Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 1183–1191
Food Chemistry
www.elsevier.com/locate/foodchem

Characterization of the components present in the active fractions of health gingers (*Curcuma xanthorrhiza* and *Zingiber zerumbet*) by HPLC–DAD–ESIMS

Sharin Ruslay [a], Faridah Abas [a], Khozirah Shaari [a,b], Zurina Zainal [a], Maulidiani [a], Hasnah Sirat [c], Daud Ahmad Israf [a], Nordin H. Lajis [a,b,*]

[a] *Laboratory of Natural Products, Institute of Bioscience, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[b] *Chemistry Department, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[c] *Chemistry Department, University of Technology Malaysia, Skudai 81310 Johor, Malaysia*

Received 4 May 2006; received in revised form 27 November 2006; accepted 19 January 2007

Abstract

Curcuma xanthorrhiza and *Zingiber zerumbet* are two of the most commonly used ingredients in Indo-Malaysian traditional medicines, health supplements and tonics. Recently, a number of products derived from the aqueous extracts of these species have appeared in the market in the form of spray-dried powder packed in sachet or bottle. On-line high performance liquid chromatography, coupled with diode array detection and electrospray ion trap tandem mass spectroscopy (HPLC–DAD–ESI–MS^n), was used to analyze the components in the antioxidant-active fractions from the rhizomes of these species. Three components were identified from *C. xanthorrhiza*, including bisdemethoxycurcumin (**1**), demethoxycurcumin (**2**) and curcumin (**3**). The active fraction from *Z. zerumbet* consisted of five components, including kaempferol 3-*O*-rhamnoside (**4**), compound **5** [kaempferol 3-*O*-(2″-*O*-acetyl)rhamnoside (**5a**) or kaempferol 3-*O*-(3″-*O*-acetyl)rhamnoside (**5b**)], kaempferol 3-*O*-(4″-*O*-acetyl)rhamnoside (**6**), kaempferol 3-*O*-(3″,4″-*O*-diacetyl)rhamnoside (**7**) and kaempferol 3-*O*-(2″,4″-*O*-diacetyl)rhamnoside (**8**). To confirm their identities, the components from *Z. zerumbet* were isolated conventionally and were analyzed by spectroscopic techniques as well as by comparison with literature data.

Keywords: *Curcuma xanthorrhiza*; *Zingiber zerumbet*; Traditional medicines; Health supplements; Tonics; Antioxidant activity; LC–DAD–ESIMS

1. Introduction

Zingiberaceae species are among the most prolific plants in the tropical rainforests. In Peninsular Malaysia, approximately 160 species from 18 genera are found, mostly growing naturally in damp, shaded parts of the lowland or hill slopes, as scattered plants or thickets (Larsen, Ibrahim, Khaw, & Saw, 1999). In the southeast Asian countries, several species are commonly used as spices, medicines, flavouring agents, as well as the source of certain dyes (Burkill, 1966). We have aimed to examine the potential of the Zingiberaceae species as new sources of active compounds because of their wide use for medicinal purposes (Habsah et al., 2000). Among these species, *Curcuma xanthorrhiza* is popularly used as an ingredient in the traditional health supplements known as "jamu" and "maajun", or individually used to remedy certain health problems. For example, the infusion of the juice from the rhizomes of *C. xanthorrhiza* is used to remedy indigestion and rheumatism, or applied to the body after childbirth, while the fresh rhizomes of *Zingiber zerumbet* are eaten as an appetizer or to cure stomachache.

* Corresponding author. Address: Laboratory of Natural Products, Institute of Bioscience, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia. Tel.: +60 3 89468082; fax: +60 3 89468080.
E-mail address: nhlajis@ibs.upm.edu.my (N.H. Lajis).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.01.067

Recently, several products derived from these two ginger species have appeared in the market in the form of spray-dried powder of the water extract, or sachets containing the dried powdered rhizomes. Antioxidant activity was selected as the initial biopotential assessment, since antioxidants have been strongly associated with the defence mechanisms of living cells against oxidative damage (Miller, 1995). Several classes of plant-derived compounds such as flavonoids, phenolics and alkaloids, have been reported to exhibit antioxidant properties (Larson, 1988).

In view of their popularity among the locals, it is important that the chemical profiles of these two species be established as the marker compounds, as well as to validate the rationale for their efficacies. To the best of our knowledge, there has not been any study to establish the profile of the active fractions from these species, although several phytochemical studies of them have been reported (Claeson et al., 1993; Itokawa, Hariyama, Funakoshi, & Takeya, 1985; Masuda, Isobe, Jitoe, & Nakatani, 1992; Nakatani, Jitoe, Masuda, & Yonemori, 1991). The water extracts of the individual species were fractionated into hexane, ethyl acetate and butanol, and the antioxidant activity of the fractions was measured. The ethyl acetate fractions from both species exhibited strongest activity in the assays performed. We report here the use of HPLC–DAD–MS/MS in accomplishing this effort.

2. Materials and methods

2.1. Chemicals and solvents

Methanol and acetonitrile were of HPLC grade (Merck, Darmstadt, Germany). Water was purified by a MilliQ system (Millipore, Bedford, MA, USA). Acetic acid (Fisher, Loughborough, UK) was used as buffer. Redistilled reagent grade hexane, dichloromethane, diethyl ether, ethyl acetate and methanol were used for the extraction and separation of compounds. Column chromatography utilized silica gel 60 (7734 and 9385, Merck, Darmstadt, Germany), while analytical TLC utilized silica gel DC-Plastikfolien 60 F_{254} (Merck, Darmstadt, Germany). Chemicals were purchased from Sigma Chemical Co. (USA), with the exception of ethanol, which was purchased from Scharlau (Barcelona, Spain).

2.2. Plant materials and sample preparation

Fresh rhizomes of *C. xanthorrhiza* and *Z. zerumbet* were purchased from a wet market in Kajang, Selangor and were identified by Mr. Shamsul Khamis. Voucher specimens (SK 145/02) were deposited at the mini herbarium of the Laboratory of Natural Products at the Institute. The rhizomes were cut into thin slices (1–1.5 mm), air-dried under a shade and ground in a mill. Ground materials (500 g) were macerated three times with water (3 × 1 l). Removal of the water with rotary evaporator under reduced pressure was followed by lyophilization, to give a brown gum. The extracts (20 g) were shaken with a mixture of water/methanol (2:1) and extracted with hexane (three times), followed by ethyl acetate (three times) and butanol (three times). Each fraction was dried under reduced pressure and then subjected to antioxidant assay. Active fractions were analyzed by HPLC–DAD–ESI–MS/MS. The samples for analysis were prepared by dissolving 10 mg of the active fraction in 10 ml of MeOH and then filtering with a C_{18}, Sep-Pak cartridge (Waters, Milford, USA). The filtrate (10 μl) was directly injected into the analytical system for analysis.

2.3. Instrumentation

The mass spectra were acquired using a ThermoFinnigan model LCQ^{DECA} (San Jose, CA) ion-trap mass spectrometer equipped with an ESI source interface and controlled by Xcalibur 1.2. Ultrahigh pure helium (He) and high purity nitrogen (N_2) were used as collision and nebulizing gases, respectively. The mass spectra were acquired in the negative ion mode. The instrument was coupled with a Surveyor HPLC binary pump, Surveyor diode array detector (DAD) (200–600 nm range; 5 nm bandwidth) and Surveyor autosampler. The separation was carried out on an Inertsil ODS-3 column (5 μm, 150 × 2.1 mm I.D., GL Sciences Inc. Japan). The profile of the gradient elution for *Z. zerumbet* was; (A) water containing 1% HOAc and (B) methanol; 0–20 min, 50% A; 20–35 min, 50–60% A; 35–40 min, 60–50% A; 40–60 min, 50% A at a flow-rate of 0.25 ml/min. The profile of the gradient elution of *C. xanthorrhiza* was: (A) water containing 0.25% HOAc and (B) acetonitrile; 0–20 min, 60% A; 20–30 min, 60–50% A; 30–40 min, 50% A; 40–50 min, 50–60% A; 50–60 min, 60% A at flow-rate 0.15 ml/min.

All chromatographic procedures were performed at ambient temperature. A data-dependent programme was used in the liquid chromatography-tandem mass spectrometry analysis so that the two most abundant ions in each scan were selected and subjected to MS/MS analysis. The spectra were obtained from the LCQ^{DECA} ESI/MS^n detector at a scan rate of 0.5 Hz, and a capillary temperature of 275 °C, with a full ion scan in the range 100–1000 amu. The collision-induced dissociation (CID) energy was adjusted to 35%. To confirm the data from HPLC–DAD–ESI–MS^n analysis, the components of the active fraction from *Z. zerumbet* were isolated and their structures were elucidated by spectroscopic methods, including MS, UV, IR and NMR spectrometry (^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC) and by comparison with the literature data (Matthes, Luu, & Ourisson, 1980; Nakatani et al., 1991). The confirmation of the LC peaks was accomplished by co-injection of the fraction with the isolates.

2.4. Antioxidant activity

The lipid peroxidation inhibition assays using ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods, as well as radical-scavenging activity, using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method were carried out according to previously described protocols (Abas, Lajis, Israf, Khozirah, & Kalsom, 2006). Results are given as means ± SEM values. All the experiments *in vitro* were conducted at least three times (for each assay) with three or more independent observations.

2.5. Isolation of the ethyl acetate fraction from Z. zerumbet

The ethyl acetate fraction (10 g) from *Z. zerumbet* crude extract was fractionated by vacuum liquid chromatography

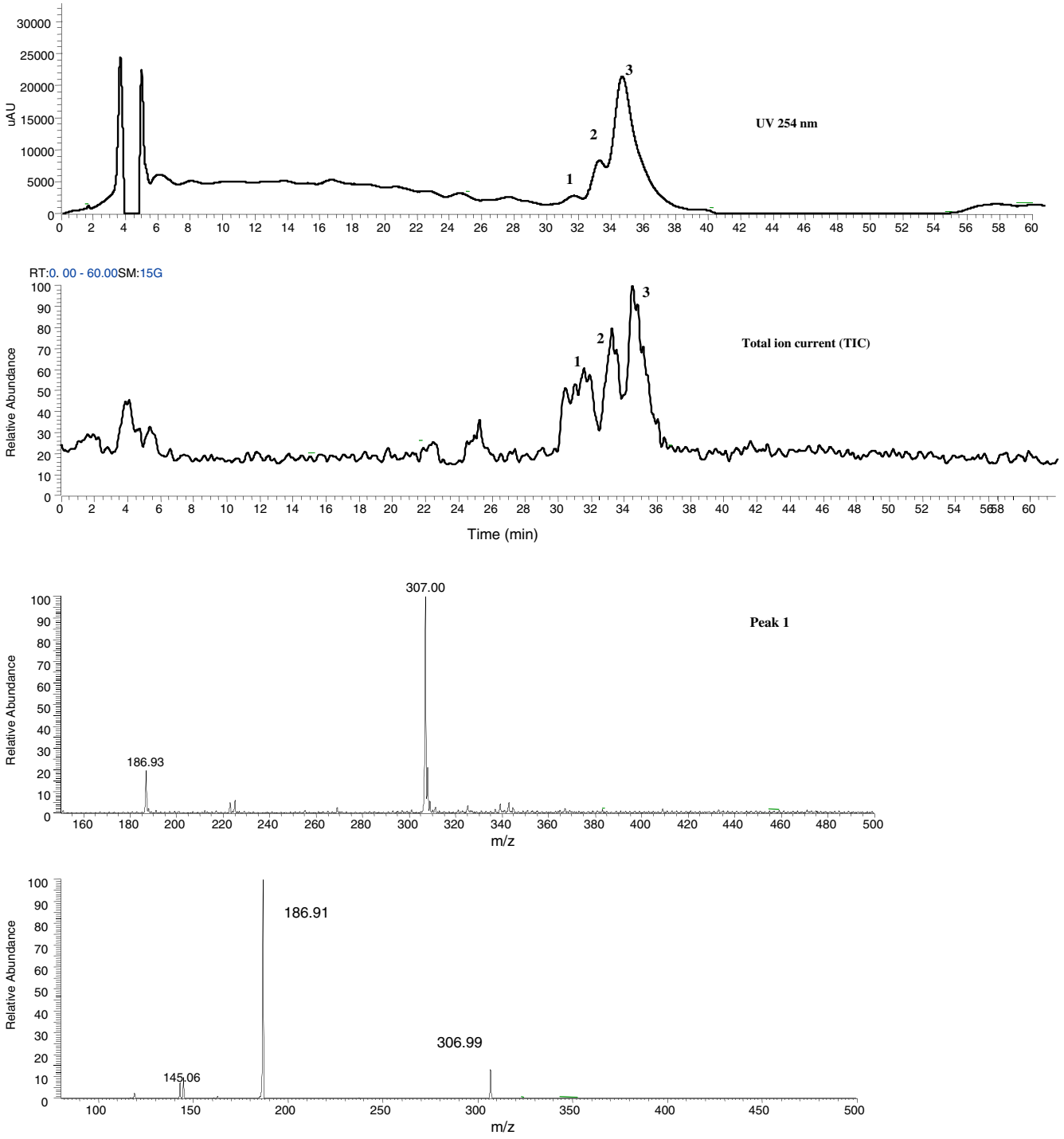


Fig. 1. The LC–DAD–MS/MS analyses of the ethyl acetate of *C. xanthorrhiza*.

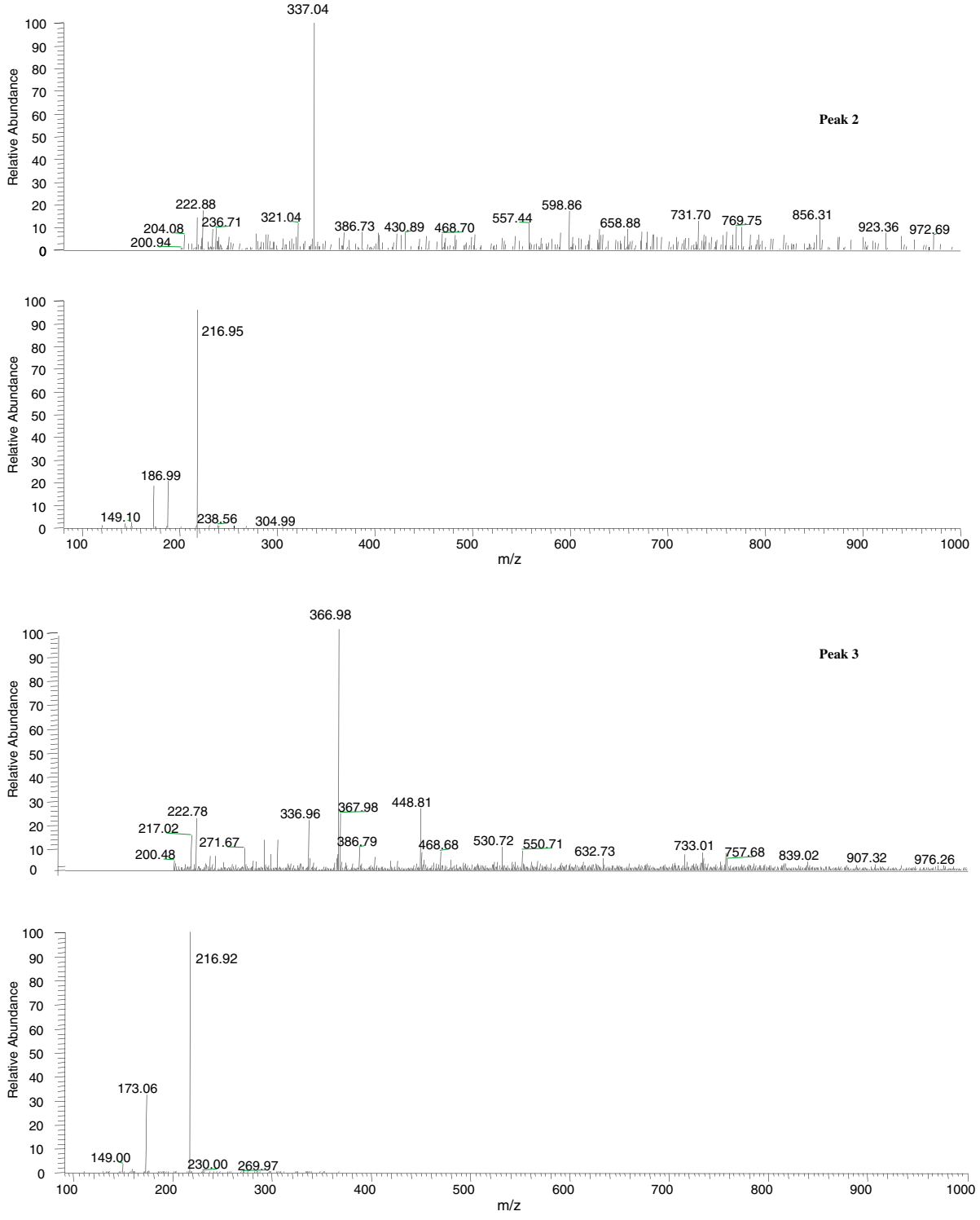


Fig. 1 (*continued*)

(VLC), using silica gel as adsorbent, and gradual elution with dichloromethane, followed by CH_2Cl_2/MeOH mixture (gradient polarity) and finally with MeOH to give 15 (100 ml) fractions. Fractions 7–9 were combined and rechromatographed on silica gel 9385 and eluted with CH_2Cl_2, followed by CH_2Cl_2/diethyl ether (gradient polarity) to give 12 fractions. Fraction 5 (350 mg) was further rechromatographed and successively (gradient polarity) eluted with CH_2Cl_2, CH_2Cl_2/diethyl ether and diethyl ether to give eight fractions. Fraction 5(1) gave compound **7** (13.5 mg), which appeared as faint yellow sticky crystals, whereas fraction 5(5) gave compound **8** (40.0 mg), which also appeared as faint yellow sticky crystals. Fractions 8 and 9 were separately rechromatographed using Sephadex

LH-20, with MeOH as eluant, to give four fractions each. Fraction 9(3) gave compound **4** (6.4 mg), obtained as a yellowish sticky solid, while fraction 8(3) was further rechromatographed on silica gel eluted with a mixture of hexane and ethyl acetate to give five fractions. Fraction 8(3/1) was evaporated to yield compound **6** (9.7 mg), obtained as a yellowish sticky solid. The structures of the isolated compounds were elucidated by spectroscopic methods, including MS, IR and NMR spectrometry (^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC) and confirmed by comparison with the literature (Matthes et al., 1980; Nakatani et al., 1991).

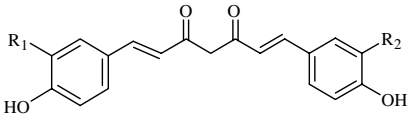
3. Results and discussion

3.1. Identification of the curcuminoids from C. xanthorrhiza

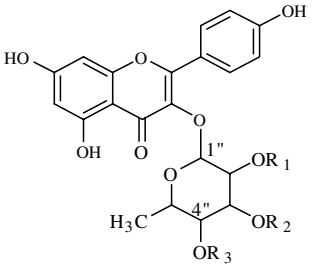
The LC–DAD and LC-total ion chromatograms of the active (ethyl acetate) fraction from *C. xanthorrhiza* are shown in Fig. 1. Peaks **1**, **2** and **3** were detected in negative mode ESI-MS/MS chromatogram. Peaks **1**, **2** and **3** showed similar UV spectra, which are characteristic of curcuminoids with λ_{max} at 425 nm. The mass spectra showed the deprotonated molecule ion masses at *m/z* 307, 337 and 367 for the peaks of **1**, **2**, and **3**, respectively. Based on their UV, MS and MS/MS data, as well as comparison with literature data (He, Lin, Lian, & Lindenmaier, 1998), they were assigned as bisdemethoxycurcumin (**1**), demethoxycurcumin (**2**) and curcumin (**3**) (Fig. 2).

3.2. Identification of flavonoid glycosides from Z. zerumbet

The LC/UV–DAD and LC–/ESI–MS/MS analyses of the ethyl acetate fraction from *Z. zerumbet* are shown in Fig. 3. All five peaks appearing in the LC–DAD chromatogram showed similar UV spectra, which are characteristic of the absorptions of the flavonol chromophores (λ_{max} 265 and 345 nm). The negative ion mode showed a deprotonated molecular ion peak at *m/z* 431 for peak **4**, which corresponds to the molecular formula, $C_{21}H_{20}O_{10}$, of sugar-substituted flavonols. Further fragmentation by collision-induced dissociation of this parent ion (*m/z* 431) gave an aglycone fragment at *m/z* 285 $[M - H - 146]^-$ after the loss of a sugar moiety. Peaks **5** and **6** showed similar deprotonated molecular ion peaks at *m/z* 473 $[M - H]^-$, which were consistent with the molecular formula $C_{23}H_{22}O_{11}$ for sugar-substituted flavonols (Masuda et al., 1992), and that they are isomeric of one another. Further fragmentation of these parent ions gave the aglycone fragment at *m/z* 285 $[M - H - 188]^-$ after the loss of acetylated sugar groups. Peaks **7** and **8** also showed a similar molecular ion peak at *m/z* 515 $[M - H]^-$, consistent with the molecular formula $C_{25}H_{24}O_{12}$, which also indicated that they were isomeric to one another. The MS/MS experiment on both peaks (*m/z* 515) gave the aglycone fragments at *m/z* 285 [M − H − 231], due to the loss of bisacetylated sugar groups. The MS and MS/MS chromatograms of each component are shown in Fig. 3. To confirm the identities of the isomers, compounds **6**, **7**, and **8** were isolated and the structures were determined by spectroscopic analyses, including MS, IR and NMR spectroscopy (^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC). The ^{1}H chemical shifts of the rhamnoside moiety of compounds **6**, **7** and **8** are presented in Table 1. The location of the acetates in this moiety is indicated by the downfield shift of the proton signals (H-2″, H-3″ and H-4″) as compared to the non-acetylated OH. Compound **5** was not isolated, due to its minor quantity. The isolates were finally used to confirm the identity of the chromatographic peaks by co-injection. The identity of the components is summarized in Fig. 2.



Bisdemethoxycurcumin (**1**): R_1=R_2=H,
Demethoxycurcumin (**2**): R_1=OMe, R_2=H,
Curcumin (**3**): R_1=R_2=OMe



	R_1	R_2	R_3
Kaempferol-3-*O*-rhamnoside (**4**)	H	H	H
Kaempferol-3-*O*-(2”- or 3”-acetyl) rhamnoside (**5**)	H	Ac	H (not isolated)
Kaempferol-3-*O*-(4”- acetyl) rhamnoside (**6**)	H	H	Ac
Kaempferol-3-*O*-(3”,4”-diacetyl) rhamnoside (**7**)	H	Ac	Ac
Kaempferol-3-*O*-(2”, 4”-diacetyl) rhamnoside (**8**)	Ac	H	Ac

Fig. 2. Curcuminoids (**1**–**3**) identified from *C. xanthorrhiza* and flavonol rhamnosides (**4**–**8**) identified from *Z. zerumbet*.

3.3. Antioxidant activity

Measurements of antioxidant activity, using ferric thiocyanate (FTC), thiobarbituric acid (TBA) and DPPH radical-scavenging methods, were carried out on the extract and fractions. Their activities were compared with the standard antioxidants, ascorbic acid, butylated hydroxyltoluene (BHT), quercetin and α-tocopherol. The FTC assay measures the amount of peroxide in initial stages of lipid peroxidation, while the TBA assay measures the extent of peroxide degradation into malondialdehyde. In these

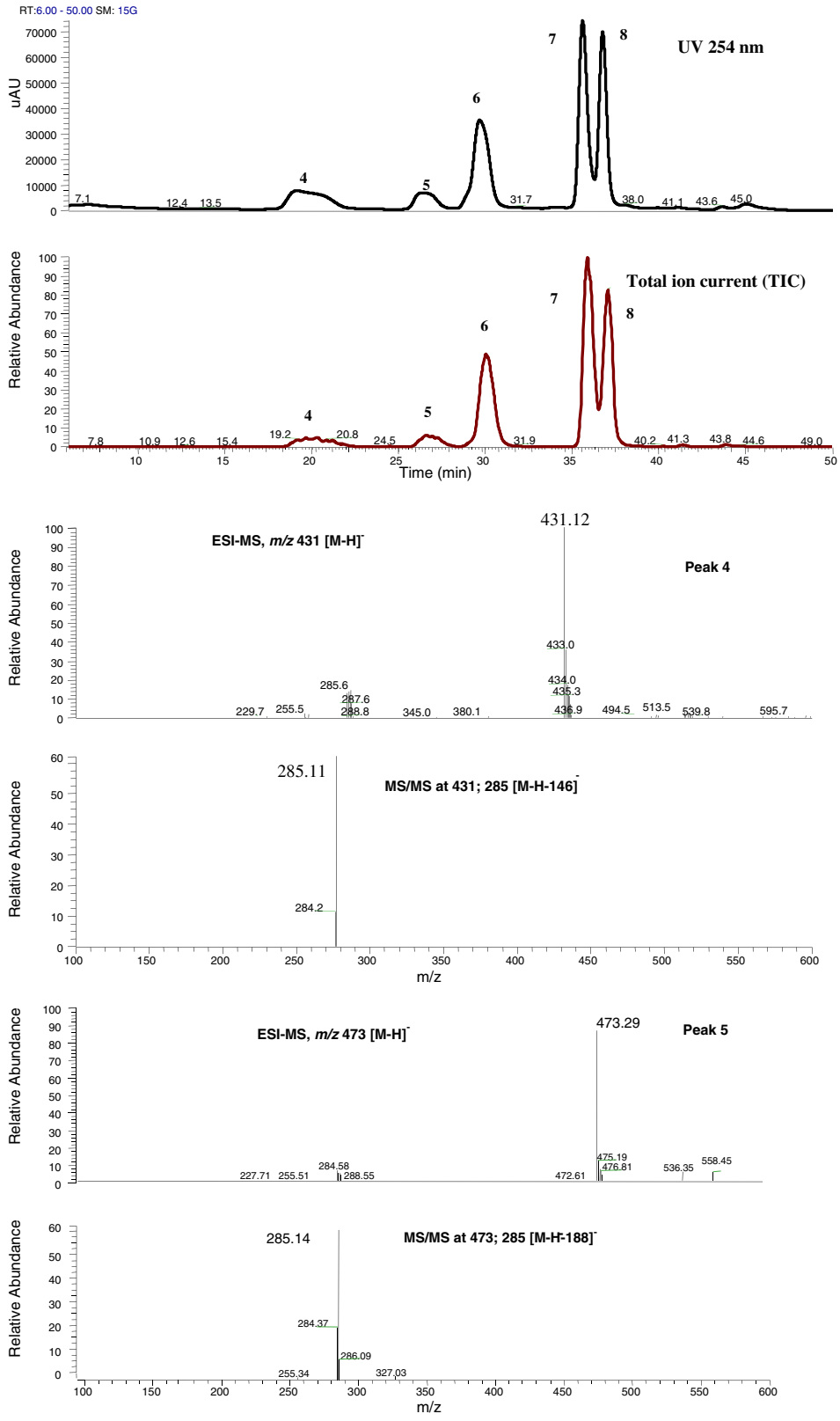


Fig. 3. The LC–DAD–MS/MS analyses of the active fractions (ethyl acetate) of *Z. zerumbet*.

assays, ethyl acetate fractions of *C. xanthorrhiza* and *Z. zerumbet* were the most significant (Table 2).

In the DPPH assay, the stable free radical (DPPH) can react with antioxidants and produce 1,1-diphenyl-2-picrylhydrazine, which gave a strong absorption band at 517 nm. As the electron became paired off in the presence of a free radical-scavenger, the absorption vanished, and this resulted in decolorization. The results from the free

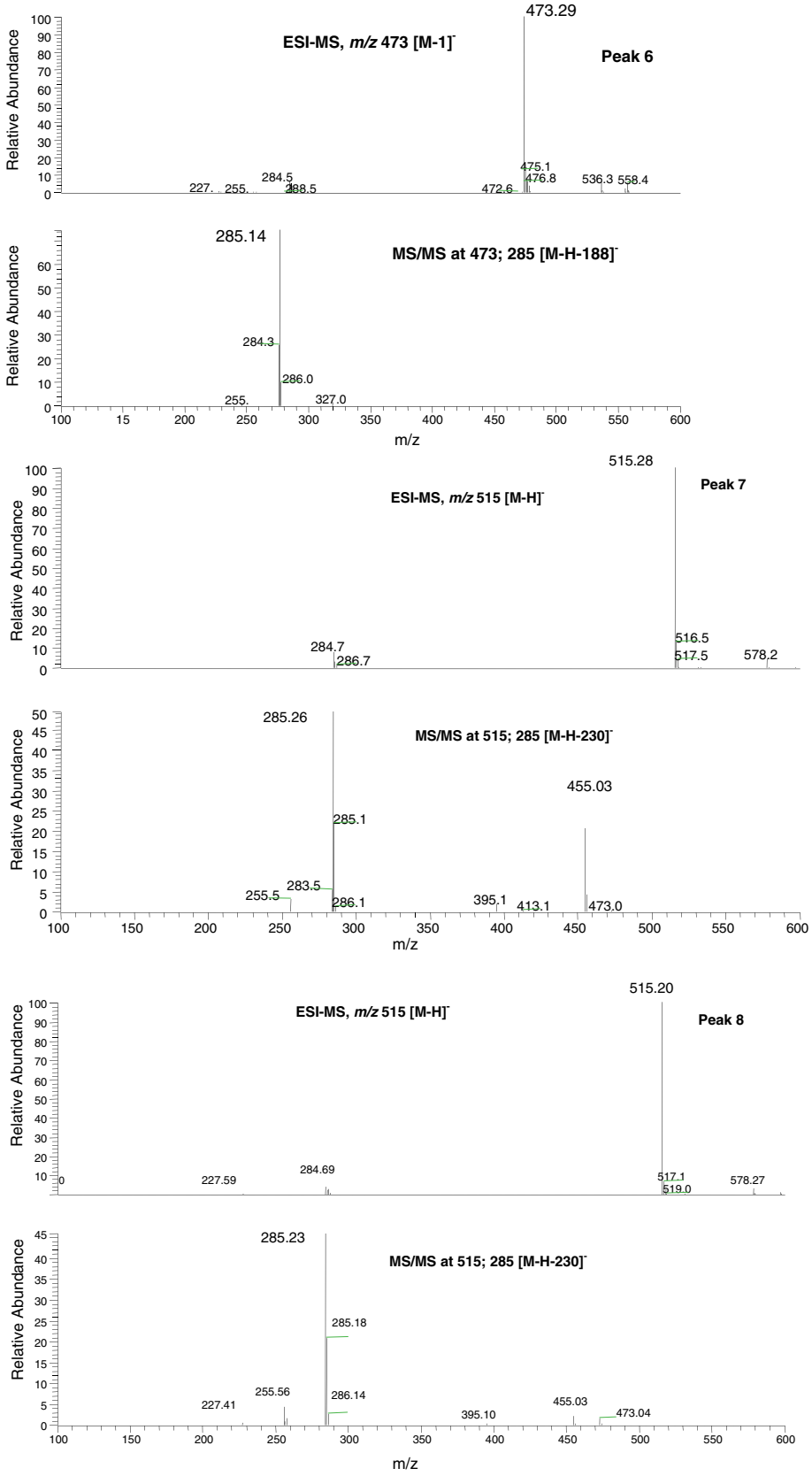


Fig. 3 (*continued*)

radical-scavenging assay of the fractions showed that the ethyl acetate fractions of *C. xanthorrhiza* and *Z. zerumbet* were the most active fractions with their IC_{50} values of 70.3 and 299 μg/ml, respectively (Table 2).

Table 1
^{1}H Chemical shifts of the rhamnoside moiety of compounds **6**, **7** and **8**

H	δ ^{1}H (**6**)	δ ^{1}H (**7**)	δ ^{1}H (**8**)
1″	5.54, br s	5.63, d ($J = 1.5$ Hz)	5.55, d ($J = 1.5$ Hz)
2″	4.24, br s	4.37, t ($J = 2.0$ Hz)	5.48, dd ($J = 9.8$, 3.6 Hz)
3″	3.89, br d ($J = 10.0$ Hz)	5.18, dd ($J = 9.8$, 3.0 Hz)	4.06, dd ($J = 9.8$, 3.6 Hz)
4″	4.85, t ($J = 9.5$ Hz)	5.02, t ($J = 9.5$ Hz)	4.78, t ($J = 9.5$ Hz)
5″	3.25, m	3.30, m	3.33, m
6″	0.81, d ($J = 6.0$ Hz)	0.84, d ($J = 6.5$ Hz)	0.84, d ($J = 6.5$ Hz)
2″-OCOMe	–	–	2.07, s
3″-OCOMe	–	2.10, s	–
4″-OCOMe	2.19, s	2.00, s	2.13, s

Table 2
Percentage inhibition of lipid peroxidation by *C. xanthorrhiza* and *Z. zerumbet* fractions using a ferric thiocyanate method

Sample	FTC (% inhibition at 200 μg/ml)	TBA (% inhibition at 80 μg/ml)	DPPH IC_{50} (μg/ml)
BHT	99.7 ± 0.002	97.7 ± 0.027	nd
Quercetin	96.8 ± 0.002	98.3 ± 0.010	8.20 ± 0.025
Vitamin E	69.3 ± 0.026	89.6 ± 0.014	nd
Vitamin C	nd	nd	8.48 ± 0.012
C. xanthorrhiza (water extract)	−6.60 ± 0.185	25.1 ± 0.129	>500
C. xanthorrhiza (hexane)	95.9 ± 0.005	98.5 ± 0.033	>500
C. xanthorrhiza (ethyl acetate)	98.4 ± 0.001	99.7 ± 0.001	62.7 ± 0.014
C. xanthorrhiza (butanol)	89.8 ± 0.005	97.6 ± 0.001	>500
Z. zerumbet (water extract)	−6.64 ± 0.045	21.5 ± 0.022	>500
Z. zerumbet (hexane)	−13.3 ± 0.026	6.74 ± 0.117	>500
Z. zerumbet (ethyl acetate)	94.5 ± 0.010	100 ± 0.001	292 ± 0.026
Z. zerumbet (butanol)	89.9 ± 0.004	96.5 ± 0.025	>500

Each experiment was carried out in triplicate and repeated three times; nd, not determined.

The LC–DAD–MS analysis indicated that the ethyl acetate fraction from *C. xanthorrhiza* contained curcumin, demethoxycurcumin and bisdemethoxycurcumin with their relative abundances being in the same respective descending order. We have encountered these three constituents in our studies of other zingiberaceous species, including *Curcuma mangga* and *Etlingera elatior*, and have determined their antioxidant activity (Abas et al., 2005; Mohamad et al., 2005). It is therefore concluded that these constituents are the responsible components for antioxidative activity in *C. xanthorrhiza*.

The constituents identified in the ethyl acetate fraction of *Z. zerumbet* include, afzelin and diacetylafzelin. These flavonoid glycosides are the derivatives of kaempferol, a 3-flavonol known to possess antioxidant property (Pietta, 2000). We have also previously investigated the antioxidant constituents of *Hedychium thyrsiforme* and identified the active constituent as methylated kaempferol (Jasril et al., 2003). Although it has been established that the presence of free 3-hydroxyl functionality in flavonoids is necessary for the enhancement of antioxidant activity in flavonoids (Abas, Shaari, Lajis, Israf, & Umi Kalsom, 2003) in this study, the glycosylated 3-hydroxyl group of the kaempferols in *Z. zerumbet* seemed to be the most active constituent.

4. Conclusions

On-line analysis using the LC–DAD–ESIMS/MS technique allowed us to identify the constituents present in the selected fractions from *C. xanthorrhiza* and *Z. zerumbet*. The constituents in the most active fraction from the water extract of *C. xanthorrhiza* were identified as curcumin, demethoxycurcumin and bisdemethoxycurcumin. The most active fraction from the water extract of *Z. zerumbet* contained a mixture of glycosylated kaempferol, as well as its isomers of mono- and diacetylated derivatives. The antioxidant properties of these classes of compound have been well established. The results from this study therefore support the use of the aqueous extracts from these species as health supplements for prevention of degenerative diseases.

Acknowledgements

The authors express their gratitude to Dr. Emerson F. Queiroz at the University of Geneva for his suggestions and comments, to the Ministry of Science, Technology and Innovation for the grant provided under the Intensified Research in Priority Areas Research Grant 06-02-06-PR (54812).

References

Abas, F., Lajis, N. H., Israf, D. A., Khozirah, S., & Kalsom, Y. U. (2006). Antioxidant and nitric oxide activities of Malay traditional vegetables. *Food Chemistry, 95*, 566–573.

Abas, F., Lajis, N. H., Shaari, K., Israf, D. A., Stanslas, J., Yusuf, U. K., et al. (2005). A new labdane diterpene glucoside from the rhizomes of *Curcuma mangga*. *Journal of Natural Products, 68*, 1090–1093.

Abas, F., Shaari, K., Lajis, N. H., Israf, D. A., & Umi Kalsom, Y. (2003). Antioxidative and radical scavenging properties of the constituents isolated from *Cosmos caudatus* Kunth. *Natural Product Science, 9*, 245–248.

Burkill, I. H. (1966). *A dictionary of the economic products of the Malay Peninsula* (Vols. 1–2). Kuala Lumpur, Malaysia: Ministry of Agriculture and Cooperative.

Claeson, P., Panthong, A., Tuchinda, P., Reutrakul, V., Kanjanapothi, D., Taylor, W. C., et al. (1993). Three non-phenolic diarylheptanoids with anti-inflammatory activity from *Curcuma xanthorrhiza*. *Planta Medica, 59*, 451–457.

He, X.-G., Lin, L.-Z., Lian, L.-Z., & Lindenmaier, M. (1998). Liquid chromatography–electrospray mass spectrometric analysis of curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa*). *Journal of Chromatography A, 818*, 127–132.

Habsah, M., Amran, M., Mackeen, M. M., Lajis, N. H., Kikuzaki, H., Nakatani, N., et al. (2000). Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. *Journal of Ethnopharmacology, 72*, 403–410.

Itokawa, H., Hariyama, F., Funakoshi, K., & Takeya, K. (1985). Studies on antitumour bisabolane sesquiterpenoids from *Curcuma xanthorrhiza*. *Chemical and Pharmaceutical Bulletin, 33*, 3388–3492.

Jasril, Mooi, L. Y., Lajis, N. H., Ali, A. M., Sukari, M. A., Rahman, A. A., et al. (2003). Antioxidant activity of the flavonoids from *Hedychium thyrsiforme* Ham. (Zingiberaceae). *Pharmaceutical Biology, 41*, 506–511.

Larsen, K., Ibrahim, H., Khaw, S. H., & Saw, L. G. (1999). *Gingers of Peninsular Malaysia*. Kota Kinabalu: Natural History Publication (Borneo).

Larson, R. A. (1988). The antioxidants of higher plants. *Phytochemistry, 27*, 918–971.

Masuda, T., Isobe, J., Jitoe, A., & Nakatani, N. (1992). Antioxidative curcuminoids from rhizomes of *Curcuma xanthorrhiza*. *Phytochemistry, 13*, 3645–3647.

Matthes, H. W. D., Luu, B., & Ourisson, G. (1980). Cytotoxic components of *Zingiber zerumbet*, *Curcuma zedoaria* and *C. domestica*. *Phytochemistry, 19*, 2643–2650.

Miller, B. (1995). *Antioxidants made simple*. Malaysia: Bruce Miller Ent. Inc. Petaling Jaya.

Mohamad, H., Lajis, N. H., Abas, F., Ali, A. M., Sukari, M. A., Kikuzaki, H., et al. (2005). Antioxidative constituents from *Etlingera elatior*. *Journal of Natural Products, 68*, 285–288.

Nakatani, N., Jitoe, A., Masuda, T., & Yonemori, S. (1991). Flavonoid constituents of *Zingiber zerumbet*. *Agricultural and Biological Chemistry, 55*, 455–460.

Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products, 63*, 1035–1042.