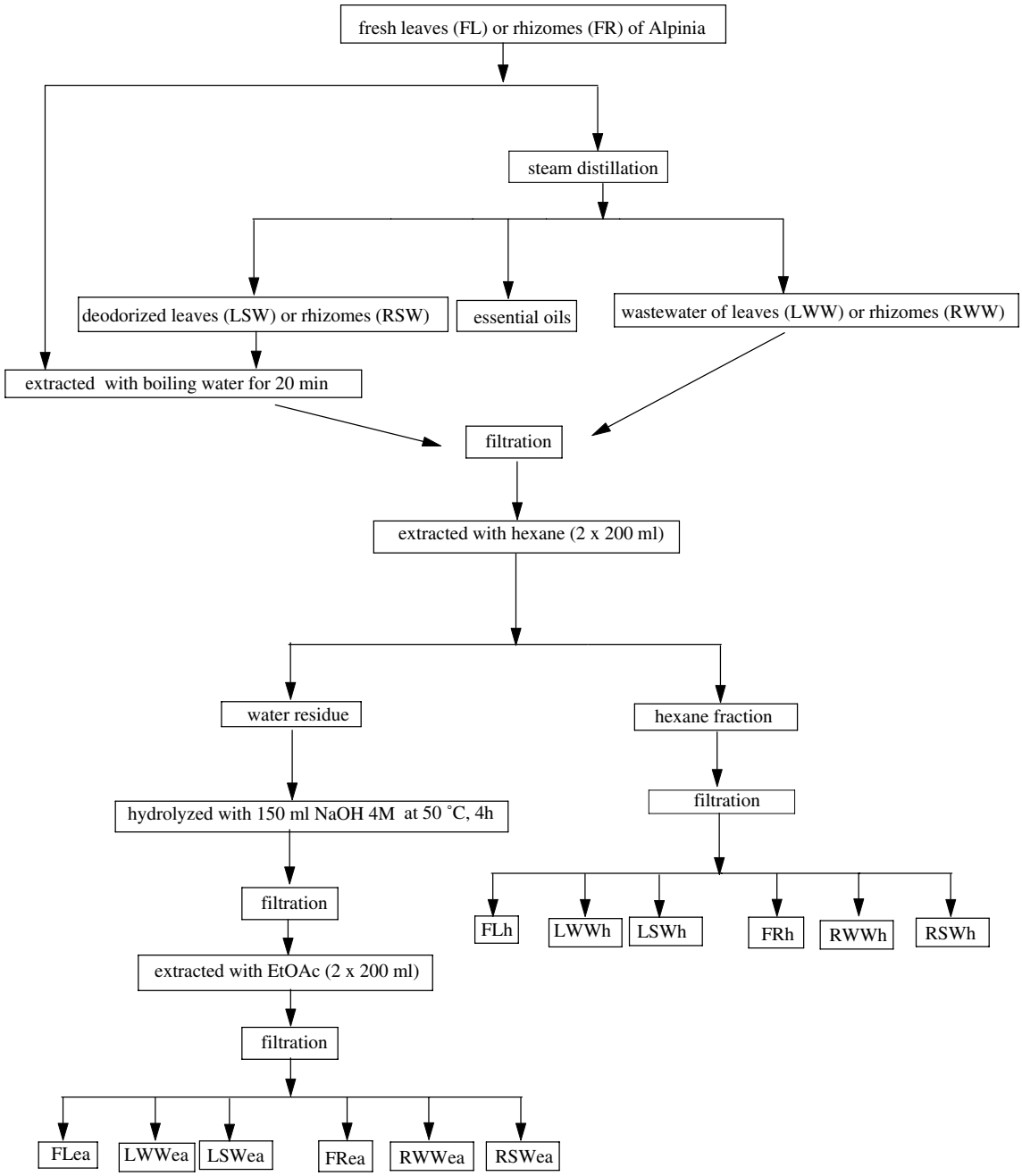

Fig. 1. Extraction scheme for preparation of the different extracts of Alpinia.

4 M at 50 °C with stirring for 4 h. The suspensions were separately filtered and the pH was adjusted to 2.0 by HCl 6 N. Afterwards, the filtrates were separately extracted with ethyl acetate (2×200 ml) and then filtered. The ethyl acetate extracts were separately dried under vacuum to give ethyl acetate extracts of fresh leaves (FLea), fresh rhizomes (FRea), leaves solid waste (LSWea) and rhizomes solid waste (RSWea). In addition, the wastewater (liquid retentate remaining after steam-distillation) of leaves (LWW) or rhizomes (RWW) were separately filtered and extracted with hexane and ethyl acetate by the same procedure mentioned above to prepare hexane extract of leaves wastewater (LWWh), hexane extract of rhizomes wastewater (RWWh), ethyl acetate extract of leaves wastewater (LWWea) and ethyl acetate extract of rhizomes wastewater (RWWea).

2.6. Antioxidant activity

2.6.1. DPPH assay

The radical scavenging activity was evaluated as described previously (Abe, Murata, & Hirota, 1998). Two milliliters of the methanol solution of Alpinia samples (25 and 50 ppm) 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). BHT 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.

2.6.2. 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 sample extracts and BHT at 1000 ppm were added. An equal amount of methanol was used for 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.

2.7. GC–MS analysis

A 1 μl aliquot of 500 ppm acetone solution of hexane and ethyl acetate extracts from all extracts of Alpinia leaves or rhizomes 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 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 at 1000 ppm 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.

2.8. 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 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.

2.9. 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 oil

Essential oils of fresh leaves or rhizomes of Alpinia were obtained as yellow oils with aromatic-spicy odor. The yield of oil extracted from leaves (0.07%, w/w) was higher than that of rhizomes (0.04%, w/w). The main components determined in leaves oil were 1,8-cineol, camphor and methyl cinnamate, whereas rhizomes oil mainly contained DDK and methyl cinnamate (Table 1). The chemical composition of the two essential oils suggests the possibility of using these oils to flavor food products such as chewing gums and sweets as well as in cosmetics, e.g., shower gels, soaps, shampoos and bath products with a fresh odor (Jirovetz, Buchbauer, Shafi, & Leela, 2003). Moreover, essential oil of Alpinia showed a strong activity on the cardiovascular system and on excitable tissues such as smooth muscle (Leal-Cardoso et al., 2004), therefore the oil may also be used in body external treatments as creams or pastes.

3.2. DDK content

Hexane extracts contained high amounts of DDK and the highest DDK quantity was found in hexane extract

Table 1
Essential oil components of fresh leaves and rhizomes of Alpinia

Compound	RI	Peak area %	
		Leaves	Rhizomes
cis-3-Hexen-1-ol	853	0.25	–
α-Pinene	932	0.27	–
Camphene	949	0.40	0.03
Benzaldehyde	961	0.39	0.30
B-Pinene	976	0.27	0.07
B-Myrcene	990	0.25	0.04
trans-3-Hexenoic acid	1002	0.37	0.03
α-Phellandrin	1006	0.06	0.04
α-Terpinene	1018	0.03	–
p-Cymene	1026	1.92	0.35
Limonene	1030	–	0.10
1,8-Cineol	1035	18.85	0.65
γ-Terpinene	1060	0.09	–
Linalool	1102	4.36	0.51
Phenylethyl alcohol	1114	0.46	0.42
Camphor	1152	11.93	2.88
Camphene hydrate	1160	0.75	0.23
Pinocarvon	1167	0.26	0.08
Borneol	1178	3.73	0.85
Terpinene-4-ol	1186	2.82	0.74
Cryptone	1193	6.63	2.04
α-Terpineol	1199	2.54	1.17
Myrtenol	1204	0.10	–
Sabinyl acetate	1208	0.26	0.32
trans-p-Menth-1-en-3-ol	1213	0.20	0.10
trans-p-Mentha-6,8-dien-2-ol	1223	0.29	–
Benzylacetone	1247	–	0.85
Cuminaldehyde	1248	1.87	–
p-Menth-1-en-3-one	1259	0.39	0.12
Phellandral	1284	0.61	0.25
Thymol	1291	0.13	0.08
Cuminalcohol	1294	0.73	0.29
Isothymol	1300	0.75	2.32
trans-Methyl cinnamate	1302	0.14	0.25
Methyl cinnamate	1332	7.59	15.04
Vanillin	1335	0.17	0.48
β-Caryophyllene	1345	0.39	0.37
α-Caryophyllene	1358	2.21	0.32
Calarene	1375	0.19	0.15
δ-Cadinene	1376	0.19	0.15
Epizonarene	1378	0.01	0.05
α-Muurolene	1382	0.02	0.09
Elemol	1386	0.04	0.12
Caryophyllene oxide	1399	1.64	0.86
γ-Eudesmol	1443	1.24	0.85
α-Eudesmol	1469	0.33	0.90
DDK	1938	2.46	21.4
Heneicosane	2102	0.02	0.52
DK	2159	0.02	0.18

RI, retention Index relative to *n*-alkanes on the DB-5 column.

of fresh rhizomes (FRh), whereas hexane extract of fresh leaves (FLh) produced the lowest amount of DDK (Fig. 2). Hexane extracts of wastewater from leaves (LWWh) and rhizomes (RWWh) had higher amounts of DDK than those of solid wastes from leaves (LSWh) and rhizomes (RSWh). DDK and DK have been used as anti-ulcerogenic and antithrombotic agents (Mpalantinos et al., 1998). Our results verified that water and solid wastes remaining after extraction of essential oil from Alpinia leaves or rhizomes contained high amounts of DDK suggesting that these wastes can be utilized as a source of DDK that may be used for medical purposes.

3.3. DPPH free radical scavenging activity

DPPH is a stable free radical that loses its purple color when accepts an electron from an antioxidant molecule (Zou, Lu, & Wei, 2004). It evidently offers a convenient, accurate and simple method for titrating the oxidizable groups of antioxidants (Blois, 1958). DPPH EC_{50} of ethyl acetate extracts from different extracts of leaves or rhizomes of Alpinia are shown in Fig. 3. Hexane extracts from leaves or rhizomes showed very weak DPPH radical scavenging activity, therefore only concentrations of ethyl acetate extracts, which were active and provided 50% inhibition to DPPH radicals were included in Fig. 3. All ethyl acetate extracts from leaves displayed lower EC_{50} values (0.07–0.10 mg/ml) when compared to those reported for ethyl acetate extracts from rhizomes (0.26-0.70 mg/ml). While no significant differences were found in DPPH EC_{50} of ethyl acetate extracts prepared from leaf samples, ethyl acetate extracts from fresh rhizomes (FRea) had the lowest DDPH EC_{50} among rhizome samples. Although the positive control (BHT) exhibited the strongest DPPH radical scavenging activity, no significant differences ($P \leqslant 0.05$) were observed among EC_{50} values of BHT, FLea and LSWea. ROS has been implicated in the pathogenesis of a variety of chronic diseases such as heart disease and rheumatism (Matthäus, 2002). Therefore, enhancing food antioxidants that scavenge ROS may be a good approach for reducing the risk of cancer and coronary heart disease (Li, Shan, Sun, Corke, & Beta, 2005). In this respect, our results indicate that disposed water and solid wastes produced during the production of essential oil from leaves or rhizomes of Alpinia showed high free radical scavenging activity proposing that these wastes may be used as a cheap source for natural antioxidants.

3.4. Antioxidant activity measured by β-carotene bleaching method

The discoloration of β-carotene is widely used to measure the antioxidant activity of plant extracts (Kumazawa et al., 2002). The method is based on minimizing β-carotene loss in an emulsion due to the oxidation of linoleic acid, which generates free radicals (Marco, 1968). The method has been recently speeded up by using 96-well microtitre plates for sample incubation and an automatic reader for simultaneous absorbance measurements (Koleva et al., 2002). Ethyl acetate extracts from all samples of Alpinia inhibited β-carotene oxidation (Fig. 4). Ethyl acetate extract of wastewater from leaves (LWWea) exhibited the highest antioxidant activity followed by those from fresh rhizomes (FRea) and solid waste from leaves (LSWea), whereas ethyl acetate extracts from fresh leaves (FLea), wastewater from rhizomes (RWWea) and solid waste from rhizomes (RSWea) showed the lowest activity.

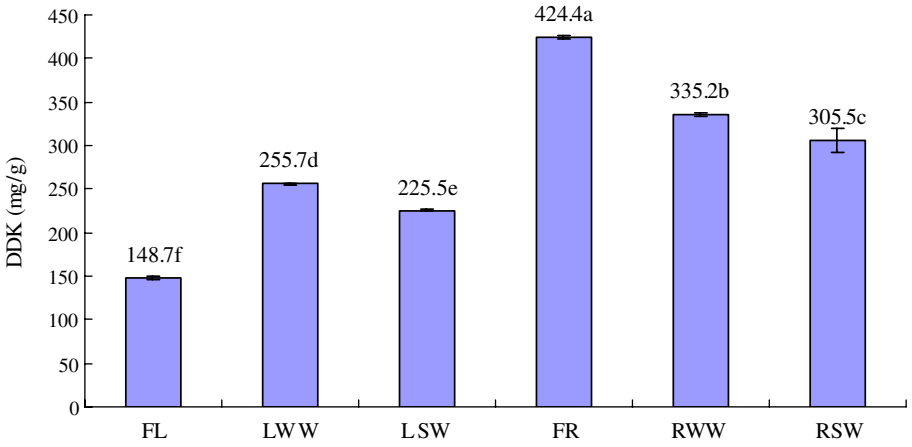

Fig. 2. DDK content in hexane extracts from various samples of Alpinia. FL, fresh leaves; LWW, leaves wastewater; LSW, leaves solid waste; FR, fresh rhizomes; RWW, rhizomes wastewater; RSW, rhizomes solid waste. Values are means of three replications ± SE. Means with the same letter are not significantly different at $P \leqslant 0.05$.

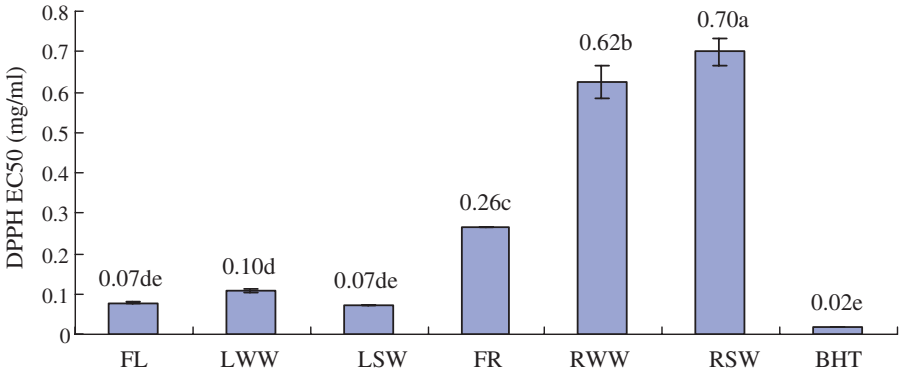

Fig. 3. DPPH EC_{50} (mg/ml) of ethyl acetate extracts from various samples of Alpinia and BHT as positive control. FL, fresh leaves; LWW, leaves wastewater; LSW, leaves solid waste; FR, fresh rhizomes; RWW, rhizomes wastewater; RSW, rhizomes solid waste. Values are means of three replications ± SE. Means with the same letter are not significantly different at $P \leqslant 0.05$.

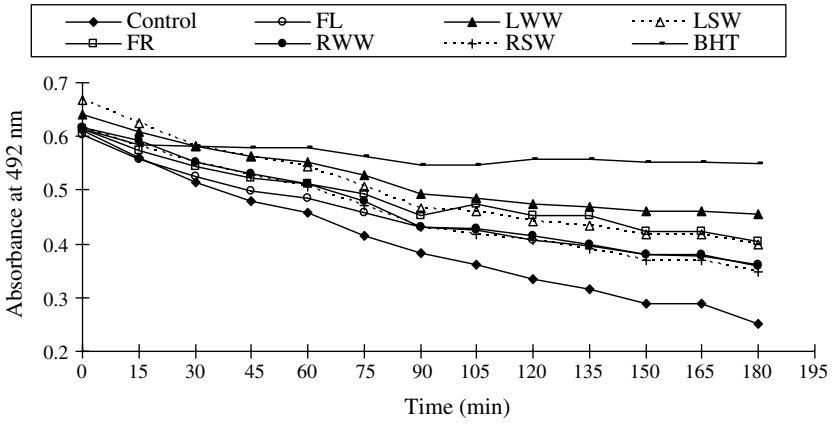

Fig. 4. Antioxidant activity of ethyl acetate extracts from various samples of Alpinia and BHT measured by β-carotene bleaching method. FL, fresh leaves; LWW, leaves wastewater; LSW, leaves solid waste; FR, fresh rhizomes; RWW, rhizomes wastewater; RSW, rhizomes solid waste.

β-Carotene in this model system undergoes rapid discoloration in the absence of an antioxidant as it loses the double bonds by oxidation. The presence of antioxidants can hinder the extent of β-carotene-bleaching by neutralizing the free radicals formed in the system (Jayaprakasha, Singh, & Sakariah, 2001).

3.5. Phenolic compounds content

Hexane and ethyl acetate extracts prepared from fresh leaves or rhizomes of Alpinia and their wastes remaining after steam distillation were analyzed by GC–MS. The ethyl acetate extracts contained a variety of compounds,

which were tentatively identified by comparison with standards and MS library. These compounds are benzoic acid, 2,3-dihydrobenzofuran, benzenepropanoic acid, *p*-hydroxybenzaldehyde, vanillin, cinnamic acid, *p*-hydroxybenzoic acid, vanillic acid, syringaldehyde, *iso*-ferulic acid, *p*-coumaric acid, syringic acid, ferulic acid, DDK and DK (Table 2). Phenolic acids are present in plants as ether- and/or ester-linked compounds requiring acidic or alkaline extraction with heat (Martens, 2002). In our study, a 4-h hydrolysis with 4 M NaOH at 50 °C released conjugated phenolics and subsequently six phenolic compounds including *p*-hydroxybenzoic acid (PHA), syringic acid (SA), vanillin (V), *p*-coumaric acid (PCA), ferulic acid (FA) and cinnamic acid (CA) were detected by HPLC. Only vanillin and cinnamic acid have been quantified in hexane extracts of leaves or rhizomes (Table 3A). Ethyl acetate extract from solid waste of leaves (LSWea) contained the highest amount of phenolic compounds, while

Table 2
Compounds detected by GC–MS in leaves and rhizomes of Alpinia

Compounds	FLh	LWWh	LSWh	FRh	RWWh	RSWh	FLea	LWWea	LSWea	FRea	RWWea	RSWea
Benzoic acid	–[a]	–	–	–	–	–	+	+	+	+	+	+
2,3-Dihydrobenzofuran	–	–	–	–	–	–	+	+	+	+	+	+
Benzenepropanoic acid	–	–	–	–	–	–	+	+	+	+	+	+
p-Hydroxybenzaldehyde	–	–	–	–	–	–	+	+	+	+	+	+
Vanillin	+[b]	+	+	–	+	–	+	+	+	+	+	+
Cinnamic acid	+	–	–	+	–	–	+	+	+	+	+	+
p-Hydroxybenzoic acid	–	–	–	–	–	–	+	+	+	+	+	+
Vanillic acid	–	–	–	–	–	–	+	+	+	+	+	+
Syringaldehyde	–	–	–	–	–	–	+	+	+	+	+	+
iso-Ferulic acid	–	–	–	–	–	–	+	+	+	+	+	+
p-Coumaric acid	–	–	–	–	–	–	+	+	+	+	+	+
Syringic acid	–	–	–	–	–	–	+	+	+	+	+	+
Ferulic acid	–	–	–	–	–	–	+	+	+	+	+	+
DDK	+	+	+	+	+	+	+	+	+	+	+	+
DK	+	+	+	+	+	+	–	+	+	–	–	+

FLh, hexane extract of fresh leaves; LWWh, hexane extract of leaves wastewater; LSWh, hexane extract of leaves solid waste; FRh, hexane extract of fresh rhizomes; RWWh, hexane extract of rhizomes wastewater; RSWh, hexane extract of rhizomes solid waste; FLea, ethyl acetate extract of fresh leaves; LWWea, ethyl acetate extract of leaves wastewater; LSWea, ethyl acetate extract of leaves solid waste; FRea, ethyl acetate extract of fresh rhizomes; RWWea, ethyl acetate extract of rhizomes wastewater; RSWea, ethyl acetate extract of rhizomes solid waste.

[a] Not detected.

[b] Detected.

Table 3
Contents of phenolic compounds in hexane extracts (A) and ethyl acetate extracts (B) from leaves and rhizomes of Alpinia

Samples	Phenolic compounds (mg/g)					
	PHA	SA	V	PCA	FA	CA
(A)						
FLh	–[a]	–	1.2 ± 0.09ab	–	–	0.6 ± 0.00c
LWWh	–	–	1.5 ± 0.23a	–	–	0.0d
LSWh	–	–	1.0 ± 0.00b	–	–	0.0d
FRh	–	–	0.0c	–	–	1.0 ± 0.08a
RWWh	–	–	1.5 ± 0.02a	–	–	0.8 ± 0.01b
RSWh	–	–	0.0c	–	–	0.0d
LSD			0.31			0.10
(B)						
FLea	7.7 ± 0.00c	4.0 ± 0.00a	0.9 ± 0.00b	1.7 ± 0.05d	32.3 ± 1.30a	2.0 ± 0.08c
LWWea	9.1 ± 0.39b	4.0 ± 0.32a	0.7 ± 0.08b	3.3 ± 0.14b	30.7 ± 1.09a	7.1 ± 0.26b
LSWea	11.5 ± 0.43a	3.3 ± 0.16bc	0.8 ± 0.06b	4.2 ± 0.17a	30.4 ± 3.25a	8.8 ± 0.30a
FRea	6.5 ± 0.49d	3.6 ± 0.00ab	6.0 ± 0.26a	2.0 ± 0.00c	6.9 ± 0.05b	2.0 ± 0.00c
RWWea	4.0 ± 0.10e	3.1 ± 0.10c	0.8 ± 0.17b	0.4 ± 0.03e	2.5 ± 0.15b	1.8 ± 0.16c
RSWea	4.5 ± 0.08e	3.2 ± 0.03bc	0.7 ± 0.05b	0.6 ± 0.00e	3.5 ± 0.03b	2.0 ± 0.08c
LSD	0.97	0.47	0.42	0.29	4.6	0.56

Values are means of three replications ± SE. Means with the same letter are not significantly different at $P \leqslant 0.05$. FLh, hexane extract of fresh leaves; LWWh, hexane extract of leaves wastewater; LSWh, hexane extract of leaves solid waste; FRh, hexane extract of fresh rhizomes; RWWh, hexane extract of rhizomes wastewater; RSWh, hexane extract of rhizomes solid waste; FLea, ethyl acetate extract of fresh leaves; LWWea, ethyl acetate extract of leaves wastewater; LSWea, ethyl acetate extract of leaves solid waste; FRea, ethyl acetate extract of fresh rhizomes; RWWea, ethyl acetate extract of rhizomes wastewater; RSWea, ethyl acetate extract of rhizomes solid waste; PHA, *p*-hydroxybenzoic acid; SA, syringic acid; V, vanillin; PCA, *p*-coumaric acid; FA, ferulic acid; and CA, cinnamic acid.

[a] Not detected.

ethyl acetate extract from wastewater of rhizomes (RWWea) had the lowest level of phenolics (Table 3B). Contents of all detected phenolic compounds were higher in leaf samples than those of rhizomes, except for vanillin which was higher in rhizomes. Ferulic and *p*-hydroxybenzoic acids were the major phenolics present in ethyl acetate extracts from leaves or rhizomes. Phenolic compounds are commonly found in plants and they have been reported to have multiple biological effects including antioxidant activity (Kuti & Konuru, 2004). Their antioxidant potential is dependent on the number and arrangement of the hydroxyl groups and the extent of structural conjugation, as well as the presence of electron-donating and electron-withdrawing substituents in the ring structure (Lapornik et al., 2005). It has been stated that a high consumption of fruits and vegetables containing phenolic antioxidants inhibits the oxidation of low density lipoprotein (LDL), and slows the process of atherosclerosis and also reduces the risk of cancer and many other diseases (Mohd Zin et al., 2006). Thus, water and solid wastes of leaves or rhizomes of Alpinia contained several phenolic compounds that give the possibility of using these wastes as phenolic-rich sources.

4. Conclusion

The present study indicates that three different products (essential oil, DDK, and phenolic compounds) can be obtained from leaves or rhizomes of Alpinia. The results also reveal that water and solid wastes produced during the production of essential oil from Alpinia leaves or rhizomes contained high amounts of DDK and phenolic compounds and showed strong antioxidant activity. There is a high possibility to use Alpinia as a multi-purpose crop. Its essential oil can be used in aromatherapy as an antispasmodic, antihypertensive and antinociceptive agent (Pinho, Coelho-de-Souza, Morais, Santos, & Leal-Cardoso, 2005). The wastewater generated during essential oil production is an interesting source to produce DDK for medical use as an antiulcerogenic and antithrombotic agent. The solid wastes including residues of leaves or rhizomes contained phenolic compounds and may be used as a source for natural antioxidants in tea preparations or in food products such as meat, dairy and bakery products. Furthermore, this is the first report describes phenolic acid profiles of fresh leaves or rhizomes of Alpinia and their wastes produced during the essential oil production.

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