Acta Pharm. **66** (2016) 387–398 Original research paper

DOI: 10.1515/acph-2016-0028

Bioactive chemical constituents of *Curcuma longa* L. rhizomes extract inhibit the growth of human hepatoma cell line (HepG2)

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Accepted February 21, 2016
Published online May 6, 2016

The present study was designed to identify the chemical constituents of the methanolic extract of *Curcuma longa* L. rhizomes and their inhibitory effect on a hepatoma cell line. The methanolic extract was subjected to GC-MS analysis to identify the volatile constituents and the other part of the same extract was subjected to liquid column chromatographic separation to isolate curcumin. The inhibition of cell growth in the hepatoma cell line and the cytopathological changes were studied. GC-MS analysis showed the presence of fifty compounds in the methanolic extract of *C. longa*. The major compounds were ar-turmerone (20.50 %), β-sesquiphellandrene (5.20 %) and curcumenol (5.11 %). Curcumin was identified using IR, ^{1}H and ^{13}C NMR. The inhibition of cell growth by curcumin (IC_{50} = 41.69 ± 2.87 μg mL^{-1}) was much more effective than that of methanolic extract (IC_{50} = 196.12 ± 5.25 μg mL^{-1}). Degenerative and apoptotic changes were more evident in curcumin-treated hepatoma cells than in those treated with the methanol extract. Antitumor potential of the methanolic extract may be attributed to the presence of sesquiterpenes and phenolic constituents including curcumin (0.051 %, 511.39 μg g^{-1} dried methanol extract) in *C. longa* rhizomes.

Keywords: *Curcuma longa* L., GC-MS analysis, curcumin, anticancer activity

Hepatocellular carcinoma (HCC) has recently acquired growing rates. HCC is responsible for ~600,000 deaths annually worldwide (1, 2). This is related to various biological predisposing factors such as hepatitis B, hepatitis C and liver cirrhosis, all of which can progress into hepatocellular carcinoma (3). Many challenges exist in the current chemotherapeutic treatment approaches, including resistance, low selectivity and high toxic margins of current chemotherapeutics (4). This has called for the need to continuously explore new chemotherapeutics that would hopefully overcome the drawbacks of the currently existing therapy. Hence, a growing interest in exploring various natural herb products as an innovative therapeutic approach began to arise (5). Natural dietary agents including

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fruits, vegetables and spices have drawn attention of the scientific community due to their demonstrated ability to suppress cancers (6, 7). Many reports have shown that dietary agents and their active principles exerted cytotoxicity toward cancer cells (8).

Curcuma (family *Zingiberaceae*) is a genus containing 70 known species that has been historically used as a spice, food preservative and coloring material. *Curcuma longa* L. is distributed throughout tropical and subtropical regions of the world. It is used in traditional medicine as a household remedy for various diseases. Also, it has been reported that *C. longa* possesses multiple pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, anticoagulant, antidiabetic and immunological ones (8, 9).

Terpenoids make a diverse class of pharmacologically active phytochemicals, including aromatic, non-aromatic, volatile and non-volatile constituents, which play a key role in traditional herbal remedies (10, 11). Among them, curcuminoids (diphenylheptanoids) include curcumin and its derivatives, demethoxycurcumin and bisdemethoxycurcumin being the major bioactive compounds in *C. longa*. Curcuminoids are yellow natural phenols that have antioxidant, anticancer, anti-inflammatory and chemotherapeutic activities (12, 13).

The main objectives of the present work were to identify bioactive chemical constituents of the crude methanolic extract of *C. longa* rhizomes using GC-MS, to isolate the major bioactive compound from the plant extract and to evaluate the potential of crude methanolic extract and curcumin on inhibition of the hepatoma cell line (HepG2) growth.

EXPERIMENTAL

General experimental procedures

Melting points were determined with a melting point apparatus SMP3 (Barloworld Scientific Ltd., UK). ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) spectra were recorded using Bruker 500 MHz (Bruker UK Ltd, UK) and JEOL-GX 270 spectrometers (Akishima, Japan), resp. Chemical shifts were expressed in δ (ppm) as a reference TMS and coupling constant (J) in Hertz. UV spectra were determined in methanol with a UV Spectronic 601 spectrophotometer (Milton Roy, USA). IR spectra (cm^{-1}) were recorded on a Perkin-Elmer 1600 spectrometer (Perkin-Elmer, USA). Silica gel 60 GF_{254} (Fluka, Switzerland) was used for analytical TLC. Sephadex LH-20 (25–100 μm, Sigma, USA) or silica gel (0.063–0.210 mm, Merck, Germany) were used for column chromatography. Spots were visualized by spraying with ethanolic $AlCl_3$ (2 %) or 10 % H_2SO_4 under UV.

Plant material

Dried rhizomes of *Curcuma longa* were purchased from a local herbal shop (Cairo, Egypt). The plant sample was identified and plant rhizomes were ground to fine powder with an electric mill for the extraction process.

Extraction and fractionation of C. longa *rhizomes*

Dried powder of *C. longa* rhizomes was extracted with 80 % methanol and then evaporated under reduced pressure using rotatory evaporator. Dried methanolic extract was

defatted with petroleum ether and then fractionated with ethyl acetate (EtOAc) and evaporated till dryness under reduced pressure. Crude methanolic extract and ethyl acetate fraction was kept away from moisture.

Phytochemical screening

Preliminary phytochemical screening of the crude methanolic extract of *C. longa* rhizomes was carried out to estimate the amount of flavonoids, phenols, tannins, alkaloids, saponins, terpenoids and steroids according to the standard procedures (14, 15).

Gas chromatography-mass spectrometry (GC-MS) analysis

The crude methanolic extract of *C. longa* was diluted with methanol (1/100, *V*/*V*) and filtered. The particle-free diluted crude extract (1 μL) was taken in a syringe and injected into a GC-MS instrument with a split ratio 1:100. The analysis was carried out using a GC (Agilent 7890A, Agilent Technologies, USA) interfaced with a mass-selective detector (MSD, Agilent 7000, Agilent Technologies) equipped with a polar Agilent HP-5ms (5 %-phenyl methyl polysiloxane) capillary column (30 m × 0.25 mm i. d., 0.25-μm film thickness). The carrier gas was helium with the linear velocity of 1 mL min^{-1} at 210 °C. Identification of chemical components was based on computer matching with NIST and WILEY libraries, as well as on comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (16).

Chromatographic isolation of curcumin

Ethyl acetate fraction of *C. longa* was subjected to column chromatography (120×4 cm) packed with silica gel 60 (0.063–0.210 mm, Merck) as a stationary phase. Elution started with $CHCl_3$, followed by $CHCl_3$/MeOH and ended with pure methanol. Fractions were collected, concentrated and examined by TLC (silica gel, solvent system $CHCl_3$/MeOH, 19:1) and by PC (Whatman No. 1, solvent 15 % acetic acid). Chromatograms were visualized under UV before and after spraying with $AlCl_3$.

The major fraction was collected and re-chromatographed on another silica sub-column. Elution started with chloroform, mixtures of chloroform and methanol, ending with pure methanol. The sub-column fractions were monitored on a thin layer chromatography plate and using the solvent system $CHCl_3$/MeOH (19:1). TLC were visualized by spraying with 10 % H_2SO_4 followed by heating at 110 °C. Fractions of similar chemical composition were collected and concentrated. The resulting fractions were further purified on a Sephadex LH-20 column using H_2O/MeOH (3:7). The major compound was purified on preparative TLC using $CHCl_3$/MeOH (19:1) and crystallized with chloroform as orange powder.

High performance liquid chromatography (HPLC) analysis

Samples of *C. longa* (MeOH extract, EtOAc extract and curcumin) were dissolved in 1 mL of HPLC grade MeOH, filtered using a filter membrane (pore size 0.45 μm, Phenex, USA) and centrifuged for 5 min at 6000 rpm before HPLC analysis.

Samples were analyzed on a Shimadzu LC-8A liquid chromatography system (Shimadzu) with LC solution software, SPD-M20A photodiode array detector (Shimadzu). A RESTEK (5 μm) C18 column was used (4.6 mm i.d. × 150 mm). Elution was carried out with a gradient solvent system at a flow rate of 1 mL min^{-1}. The mobile phase consisted of acetonitrile (B) and 1 % formic acid in water (A). The LC time program was as follows: 5–20 % B (10 min), 20–30 % B (10 min), 30–50 % B (10 min), 50–98 % B (20 min), 98–98 % B (5 min) and 98–5 % B (5 min). The sample was injected into a volume of 20 μL and the eluate was monitored at 210 and 425 nm for detection of major chemical constituents in *C. longa* extracts.

The concentration of curcumin was calculated using the calibration curve of isolated curcumin with concentration ranging from 75 to 1000 μg mL^{-1}. Regression coefficient for the curcumin calibration curve was 0.992 within this concentration range. The concentration of curcumin was expressed as μg g^{-1} in dry MeOH extract.

Cell culture

Human hepatocellular carcinoma HepG2 cell line (kindly provided by the Biology Department of the American University in Cairo, Egypt) was used in testing the anticancer activity of the methanolic extract of *C. longa* and curcumin. Briefly, HepG2 cells were maintained in 75-cm^3 culture flasks (Greiner bio-one GmbH, Germany) containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 4.5 g L^{-1} glucose and 10 g L^{-1} L-glutamine (Bio Whittaker, Combrex Company, Belgium), 100 mL L^{-1} fetal calf serum (FCS), 10 g L^{-1} penicillin/streptomycin and 1 g L^{-1} fungizone (250 mg L^{-1}, Gibco-BRL Life Technologies, USA). The HepG2 cell line was cultured at 37 °C in a 5 % CO_2 incubator for 72 hours. When the growth was in the confluence phase, the cell line was resuspensed from the culture flask by 0.025 % trypsin (Sigma) and the culture flask was gently shaken to ensure that a single-cell suspension was obtained. Complete culture medium was renewed every 3 days and cells were passaged every 6–10 days. The exact cell count was recorded in 50 μL aliquots after mixing with an equal volume of trypan blue (5 g L^{-1}, Biochrom KG, Berlin, Germany). The cells were counted using a Neubauer hemocytometer (Marienfeld, Germany). Cell viability was tested by the trypan blue dye exclusion method (17).

Neutral red cytotoxicity assay

Neutral red cytotoxicity assay based on the initial protocol described by *Repetto et al.* (18) was carried out. Cells from the mother flask were seeded in a 24-well microtitre plate (Corning, USA) (1×10^6 cells per well). The plates were incubated at 37 °C in 5 % CO_2 for 24 h to achieve monolayer confluence. Culture media containing different concentrations of the methanolic extract of *C. longa* and curcumin (15, 30, 60, 125, 250, 500, 1000 and 2000 μg mL^{-1}) were added in triplicate. Medium without a sample acted as untreated control. The dye-medium was removed and the plates were washed with PBS (pH = 7.4). Five hundred μL of acetic acid-ethanol (1 mL glacial acetic acid in 100 mL 50 % ethanol) was added and the plates were kept for 15 min at room temperature to extract the dye. They were then shaken for a few seconds so that complete dissolution was achieved. The absorbance of the extracted dye was measured using a 540 nm filter (Spectra max 190 Microplate Reader, Molecular Devices, USA). The inhibition percentage was calculated.

Cytopathological diagnosis

The same population of cells was trypsinized and washed with phosphate buffered saline (pH = 7.4) and collected into a centrifuge tube. Collected sample was centrifuged at a rate of 1200–1500 rpm for 15 minutes using Shandon Cytospin (Thermo Fisher Scientific, USA). The sediment was smeared on slides pretreated with 3-amino-propyl-triethoxysaline (3-APTES, Sigma-Aldrich Ireland Ltd, Ireland). Slides were fixed immediately in 95 % ethanol for 24 hours and then stained with hematoxylin and eosin.

Data analysis

Experiments were carried out in triplicate and the data were presented as a mean ± standard error of the mean (SEM). Dose-response curves were plotted, and 50 % inhibitory concentration (IC_{50}) was calculated using the Statistical Package for the Social Sciences (SPSS) for Windows 13.0.

RESULTS AND DICUSSION

Preliminary phytochemical screening

Preliminary phytochemical screening of the crude methanolic extract of *C. longa* rhizomes revealed the presence of a large amount of flavonoids, phenols and terpenoids. Also, a moderate amount of tannins, alkaloids, saponins and steroids was detected. On the other hand, Pawar *et al.* (19) and Gupta *et al.* (20) recently reported that terpenoids and curcuminoid compounds represent major components in the *Curcuma* species.

GC-MS analysis of C. longa *rhizome methanolic extract*

GC-MS analysis of the methanolic extract of *C. longa* showed the major chemical constituents shown in Table I and Figs. 1 and 3. Identification of the components of the extract was carried out based on their retention time (t_R), molecular formula (MF), relative molecular mass (M_r) concentration (%) and mass fragmentation pattern. Our data showed a total of 50 compounds identified in the volatile part of methanolic extract. Major compounds (in %) were ar-turmerone (20.50), β-sesquiphellandrene (5.20), curcuminol (5.11), 2-methoxy-4-vinylphenol (4.32), *cis*-β-elemenone (3.98), isolongifolol (2.83), geranyl-*p*-cumene (2.77), β-turmerone (2.58), β-curcumene (2.50), β-vatirenene (2.37), β-caryophyllene (2.27), α-curcumene (2.24), furanodiene (2.23), γ-curcumene (2.22), α-turmerone (1.84) and α-cedrene (1.80). These results revealed that the methanolic extract of *C. longa* rhizomes contains a mixture of compounds, especially sesquiterpene compounds such as germacrone, furanodiene, curcumene, turmerone, cedrene, curlone and β-elemene. Many of these compounds were major volatile components in essential oils and natural phytochemicals of *C. longa* known to have many biological activities (21, 22).

Structure elucidation of curcumin

Curcumin was isolated by liquid column chromatography and proved to be water insoluble and methanol soluble [m.p. 185–187 °C (lit. m.p. 184–186 °C); IR ν_{max}: 3425 (OH),

Table I. GC-MS analysis of methanolic extract of C. longa

Compd.	t_R	M_r	MF	Peak area (%)	Compound name
1	3.70	134	$C_{10}H_{14}$	0.29	*o*-cymene
2	4.22	194	$C_{12}H_{18}O_2$	1.58	*L-trans*-chrysanthenyl acetate
3	5.63	90	$C_4H_{10}O_2$	0.51	*DL*-2,3-butanediol
4	5.78	166	$C_{11}H_{18}O$	0.83	5-isopropenyl-1,2-dimethylcyclohexan-2-enol
5	8.73	134	$C_{10}H_{14}$	0.30	*p*-cymene
6	9.59	156	$C_{10}H_{20}O$	0.19	(2,4,6-triethylcyclohexyl)methanol
7	10.70	152	$C_{10}H_{16}O$	0.32	α-thujone
8	10.95	150	$C_{11}H_{18}$	0.19	2-ethenyl-1,1-dimenthyl-3-methylene-cyclohexane
9	11.11	152	$C_{10}H_{16}O$	0.47	*cis*-sabinol
10	11.64	152	$C_{10}H_{16}O$	0.68	*cis-p*-menth-2,8-dienol
11	12.06	150	$C_{10}H_{14}O$	0.23	2-isopropylidene-3-methylhexa-3,5-dienal
12	12.19	150	$C_{10}H_{14}O$	0.25	thymol
13	12.39	150	$C_9H_{10}O_2$	4.32	2-methoxy-4-vinyl phenol
14	12.86	164	$C_{10}H_{12}O_2$	0.26	*m*-eugenol
15	12.97	120	C_9H_{12}	0.35	hemellitol
16	13.05	176	$C_{13}H_{20}$	1.17	benzene-2-methyl-1,4-bis(1-methylethyl)
17	13.22	204	$C_{15}H_{24}$	2.50	β-curcumene
18	13.33	204	$C_{15}H_{24}$	1.80	α-cedrene
19	13.47	202	$C_{15}H_{22}$	2.22	γ-curcumene
20	13.54	204	$C_{15}H_{24}$	2.27	β-caryophyllene
21	13.63	202	$C_{15}H_{22}$	1.20	ar-curcumene
22	13.71	204	$C_{15}H_{24}$	0.31	β-himachalene
23	13.75	202	$C_{15}H_{22}$	2.24	α-curcumene
24	13.91	204	$C_{15}H_{24}$	0.29	β-elemene
25	14.07	204	$C_{15}H_{24}$	1.92	α-cubebene
26	14.18	204	$C_{15}H_{24}$	2.80	β-cedrene
27	14.32	204	$C_{15}H_{24}$	2.63	β-bisabolene
28	14.50	204	$C_{15}H_{24}$	5.20	β-sesquiphellandrene
29	14.67	220	$C_{15}H_{24}O$	0.19	*cis-Z*-α-bisabolene epoxide
30	14.77	202	$C_{15}H_{22}$	2.37	β-vatirenene
31	14.88	222	$C_{15}H_{26}O$	0.53	agarospirol
32	14.95	218	$C_{15}H_{22}O$	2.58	β-turmerone
33	15.02	218	$C_{15}H_{22}O$	0.29	curlone
34	15.19	218	$C_{15}H_{22}O$	1.90	germacrone
35	15.31	218	$C_{15}H_{22}O$	1.84	α-turmerone
36	15.38	216	$C_{15}H_{20}O$	1.55	curzerene
37	15.50	234	$C_{15}H_{22}O_2$	0.21	dihydrocostunolide
38	15.63	216	$C_{15}H_{20}O$	2.23	furanodiene

39	16.02	216	$C_{15}H_{20}O$	20.50	Ar-turmerone
40	16.17	234	$C_{15}H_{22}O_2$	1.07	isocurcumenol
41	16.26	236	$C_{15}H_{24}O_2$	0.87	curdione
42	16.34	234	$C_{15}H_{22}O_2$	5.11	curcumenol
43	16.52	222	$C_{15}H_{26}O$	1.27	7-epi-*cis*-sesquisabinene hydrate
44	16.65	222	$C_{15}H_{26}O$	2.83	(–)-isolongifolol
45	16.78	242	$C_{18}H_{26}$	2.77	geranyl-*p*-cumene
46	16.89	218	$C_{15}H_{22}O$	3.98	*cis*-β-elemenone
47	16.97	220	$C_{15}H_{24}O$	1.78	isoshyobunone
48	17.19	234	$C_{15}H_{22}O_2$	0.17	dihydrocostunolide
49	17.17	232	$C_{15}H_{20}O_2$	1.72	dehydrosaussurea lactone
50	17.24	236	$C_{15}H_{24}O_2$	1.84	corymbolone

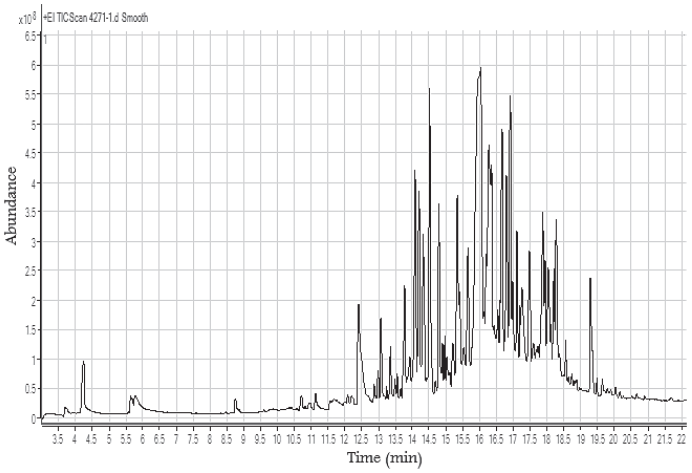

Fig. 1. GC-MS chromatogram of *C. longa* rhizomes methanolic extract. The most abundant peaks: 2-methoxy-4-vinylphenol (t_R = 12.39 min), β-sesquiphellandrene (t_R = 14.50 min), ar-turmerone (t_R = 16.02 min), curcuminol (t_R = 16.34 min), *cis*-β-elemenone (t_R = 16.89 min)

2927, 2853, 1627, 1600, 1508, 1464, 1427, 1275, 1202, 1182, 1105, 965 cm^{-1}; ^1H NMR (DMSO): 9.68, 7.53 (*d, J* = 15.8 Hz, 2H), 7.29 (*brs*, 2H), 7.11 (*dd, J* = 8.0 Hz, 2H), 6.79 (*d, J* = 8.0 Hz, 2H), 6.74 (*d, J* = 15.7 Hz, 2H), 3.94 ppm (*s*, 6H) ppm; ^{13}C NMR (DMSO, 500 MHz): 183.74, 149.88, 148.52, 141.24, 126.86, 123.65, 121.61, 116.23, 111.86, 101.59, 56.20 ppm].

IR spectrum indicated the presence of a hydroxyl group at 3425 cm^{-1}, a carbonyl group at 1627 cm^{-1} and two bands at 1524 and 1509 cm^{-1} for aromatic double bonds (C=C). The ^1H NMR spectrum data of this compound exhibited two broad singlets at δ 6.79 and 7.53 ppm assigned to H-3/H-3′ and H-4/H-4′ protons, respectively. The *ortho*-coupled protons of the aromatic ring appeared as doublets at δ 6.79 and 7.11 ppm being assignable to H-9/H-9′ and H-10/H-10′, respectively. A singlet corresponding to two methoxy groups (OCH_3) was observed at δ 3.94 ppm. This was confirmed by the presence of a signal at δ 56.20 ppm in the

^{13}C NMR spectrum. Also, the characteristic signals of other carbons of curcumin were observed in ^{13}C NMR at 101.59 (C-1), 111.86 (C-6), 116.23 (C-9), 121.61 (C-3), 123.65 (C-10), 126.86 (C-5), 141.24 (C-4), 148.52 (C-7), 149.88 (C-8) and 183.74 (C-2) ppm. Therefore, this compound was characterized as curcumin [1,7-*bis*(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], as shown in Fig. 3 (23, 24).

HPLC-DAD fingerprint of C. longa *extracts and pure curcumin*

HPLC-DAD analysis of crude methanolic extract, ethyl acetate fraction and pure curcumin was performed at two wavelengths, 210 and 425 nm, as shown in Fig. 2. The methanolic extract exhibited several peaks at 210 nm, which revealed that it contained many phenolic compounds whereas three major peaks assigned to curcuminoid group

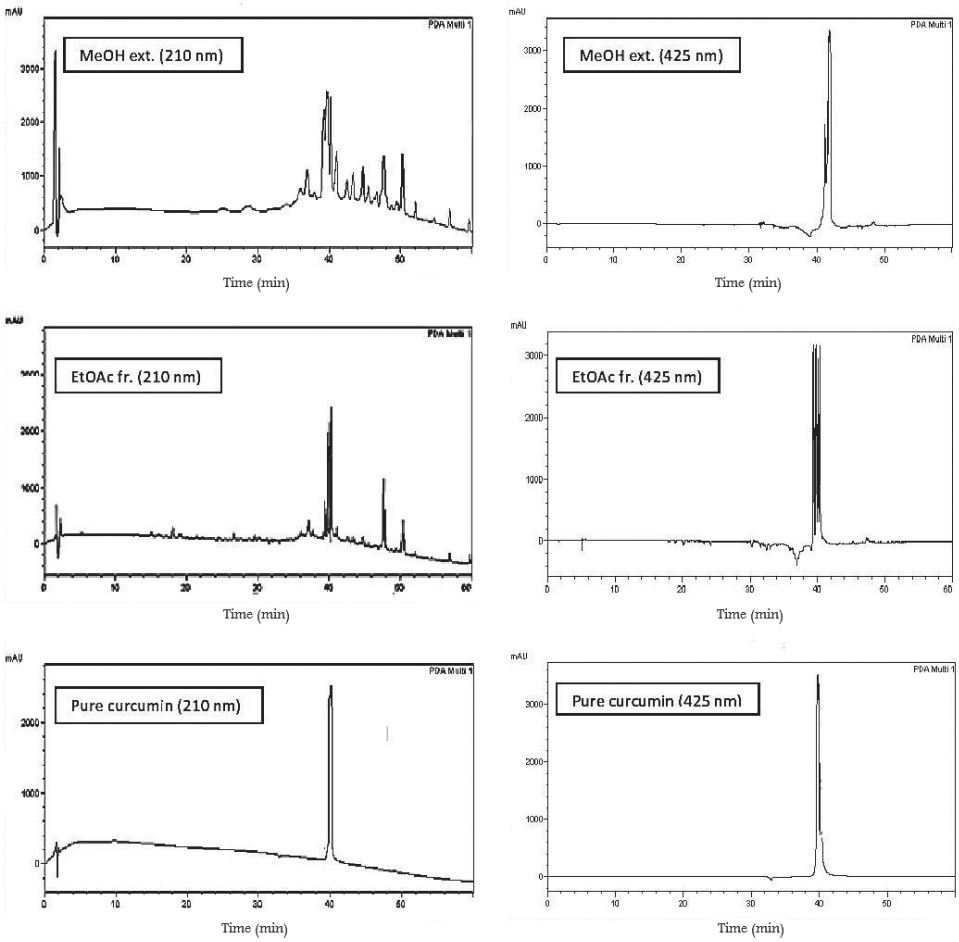

Fig. 2. HPLC chromatogram of methanolic extract, ethyl acetate fraction and pure curcumin.

appeared at 425 nm with 40–42 min retention time in MeOH and EtOAc extracts. On the other hand, pure curcumin appeared at t_R of 40 min with a concentration of 0.051 % (511.39 μg g^{-1}) in dry methanolic extract of *C. longa* rhizomes. These results are in full agreement with the previous studies, which reported that curcuminoids of curcuma species can be defined as phenolic compounds, including three compounds, namely curcumin (60–80 %), desmethoxycurcumin (15–30 %) and bisdesmethoxycurcumin (2–6 %) (24, 25).

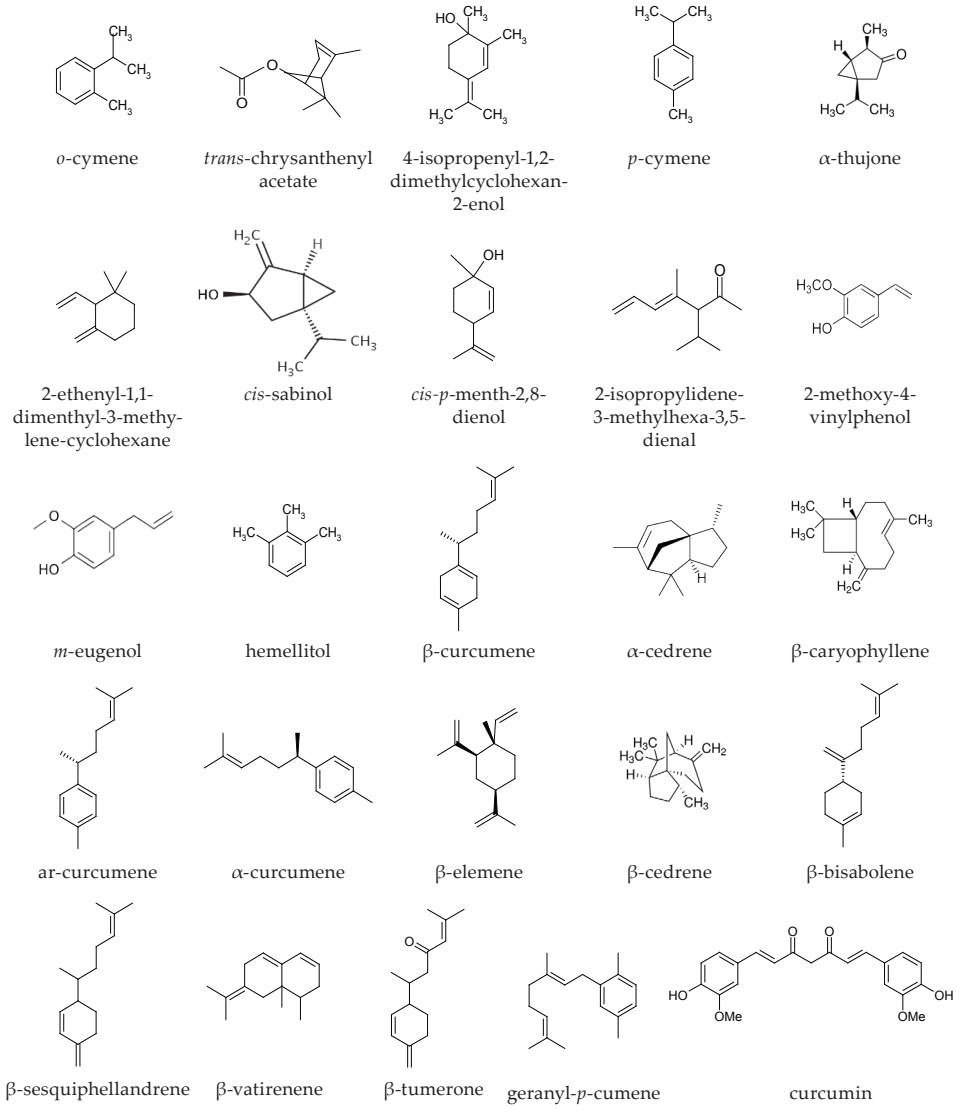

Fig. 3. Structures of some compounds identified in *C. longa* rhizomes methanolic extract.

Cytotoxicity assay and cytopathological changes

The present results show that both the methanolic extract of *C. longa* and curcumin itself induced cytotoxicity in the HepG2 cell line in a concentration dependent manner. The percentage of inhibition of cell viability of treated groups relative to untreated control is shown in Fig. 4. As expected, the IC_{50} value of pure curcumin was 41.69 ± 2.87 μg mL^{-1}, while the analogous value for methanolic extract of *C. longa* rhizomes was 196.12 ± 3.98 μg mL^{-1}.

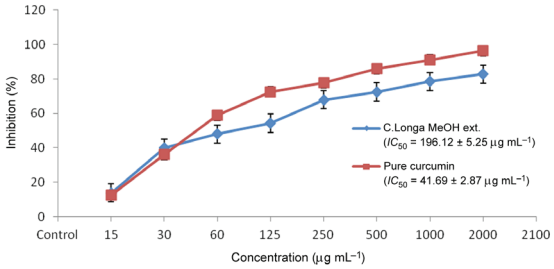

Fig. 4. Percentage inhibition of proliferation of HepG2 cell line for curcumin and methanolic extract of *C. longa* rhizomes (mean ± SD, n = 3).

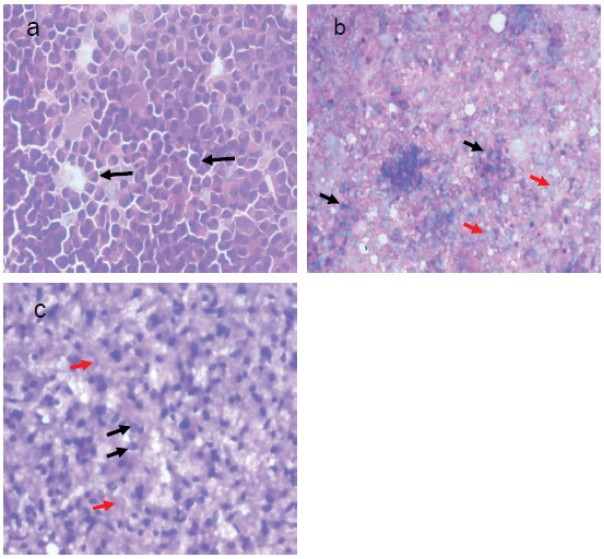

Fig. 5. Cytospin smear stained with H&E (× 400) prepared from: a) untreated control HepG2 cells showing a large number of malignant hepatocytes forming sheets, clusters of cells with enlarged nuclei and increased nucleocytoplasmic ratio (arrow), b) HepG2 cell treated with pure curcumin showing a large number of small shrinking hepatocytes with central nuclei and with scattered apoptotic changes (black arrow) with scattered (few) malignant hepatocytes (red/grey arrow). c) HepG2 cell treatment with *C. longa* methanolic extract showing a moderate number of small shrinking hepatocytes with central nuclei and with scattered apoptotic changes (red/grey arrow) with a small number of malignant hepatocytes (black arrow).

Concurrently, the cytotoxicity findings were confirmed by cytopathological changes in cytospin smear on the same cell population. Examination of smears prepared from HepG2 cells without treatment showed, using a light microscope, a large number of malignant polyhedrally-shaped hepatocytes forming acini and clusters of cells with enlarged nuclei and increased nucleocytoplasmic ratio.

HepG2 cell line cells treated with pure curcumin showed moderate to marked number of apoptotic cells; apoptotic changes appeared in the form of reduction in cell volume, cell shrinkage, reduction in chromatin condensation and formation of numerous clear cytoplasmic vacuoles and blebs. In addition, structural alterations of the degenerated cells with necrotic debris were observed, which were not seen in untreated HepG2 cells. Hepatoma cell line cells (HepG2) treated with the *C. longa* methanol extract showed a slight to moderate number of apoptotic cells, and degenerated cells as shown in Fig. 5.

Thus, *in vitro* cytotoxic activities induced by the *C. longa* methanolic extract and curcumin shown though cytopathological changes in the same cell population were comparable. The methanolic extract of *C. longa* induced inhibition of cell growth as well as apoptotic changes in the HepG2 cell line, which were attributed to the presence of multiple bioactive sesquitepenoidal and phenolic compounds in this extract. The isolated curcumin showed the same pattern of changes.

CONCLUSIONS

The results presented herein show that the methanolic extract of *C. longa* rhizomes contains a mixture of compounds especially sesquiterpenes and curcuminoids (especially curcumin) as major bioactive compounds. They exerted anticancer potential and induced inhibition of cell growth *via* apoptotic changes. Hence, the rhizomes of *C. longa* can be used as a natural source of anticancer substances and can serve as a source of quality raw material for pharmaceutical industries. This contributes to the scientific evidence for the use of this medicinal plant in traditional medicine.

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