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Research Article

Correlation between Chemical Composition of *Curcuma domestica* and *Curcuma xanthorrhiza* and Their Antioxidant Effect on Human Low-Density Lipoprotein Oxidation

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The antioxidant activity of the curcuminoids of *Curcuma domestica* L. and *C. xanthorrhiza* Roxb. and eight compounds which are prevalent constituents of their rhizome oils were investigated in an effort to correlate human low-density lipoprotein (LDL) antioxidant activity with the effect of the herbs and their components. The antioxidant activity was examined using thiobarbituric acid reactive substances (TBARSs) assay with human LDL as the oxidation substrate. The methanol extracts and rhizome oils of *C. xanthorrhiza* and *C. domestica* showed strong inhibitory activity on copper-mediated oxidation of LDL. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin, isolated from the methanol extracts of both plants, exhibited stronger activity than probucol (IC_{50} value 0.57 μmol/L) as reference, with IC_{50} values ranging from 0.15 to 0.33 μmol/L. Xanthorrhizol, the most abundant component (31.9%) of the oil of *C. xanthorrhiza*, showed relatively strong activity with an IC_{50} value of 1.93 μmol/L. The major components of *C. domestica*, ar-turmerone (45.8%) and zerumbone (3.5%), exhibited IC_{50} values of 10.18 and 24.90 μmol/L, respectively. The high levels of curcuminoids in the methanol extracts and xanthorrhizol, ar-turmerone and zerumbone in the oils, and in combination with the minor components were responsible for the high LDL antioxidant activity of the herbs.

1. Introduction

Curcuma (Zingiberaceae) is a large genus of rhizomatous herbs distributed in tropical and subtropical regions especially in India, Thailand, the Malay Archipelago, Indochina, and Northern Australia. Many species have been cultivated, and their powdered rhizomes have been widely used as flavours in native dishes and ingredients in many traditional medicines to treat various ailments [1]. The more popular and economically more important species, *C. domestica* L. and *C. xanthorrhiza* Roxb., are more widely used as condiments than for their medicinal purposes. Many phytochemical studies on the extracts and essential oils of several *Curcuma* species, especially *C. longa*, have identified curcuminoids and sesquiterpenoids as the major components [2–6], and these compounds have been identified as the major groups of antioxidants in the plants [7, 8].

The antioxidant activity of *Curcuma* species, especially *C. longa*, has been measured by various chemical methods such as DPPH radical scavenging activity assay, superoxide anion radical scavenging activity assay, ferric reducing/antioxidant power (FRAP) assay, and metal chelating activity assay [8, 9]. Inhibitory activity of curcumin from *C. longa* and its analogues against free radical initiated peroxidation of human low-density lipoprotein (LDL) [10] and lipid peroxidation and protein oxidation in rat liver mitochondria have been reported [11]. The need to use different methods of antioxidant capacity measurement is due to the various mechanisms of antioxidant action. Determination of the antioxidant activity of plant extracts and compounds often

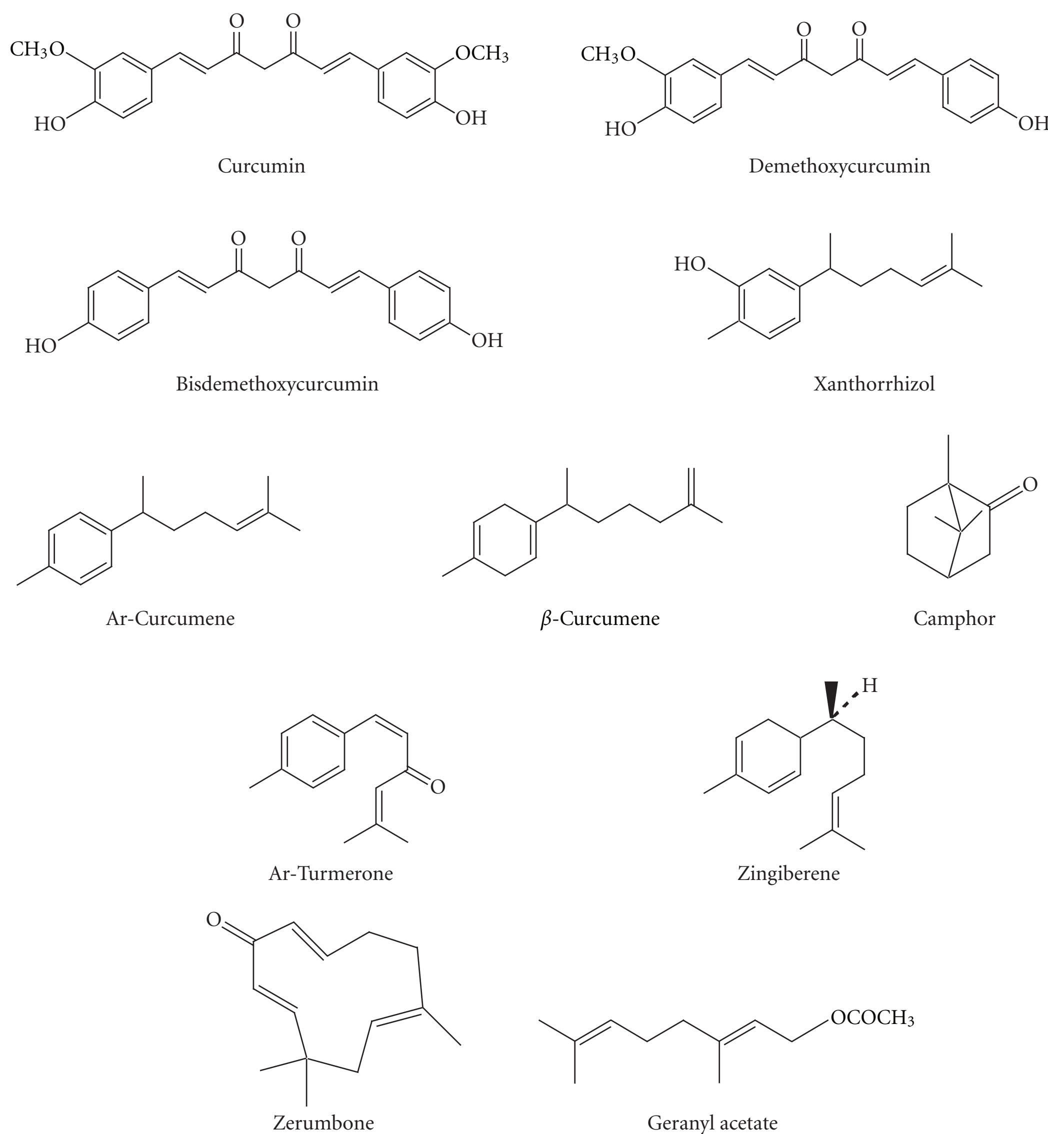

Figure 1: Structures of major compounds from *Curcuma domestica* and *Curcuma xanthorrhiza*.

gave different results as the methods used are based on different reaction mechanisms [12]. Although the chemical constituents of *Curcuma* species have been reported and their antioxidant activity has been demonstrated, there has been little effort to correlate the chemical constituents and their antioxidant activity, and the actual substances contributing to the antioxidant activity have not been identified. Direct evidence of therapeutic benefits of the plants and their compounds in cardiovascular disorders remains sparse, and data on LDL oxidation have been few.

In a search for sources of natural cardiovascular protective agents for pharmaceutical, food, and nutraceutical applications, we investigated the antioxidant effect of the methanol extracts and essential oils of *C. domestica* and *C. xanthorrhiza*. The antioxidant activity was determined against copper-mediated isolated human LDL oxidation. Three major curcuminoids, that is, curcumin, demethoxycurcumin, and bisdemethoxycurcumin, were isolated from the methanol extract of the rhizomes of both herbs. The chemical composition of the oils was analysed by GC and GC-MS. The antioxidant activity of the three curcuminoids and eight known constituents (xanthorrhizol, ar-turmerone, camphor, geranyl acetate, zerumbone, β-curcumene, zingiberene, and ar-curcumene) of the essential oils of the *Curcuma* species were also investigated in an effort to correlate the effectiveness of the herbs with those of their components. The structures of the major compounds are shown in Figure 1.

2. Materials and Methods

2.1. Chemicals and Reagents. The chemicals used in this study were of analytical grade that include methanol, dimethyl sulfoxide (DMSO) and sodium citrate (Merck, Darmstadt, Germany). Camphor, geranyl acetate, zerumbone, β-curcumene, zingiberene, ar-curcumene OptiPrep,

Sudan Black B, phosphate buffer saline (PBS) tablet, probucol, and Protein Kit were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Subcell electrophoresis and agarose gel were obtained from Bio-Rad, USA. TBARS kit was purchased from ZeptoMetrix Corporation, New York. Melting points were determined using Electrothermal Melting Point Apparatus Model 9100. Ultraviolet (UV) spectra were obtained on Shimadzu UV-1601 spectrophotometer (Shimadzu Corporation, Japan). Molecular weights of the compounds were recorded by ESIMS using ESI-TOFF MS (Bruker MicroToF-Q 86, Switzerland). IR spectra were recorded on a Perkin-Elmer GX spectrophotometer (Massachusetts, USA). The ^{1}H and ^{13}C spectra were carried out on a JOEL NMR 400 MHz (JOEL Ltd., Japan) with TMS as internal standard.

2.2. Plant Materials and Sample Preparation. The rhizomes of *Curcuma domestica* and *C. xanthorrhiza* were collected from Kuala Selangor in peninsular Malaysia in the months of March and July 2008. The voucher specimens of *C. domestica* (B-29789) and *C. xanthorrhiza* (B-29783) were identified by Dr. Abdul Latiff Mohamad of Universiti Kebangsaan Malaysia (UKM) and deposited at the Herbarium of UKM, Bangi, Malaysia. The fresh rhizomes of *C. domestica* and *C. xanthorrhiza* were allowed to dry under shade. Five hundred g of dried material of each plant sample were ground and macerated in methanol at the ratio of 1 : 10 (w/v). The extracts were filtered through Whatman No. 1 filter paper, and the entire extraction process was repeated twice on the residue. The filtrates were combined and the methanol was removed under reduced pressure to obtain extracts of *C. domestica* and *C. xanthorrhiza* at 35.2 and 17.0% yields, respectively (calculated based on dry weight). Each of the extracts was shaken with n-hexane to remove much of the volatile oils and fatty components, and the resultant extract was then subjected to antioxidant assay.

2.3. Isolation of Curcuminoids. The defatted rhizome extract (14 g) of *C. domestica* was fractionated by vacuum liquid chromatography (VLC) on silica gel type H (10–40 μm, 7 × 30 cm) and eluted with a gradient system of hexane : $CHCl_3$ (10 : 0–1 : 9, v/v) and $CHCl_3$: MeOH (10 : 0–0 : 10, v/v); repeated silica gel column (40–63 μm, 3 × 60 cm) was eluted consecutively with toluene : $CHCl_3$: MeOH (75 : 15 : 5, v/v) and a gradient system of hexane : EtOAc (10 : 0–1 : 9, v/v) followed by recrystallization from EtOAc : hexane to yield curcumin (500 mg, 3.6%), demethoxycurcumin (200 mg, 1.4%), and bisdemethoxycurcumin (300 mg, 2.1%). The procedure for isolation of the compounds from *C. xanthorrhiza* was similar to that performed on *C. domestica.* Thirteen g of the defatted methanol extract of *C. xanthorrhiza* resulted in the isolation of curcumin (300 mg, 2.3%), demethoxycurcumin (250 mg, 1.9%), and bisdemethoxycurcumin (100 mg, 0.8%).

2.3.1. Curcumin. Orange crystals, mp 184°C. HRESIMS: *m/z*: 391.0585 $[M + Na]^+$ 759.1357 $[2M + Na]^+$ which corresponded to $C_{21}H_{20}O_6$. NMR ^{1}H (acetone, 600 MHz): δ 7.60 (2H, *d*, $J = 15.6$ Hz, H-1, H-7), 7.32 (2H, *d*, $J = 1.8$ Hz, H-9, H-15), 7.16 (2H, *dd*, $J = 1.8, 8.0, 1.8$ Hz, H-13, H-19), 6.88 (2H, *d*, $J = 7.8$ Hz, H-12, H-18), 6.70 (2H, *d*, $J = 16.2$ Hz, H-2, H-6), 5.96 (1H, *s*, H-4), 3.91 (6H, *s*, OMe); NMR ^{13}C (acetone, 600 MHz): δ 55.5 (C-OMe), 100.7 (C-4), 110.8 (C-9, C-15), 115.4 (C-12, C-18), 121.5 (C-2, C-6), 122.9 (C-13, C-19), 127.3 (C-8, C-14), 140.5 (C-1, C-7), 147.9 (C-10, C-16), 149.2 (C-11, C-17), 183.6 (C-3, C-5).

2.3.2. Demethoxycurcumin. Orange crystals, mp 172°C. Its molecular formula was $C_{20}H_{18}O_5$ as indicated by HRESIMS: *m/z*: 337.0826 $[M\text{-}H]^-$, 675.1758 $[2M\text{-}H]^-$. NMR ^{1}H (acetone, 400 MHz): δ 7.62 (1H, *d*, $J = 8.0$ Hz, H-9), 7.59 (1H, *d*, $J = 15.9$ Hz, H-1), 7.58 (1H, *d*, $J = 15.4$ Hz, H-7), 7.56 (1H, *d*, $J = 8.4$ Hz, H-13), 7.34 (1H, *s*, H-15), 7.19 (1H, *d*, $J = 8.0$ Hz, H-19), 6.91 (3H, *d*, $J = 8.4$ Hz, H-10, H-12, H-18), 6.87 (1H, *d*, $J = 16.4$ Hz, H-2), 6.74 (1H, *d*, $J = 16.1$ Hz, H-6), 5.97 (1H, *s*, H-4), 3.91 (3H, *s*, OMe); NMR ^{13}C (acetone, 400 MHz): δ 56.3 (C-OMe), 101.8 (C-4), 111.5 (C-15), 116.3 (C-18), 116.9 (C-10, C-12), 122.1 (C-2), 122.3 (C-6), 123.9 (C-19), 127.7 (C-8), 128.2 (C-14), 131.1 (C-9, C-13), 141.1 (C-7), 141.5 (C-1), 148.8 (C-17), 150.1 (C-16), 160.6 (C-11), 184.5 (C-5), 184.6 (C-3).

2.3.3. Bisdemethoxycurcumin. Orange crystals, mp 222°C. HRESIMS for $C_{19}H_{16}O_4$ *m/z*: 307.0757 $[M\text{-}H]^-$. NMR ^{1}H (acetone, 400 MHz): δ 7.61 (4H, *d*, $J = 8.4$ Hz, H-9, H-13, H-15, H-19), 7.55 (2H, *d*, $J = 15.7$ Hz, H-1, H-7), 6.86 (4H, *d*, $J = 8.0$ Hz, H-10, H-12, H-16, H-18), 6.73 (2H, *d*, $J = 15.8$ Hz, H-2, H-6); NMR ^{13}C (acetone, 400 MHz): δ 100.7 (C-4), 116.0 (C-10, C-12, C-16, C-18), 121.4 (C-2, C-6), 127.4 (C-9, C-13, C-15, C-19), 130.2 (C-8, C-14), 140.6 (C-1, C-7), 161.3 (C-11, C-17), 183.5 (C-3, C-5).

2.4. Preparation of Essential Oils. The fresh rhizomes of *C. domestica* and *C. xanthorrhiza* were washed, comminuted, and hydrodistilled in Clevenger-type apparatus for 8 h. The oily layers obtained were separated and dried over anhydrous magnesium sulphate. The percentage yields of oils based on dry weight for *C. domestica* and *C. xanthorrhiza* were 2.9 and 4.5%, respectively.

2.5. Analysis of the Essential Oils. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses were used for the identification of the essential oil components. The components were separated using a Shimadzu GC-2010 equipped with a flame ionizing detector (FID) and a DB-5 (30 m × 0.25 mm, 1 μm film thickness) capillary column. One μL of each sample, dissolved in ethyl acetate, was injected automatically in spilt mode (autoinjector Shimadzu AOC-20i), using pressure-controlled nitrogen as a carrier gas at a linear velocity of 50 cm^3/min. The temperature of the injector and the detector was maintained at 250°C. The oven temperature was programmed from 75°C for 10 min, then at 3°C/min to 250°C and held for 5 min. The oils were also examined using stationary phase SE-30 (30 m × 0.25 mm, 0.25 μm film thickness) under the following program conditions; initial temperature 60°C for 10 min, then

3°C/min to 230°C for 1 min. Peak areas and retention times were measured by computerized integration. The relative amounts of individual components were calculated based on the peak areas obtained without a flame ionization detector (FID) response factor correction. The linear retention indices of the components relative to n-alkanes were also determined. The oils were also analyzed using a Hewlett Packard GC-MSD 5890 series II; EI electron impact mode with electron energy 70 eV, scan time 1.5 s, and mass range 40–500 Da using a BPX5 (25 m × 0.25 mm × 0.25 μm film thickness) capillary column. Similar conditions were used as described in GC programs. Components were identified by comparing their relative retention indices with those in the literature, their mass spectral data with the existing Wiley library, and cochromatography of some components with authentic components on the DB-5 capillary column [13].

2.6. Isolation of Pure Compounds from the Essential Oils. The essential oils of *C. xanthorrhiza* and *C. domestica* were subjected to repeated column chromatography on silica gel (230–400 mesh) eluted with hexane-ethyl acetate (1 : 1, v/v), hexane-ethyl acetate (3 : 7, v/v), and 100% ethyl acetate. Xanthorrhizol in 18% yield and ar-turmerone in 30% yield were obtained from the essential oils of *C. xanthorrhiza* and *C. domestica*, respectively. The compounds were identified by spectroscopic techniques and by comparison with published data [14, 15].

2.7. Human LDL Isolation. The use of human whole blood in this study was approved by the Ethics Committee of Universiti Kebangsaan Malaysia (UKM) (approval no. FF-120-2007). All subjects were healthy volunteers aged 24–70 yrs, normolipidemic, nonsmokers, having not taken any medications including vitamin supplements within the last 2 weeks, and fasting for the last 8 h. Venous blood was drawn from the volunteers, and 9 volumes of blood were added into 1 volume of 3.8% (w/v) sodium citrate solution as an anticoagulant. Plasma was obtained by centrifugation at 2000 g for 20 min. LDL was isolated by density gradient ultracentrifugation using a method developed by Graham et al. [16] with slight modification using OptiPrep as the density gradient medium. Briefly, 3.2 mL of plasma was mixed with 0.8 mL of OptiPrep (60% iodixanol) to give a final iodixanol concentration of 12% (v/v); 4 mL of this was layered under 4 mL of 6% iodixanol in saline in an 8.9 mL Opti Seal tube [17]. The tube was topped up with saline and ultracentrifuged at 402 000 g at 16°C for 3 h 10 min in a Ti. 70.1 rotor. The subfractions of lipoprotein were labelled as VLDL (very low-density lipoprotein), LDL, GAP (mixture of LDL and HDL), and HDL (high-density lipoprotein). The brightly coloured LDL band was located approximately one-third of the way down the tube and was isolated using a pasteur pipette. LDL was characterized by measuring the amount of protein by the Bradford protein assay using bovine serum albumin as standard [18]. LDL was diluted with phosphate-buffered saline (PBS)(pH 7.4) to a final concentration of 200 μg protein/mL prior to oxidation analysis. The purity of LDL was evaluated by using a UV spectrophotometer as described by Galle and Wanner [19] and agarose gel electrophoresis as described by Noble [20]. The electrophoretic mobility of LDL was measured using agarose gels. Samples were electrophoresed at a constant of 45 mA/gel for 45 min, then oven dried at 85°C and stained with Sudan Black for 20 min.

2.8. Oxidation of LDL. LDL (200 μg protein/mL) was oxidised by exposing it to 10 μM $CuSO_4$ at 37°C for 5 h [17]. This incubation was also carried out in the presence of serial dilutions (5, 2.5, 1.25, 0.63, 0.31, and 0.16 μg/μL) of each sample in DMSO. The oxidation of LDL was terminated by rapid freezing. Samples intended for TBARS (thiobarbituric acid reactive substances) analysis were kept at −20°C for a maximum of 48 h. The methanol extracts and essential oils of *C. xanthorrhiza* and *C. domestica,* isolated curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) and the essential oil standards (xanthorrhizol, ar-turmerone, ar-curcumene, zerumbone, camphor β-curcumene, zingiberene, and geranyl acetate), and probucol (as a positive control) were added to LDL directly before incubation.

2.9. TBARS Assay. The inhibition of copper-catalysed LDL oxidation was determined using TBARS assay [21]. Five μL of the sample was added to a cuvette containing 945 μL of LDL and 50 μL of $CuSO_4$ and incubated at 37°C for 5 h. A mixture containing LDL and $CuSO_4$ was used as control, and blank experiment consisted of LDL and 0.5% DMSO. The total volume of the mixture was 1 mL. The final concentrations of the sample in the mixture were 25.0, 12.5, 6.25, 1.13, 1.56, and 0.78 μg/mL. Further dilutions were carried out for active samples to obtain the final concentrations of 0.39, 0.20, and 0.10 μg/mL. Probucol was used as the positive control in the assay. The final concentration of DMSO in the reaction mixtures was less than 0.5% to eliminate the effect of the solvent on the reaction as evidenced by control experiments. After the incubation, sodium dodecyl sulphate (SDS) and thiobarbituric acid (TBA) were added to the mixture followed by incubation at 95°C for 1 h to increase the peroxidation. The mixture was bathed with ice for 10 min to cool down and stop the peroxidation process. The precipitate formed was removed by centrifugation at 3000 rpm for 15 min. Malondialdehyde (MDA) in the supernatant was determined at 532 nm. TBARS are expressed in terms of MDA equivalents, and the results are expressed as nmoles of MDA/mg LDL protein. MDA standard was used to construct a standard curve [17].

The percentage inhibition of LDL oxidation was calculated as follows:

$$1 - \left(\frac{\text{oxidation of sample}}{\text{oxidation of control}}\right) \times 100. \quad (1)$$

2.10. Statistical Analysis. All the data are presented as means ± standard error median (SEM) from triplicate experiments

and were analysed using *Statistical Package for the Social Sciences* (SPSS) software version 17.0. A one-way analysis of variance (ANOVA) was used for multiple comparison. The concentration of the compounds required to inhibit 50% oxidation (IC_{50}) for active extract was determined using Probit programme. $P < 0.05$ was considered to be statistically significant.

3. Results and Discussion

3.1. Isolation and Identification of Curcuminoids from C. domestica and C. xanthorrhiza. It was found that *Curcuma domestica* has higher levels of total curcuminoids (7.1%) compared to *C. xanthorrhiza* (5.0%). The high percentage of curcuminoids isolated from both plants indicates that they are good sources for the isolation of curcuminoids. Curcumin was found to be the major compound in both species, where its concentration was higher in *C. domestica* (3.6%) than in *C. xanthorrhiza* (2.3%). Bisdemethoxycurcumin was also present at higher concentration in *C. domestica* (2.1%) than in *C. xanthorrhiza* (0.8%), but the latter contained higher amount of demethoxycurcumin (1.9%). The purity of the isolated compounds were confirmed by melting point determination. The structures of the compounds were elucidated by spectroscopic techniques including NMR and MS spectroscopy and confirmed by comparison with the literature values [22].

3.2. Chemical Composition of the Essential Oils. The chemical composition of the essential oils of *Curcuma xanthorrhiza* and *C. domestica* which showed strong inhibitory activity on LDL peroxidation was investigated in an effort to correlate the constituents of the oils and their antioxidant activity. The list of constituents identified in the oils is shown in Table 1 in order of elution on a DB-5 type column. The chemical components of the essential oils of *Curcuma xanthorrhiza* and *C. domestica* have been previously reported by us [4]. A comparison between the oils of the present study, with those reported by us previously showed that more compounds have been identified in the present study and there were some compositional differences and considerable variation in the levels of some individual constituents. The rhizome oil of *C. xanthorrhiza* was characterised by the presence of a high concentration of bisabolene-type sesquiterpenes and their oxygenated derivatives which accounted for more than 92% of the oil. The most abundant component was the sesquiterpene phenol, xanthorrhizol (32%). The other major compounds present in the oil were β-curcumene (17.1%), zingiberene (13.2%), β-bisabolol (3.5%), and ar-curcumene (2.6%). The major components of the rhizome oils of *C. domestica* were ar-turmerone (45.8%), curcumenol (18.2%), and geranyl acetate (2.5%). The chemical composition of the oil was qualitatively similar to those reported by other workers, although there were some variations in the composition and levels of individual constituents of the oils, suggesting the existence of chemical varieties [2, 3, 9]. However, the variations may also be due to environmental factors such as the growth conditions and postharvest handling and processing.

3.3. LDL Antioxidant Activity. The methanol extracts and essential oils of *Curcuma domestica* and *C. xanthorrhiza* were investigated for their ability to inhibit copper-mediated oxidation on isolated human LDL. The methanol extracts of *C. xanthorrhiza* and *C. domestica* showed strong inhibition of LDL oxidation at 6.25 μg/mL, exhibiting greater than 84% inhibition (Table 2). The essential oils of the plants showed weaker activity than their methanol extracts, exhibiting >78% inhibition at 25.0 μg/mL (Table 3). The results demonstrated that the methanol extracts and the oils inhibited copper-mediated oxidation of LDL in a dose-dependent manner; that is, as the concentration of the samples increased, the percentage inhibition of LDL peroxidation increased. The IC_{50} values of the methanol extracts and the oils with LDL antioxidant activity are shown in Tables 2 and 3.

The antioxidant activity of the isolated curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) and the 8 standard compounds (xanthorrhizol, ar-turmerone, ar-curcumene, β-curcumene, zerumbone, zingiberene, camphor, and geranyl acetate) that are the major components of the oils were also investigated. The three curcuminoids showed strong inhibition on LDL peroxidation, with curcumin and demethoxycurcumin showing comparable antioxidant activity and more potent than bisdemethoxycurcumin (Table 2). The three curcuminoids showed strong inhibition on LDL peroxidation with IC_{50} values of 0.15, 0.16, and 0.36 μmol/L, respectively, lower than that of probucol (0.57 μmol/L), a potent inhibitor of copper-catalysed LDL peroxidation [23]. The high antioxidant activity of the three curcuminoids was consistent with previous results obtained using three different bioassay models, that is, the linoleic acid auto-oxidation model, rabbit erythrocyte membrane ghost system, and liver microsome system [7]. The present study was in accordance with previous studies which indicated that the absence of one methoxy group (demethoxycurcumin) on the phenyl ring did not have effect, but the absence of both methoxy groups (bisdemethoxycurcumin) resulted in decreased antioxidant activity in curcuminoids. The phenolic hydroxyl and the methoxyl groups on the phenyl ring and the 1,3-diketone system are important structural features for antioxidant activity [6].

Xanthorrhizol was found to be the most active compound in the oil of *C. xanthorrhiza*, with IC_{50} value of 1.93 μmol/L (Table 3). Ar-turmerone and zerumbone were the compounds in the oil of *C. domestica* that exhibited strong inhibition of LDL peroxidation, with IC_{50} values of 10.18 and 24.90 μmol/L, respectively. The other compounds, ar-curcumene, camphor, and geranyl acetate, showed relatively weak activity. The inhibition of LDL peroxidation by xanthorrhizol, ar-turmerone, and zerumbone was dose dependant. Structure-activity analysis indicated that nonoxygenated bisabolene-type sesquiterpenes exhibited weak antioxidant activity. The strong antioxidant effect of xanthorrhizol is most likely due to the presence of a phenolic hydroxyl group on the bisabolene skeleton. The antioxidant effect appears to be due to the ability of the compounds to chelate Cu^{2+} ion and thus may inhibit the

Table 1: Chemical constituents of the rhizome oils of *Curcuma domestica* and *Curcuma xanthorrhiza*.

Compound	Percentage		RI	Method of identification
	C. domestica	*C. xanthorrhiza*		
α-Thujene	—	tr	931	a, b
α-Pinene	—	0.3	940	a, b, c
Camphene	—	0.7	956	a, b, c
β-Pinene	—	0.1	976	a, b, c
Cis-Pinane	—	0.1	986	a
Myrcene	—	0.1	991	a, b, c
α-Phellandrene	1.1	tr	1009	a, b, c
α-Terpinene	0.2	0.1	1016	a, b, c
p-Cymene	0.2	—	1029	a, b, c
1,8-Cineole	0.3	0.1	1034	a, b, c
(Z)-β-Ocimene	—	0.1	1037	a, b, c
γ-Terpinene	—	tr	1063	a, b, c
Terpinolene	0.3	—	1090	a, b, c
6,7-Epoxymyrcene	—	tr	1093	a
Camphor	—	5.4	1156	a, b, c
Cis-dehydro-β-terpineol	—	0.3	1160	a, b
α-Terpineol	—	0.3	1167	a, b, c
Terpinen-4-ol	—	0.2	1177	a, b, c
Ethyl-4E-octenoate	—	0.1	1187	a
Dihydro citronellol acetate	—	0.1	1321	a
α-Cubebene	—	0.1	1351	a, b
(Z)-β-Damascenone	—	0.1	1364	a
n-Undecanol	—	tr	1370	a, b
Geranyl acetate	2.5	tr	1382	a, b, c
β-Cubebene	—	tr	1388	a, b
β-Elemene	1.5	—	1389	a, b
Methyl perillate	—	0.1	1394	a
(Z)-Isoeugenol	—	0.2	1407	a, b, c
α-Cis-bergamotene	0.9	0.6	1414	a, b
Methyl undecanoate	—	0.1	1428	a
β-Humulene	—	0.1	1439	a, b, c
(Z)-β-Farnesene	—	0.2	1443	a, b
(E)-caryophyllene	0.3	—	1444	a, b, c
γ-elemene	—	0.4	1448	a, b
(E)-β-farnesene	0.2.0	1.2	1457	a, b
α-Humulene	0.1	—	1460	a
(E)-Ethyl cinnamate	—	tr	1467	a, b, c
Ar-Curcumene	1.2	13.2	1493	a, b, c
γ-Curcumene	0.4	2.6	1481	a, b
β-Bisabolene	0.2	0.6	1505	a, b
Zingiberene	1.7	—	1506	a, b, c
(Z)-γ-Bisabolene	—	2.6	1516	a, b
β-Curcumene	0.8	17.1	1523	a, b, c
β-Sesquiphellandrene	1.9	0.4	1538	a, b
1,10-Decanediol	—	0.4	1549	a, b
(Z)-Isoeugenol acetate	0.3	1.2	1567	a, b
Ar-Turmerol	0.3	—	1571	a, b, c
Caryophyllene oxide	0.3	0.5	1581	a, b, c
Thujopsan-2-α-ol	—	0.3	1587	a
Ar-Dihydro-turmerone	1.0	—	1592	a, b

Table 1: Continued.

Compound	Percentage		RI	Method of identification
	C. domestica	*C. xanthorrhiza*		
Sesquithuriferol	—	0.2	1605	a
Geranyl isovalerate	0.3	—	1607	a
1,10-Di-epi-cubenol	—	0.4	1619	a
(*Z*)-Dihydromyrcene-1,6-diol	3.6	—	1621	a, b
10-Epi-γ-eudesmol	0.4	—	1624	a
Citronellyl pentanoate	—	5.7	1626	a, b
1-Epi-cubenol	0.8	—	1629	a
γ-Eudesmol	0.2	—	1632	a, b
Cis-cadin-4-en-7-ol	—	0.8	1637	a
Hinesol	0.4	—	1642	a, b
Cubenol	0.3	0.5	1647	a, b
β-Eudesmol	1.3	—	1651	a, b
α-Eudesmol	—	0.8	1654	a, b
(*E*)-Dihydromyrcene-1,6-diol	1.3	—	1655	a, b
(*E*)-Amyl cinnamic alcohol	—	0.7	1661	a
14-Hydroxy-9-epi-(*E*)-caryophyllene	0.4	—	1670	a
(*E*)-citronellyl tiglate	—	0.9	1668	a
β-Bisabolol	—	3.5	1687	a, b
Ar-Turmerone	45.8	—	1689	a, b, c
Ar-Curcumen-15-al	—	0.8	1712	a
14-Hydroxy-α-humulene	0.2	—	1714	a
1-Phenyl-hepta-1,3,5-triyne	—	0.3	1721	a
Curcumenol	18.2	—	1724	a, b
4-Hydroxy-3-methoxy-cinnamaldehyde	—	0.9	1728	a
Zerumbone	1.4	—	1730	a, b, c
Chamazulene	—	0.3	1732	a
(*E*, *Z*)-Farnesol	—	0.1	1745	a, b
α-Bisabolol oxide A	—	0.2	1749	a
α-Amyl-cinnamyl acetate	0.2	—	1758	a
β-Bisabolen-12-ol	0.3	—	1762	a
γ-Curcumen-15-al	1.1	—	1767	a, b
Xanthorrhizol	—	31.9	1768	a, b, c
γ-Eudesmol acetate	1.6	—	1782	a, b
Butyl dodecanoate	—	0.2	1786	a
8-Cedren-14-ol acetate	0.8	—	1789	a, b
α-Eudesmol acetate	2.3	—	1795	a, b
Total	96.3	97.7		

Percentages were calculated on the basis of results obtained on column DB 5; RI: retention index; a: retention index; b: mass fragmentation; c: co-chromatography with authentic samples.

initiation of LDL oxidation and free radical formation at the lipoprotein [24].

3.4. Correlation between Antioxidant Activity and the Chemical Contents. Based on the results of the LDL antioxidant assays on the methanol extracts, essential oils, isolated compounds, and standard samples, relationships between the antioxidant activity and the chemical contents can be deduced. The results suggest that the strong antioxidant activity of the methanol extracts of the plants is due to the presence of high amounts of curcuminoids. The higher activity of the methanol extract of *C. domestica* (IC_{50}, 0.31 μg/mL) than *C. xanthorrhiza* (IC_{50}, 0.78 μg/mL) could possibly be due to the higher level of curcuminoids in the

TABLE 2: Percentage inhibition and IC_{50} value (μg/mL) of the methanol extracts and curcuminoids of *Curcuma domestica* and *C. xanthorrhiza* on human LDL peroxidation.

Sample	Concentration (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)
Curcuma domestica	6.25	84.14 ± 0.13	0.31 ± 0.01
	3.13	83.77 ± 0.13	
	1.56	73.18 ± 0.78	
	0.78	68.73 ± 0.79	
	0.39	50.45 ± 0.20	
Curcuma xanthorrhiza	12.5	91.94 ± 0.92*	0.78 ± 0.03
	6.25	88.04 ± 0.52	
	3.13	77.82 ± 0.92	
	1.56	62.23 ± 0.13	
	0.78	50.89 ± 1.05	
Demethoxycurcumin	1.56	98.81 ± 0.10*	0.11 ± 0.01 (0.16 μM)
	0.78	93.57 ± 0.56	
	0.39	84.23 ± 1.38	
	0.20	64.42 ± 3.10	
	0.10	47.83 ± 3.25	
Bisdemethoxycurcumin	1.56	93.20 ± 0.59*	0.05 ± 0.01 (0.33 μM)
	0.78	88.71 ± 0.98	
	0.39	80.87 ± 1.67	
	0.20	71.90 ± 2.45	
	0.10	56.01 ± 0.71	
Curcumin	1.56	90.58 ± 0.82*	0.06 ± 0.01 (0.15 μM)
	0.78	89.09 ± 0.95	
	0.39	78.25 ± 1.90	
	0.20	58.15 ± 2.89	
	0.10	50.39 ± 3.43	
Probucol	5.00	76.3 ± 0.6	0.30 ± 0.1 (0.57 μM)
	2.50	74.2 ± 0.2	
	1.25	70.3 ± 0.1	
	0.63	62.1 ± 1.6	
	0.31	48.9 ± 0.1	

Probucol was used as a positive control. Values are presented as mean ± SEM ($n = 3$). $^*P < 0.05$ as compared with the control. IC_{50} values in μM are in parentheses.

former (Table 2). The strong antioxidant activity of the rhizome oil of *C. xanthorrhiza* may be related to the high level of xanthorrhizol (32%), although other constituents may also contribute to the antioxidant activity of the oil (Table 3). Previous studies have indicated that xanthorrhizol strongly inhibited platelet aggregation induced by arachidonic acid, collagen, and ADP [25] and had antimicrobial activity against *Candida* species, filamentous fungi and food-borne pathogens, *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*, (MRSA) [26, 27]. It also nonselectively inhibited DNA, RNA, and protein synthesis and exhibited antiproliferative activity on different cancer cell lines [28]. The presence of high levels of ar-turmerone (45.8%) and zerumbone (1.4%) in the rhizome oil of *C. domestica* could explain its strong inhibition on LDL peroxidation. The strong antioxidant activity of the oil may be possibly due to the synergy between these compounds with the other constituents of the oil. Previous studies have indicated that ar-turmerone isolated from *C. longa* inhibited collagen- and arachidonic-induced platelet aggregation [29], exhibited immunomodulatory activity [30], and induced the apoptotic activity in various cell lines [31]. Antitumor activity of zerumbone isolated from *Zingiber zerumbet* against various cancer cell lines has been widely investigated [32, 33]. Zerumbone has also showed anti-inflammatory and antinociceptive activities [34].

Table 3: Percentage inhibition and IC_{50} value (μg/mL) of the essential oils of *Curcuma domestica* and *C. xanthorrhiza* and the essential oil standards on human LDL peroxidation.

Sample	Concentration (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)
	25	78.8 ± 1.2*	
	12.5	56.4 ± 1.0	
Curcuma domestica	6.25	44.8 ± 0.4	7.8 ± 0.2
	3.13	27.5 ± 1.5	
	1.56	20.0 ± 0.8	
	25	86.9 ± 2.3*	
	12.5	83.2 ± 1.2	
Curcuma xanthorrhiza	6.25	64.0 ± 1.3	2.2 ± 0.1
	3.13	57.8 ± 4.6	
	1.56	49.4 ± 0.2	
	12.5	98.0 ± 0.7*	
	6.25	87.5 ± 2.0	
Xanthorrhizol	3.13	84.5 ± 2.5	0.4 ± 0.1 (1.9 μM)
	1.56	77.5 ± 0.8	
	0.78	64.6 ± 1.7	
	12.5	85.6 ± 0.7*	
	6.25	72.8 ± 1.0	
Ar-Turmerone	3.13	62.2 ± 1.7	2.2 ± 0.1 (10.2 μM)
	1.56	41.7 ± 1.2	
	0.78	24.6 ± 0.8	
	25	69.9 ± 0.3*	
	12.5	58.1 ± 0.3	
Zerumbone	6.25	55.9 ± 0.7	5.4 ± 0.4 (24.9 μM)
	3.13	49.0 ± 0.3	
	1.56	27.6 ± 2.9	
Geranyl acetate	25	26 ± 2.6	
Zingiberene	25	16 ± 1.9	
Ar-Curcumene	25	4.6 ± 1.2	—
β-Curcumene	25	7.8 ± 1.5	
Camphor	25	11.2 ± 0.4	—

Values are presented as mean ± SEM ($n = 3$). *$P < 0.05$ as compared with the control. IC_{50} values in μM are in parentheses.

4. Conclusion

The methanol extracts and essential oils of *C. xanthorrhiza* and *C. domestica* showed high LDL antioxidant activity. The high antioxidant activity of the methanol extracts of the plants could be due to the high amounts of curcuminoids present. The high levels of xanthorrhizol and ar-turmerone in the rhizome oils of *C. xanthorrhiza* and *C. domestica*, respectively, could explain their strong inhibition on LDL peroxidation.

Conflict of Interests

The authors declare that they have no conflict of interests.

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