Phytochemical and Biological Investigations of *Curcuma longa*

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ABSTRACT: Seven compounds, turmeronol-A (**1**), turmeronol-B (**2**), 3,4-dimethoxycinnamic acid (**3**), 4-hydroxy-3-methoxycinnamic acid (**4**), 4-hydroxybenzaldehyde (**5**), 2,3,5,6-tetrahydroxyarturmerone (**6**) and 4-hydroxy-bisabola-2,10-diene-9-one (**7**) have been isolated from the carbon tetrachloride soluble fraction of a methanol extract of the rhizomes of *Curcuma longa*. Careful interpretation of the NMR data and comparison with turmeronol A (**1**) allowed tentative identification of compound **6** as 2,3,5,6-tetrahydroxy-arturmerone, which appears to be a new molecule. The *n*-hexane, carbon tetrachloride and chloroform extractives of the rhizomes when subjected to antimicrobial screening and brine shrimp lethality bioassay demonstrated mild to moderate antimicrobial activity and strong cytotoxicity with LC_{50} value 1.56 μg/ml.

Key words: *Curcuma longa*, Zingiberaceae, turmeronol-A, turmeronol-B, 2,3,5,6-tetrahydroxy-arturmerone and 4-hydroxybisabola-2,10-diene-9-one, antimicrobial, cytotoxicity.

INTRODUCTION

Curcuma longa (Family- Zingiberaceae, Bengali name- Halud) is a perennial herb with pulpy, orange, tuberous roots that grows to about 2 feet in length and is cultivated extensively in India, China, Bangladesh and other Asian countries with a tropical climate. The isolated compound, curcumin, has been reported to show anti-inflammatory, antioxidant and chemopreventive activities.[1] Turmerin, a protein from *C. longa*, is known to exhibit cytotoxicity and myotoxicity of multitoxic phospholipase A_2.[2] Several sesquiterpenes such as wenyujinlactone A, neolitamone A, zedoarondiol, isozedoarondiol, aerugidiol, curcumol, curdione, (1R,10R)-epoxy-(-)-1, 10-dihydrocurdine[3] and parviflorene F[4] and some curcuminoids such as curcumin, demethoxycurcumin and bisdemethoxycurcumin have been reported from the genus *Curcuma*.[5]

We, herein, describe the re-isolation of turmeronol-A (**1**), turmeronol-B (**2**) and 4-hydroxybisabola-2,10-diene-9-one (**7**) and first report of 3,4-dimethoxycinnamic acid (**3**), 4-hydroxy-3-methoxycinnamic acid (**4**), 4-hydroxybenzaldehyde (**5**) and 2,3,5,6-tetrahydroxy-arturmerone (**6**) from a carbon tetrachloride soluble fractions of methanol extract of *C. longa*.

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MATERIALS AND METHODS

General experimental procedure. The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument. The ^{13}C NMR spectra were obtained on the same instrument at 100 MHz. For NMR studies deuterated chloroform was used as solvent and the δ values for ^{1}H and ^{13}C spectra were referenced to the residual non-deuterated solvent signals.

Plant material. Rhizomes of *C. longa* were collected from Savar, Dhaka in January 2004. A voucher specimen for this collection has been deposited in the herbarium of the Department of Botany, University of Dhaka.

Extraction and isolation. The air dried and powdered rhizome (533 g) of *C. longa* was soaked in 2.5 L of methanol for 7 days accompanying occasional shaking and stirring. The whole mixture was then filtered through a cotton plug followed by Whatman number 1 filter paper and the filtrate thus obtained was concentrated at 40°C with a rotary evaporator. A portion (5.0 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method[6] which afforded of *n*-hexane (515.0 mg), carbon tetrachloride (1.10 g), chloroform (1.25 g) and aqueous soluble fractions (2.00 g).

An aliquot (550.0 mg) of the carbon tetrachloride soluble fraction was subjected to column chromatography over silica gel (Kiesel gel 60H, mesh 70-230) and the column was eluted with *n*-hexane followed by mixtures of *n*-hexane and ethyl acetate in order of increasing polarities. A total of 128 test tubes were collected, each 20 ml. Preparative thin layer chromatography (PTLC) of column fraction 34 over silica gel (Silica gel PF_{254}) using 12.5% ethyl acetate in toluene gave compound **1**. Similar purification of column fraction 25 using 4% ethyl acetate in toluene afforded compound **2**. On the other hand, PTLC of column fraction 100 with 30% ethyl acetate in toluene yielded compound **3** while similar treatment of fraction 86 using 25% ethyl acetate in toluene provided compound **4**. Similarly, PTLC of column fraction 7 with 7% ethyl acetate in toluene provided compounds **5**, **6** and **7**.

Turmeronol-A (1). Amorphous white powder, 2.5mg (0.05% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (1H, *d, J*=8.0 Hz, H-6), 6.69 (1H, *dd, J*=8.0 Hz, 1.8 Hz, H-5), 6.64 (1H, *d*, *J*=1.8 Hz, H-3), 6.01 (1H, *s*, H-12), 3.23 (1H, m, H-8), 2.69 (1H, *dd*, *J*=15.7 Hz, 8.0 Hz, H_b-10), 2.58 (1H, *dd*, *J*=15.7 Hz, 6.0 Hz, H_a-10), 2.19 (3H, *s*, H-7), 2.09 (3H, br. *s*, H-14), 1.84 (3H, br. *s*, H-15), 1.22 (3H, *d*, *J*=6.9 Hz, H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ 121.3 (C-1), 155.2 (C-2), 113.5 (C-3), 146.0 (C-4), 118.8 (C-5), 131.0 (C-6), 15.3 (C-7), 35.3 (C-8), 22.0 (C-9), 52.6 (C-10), 200.0 (C-11), 124.1 (C-12), 153.7 (C-13), 27.7 (C-14), 20.8 (C-15).

Turmeronol-B (2). White amorphous powder, 3.0 mg (0.06% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (1H, *d*, *J*=8.0 Hz, H-5), 6.73 (1H, br.*s*, H-2), 6.70 (1H, br.*d*, *J*=8.0 Hz, H-6), 6.01 (1H, br.*s*, H-12), 3.55 (1H, m, H-8), 2.29 (2H, br.*d, J*=6.7 Hz, H_a-10 & H_b-10), 2.19 (3H, *s*, H-7), 2.10 (3H, *s*, H-14), 1.84 (3H, *s*, H-15) and 1.22 (3H, *d*, *J*=6.8 Hz, H-9).

3,4-Dimethoxycinnamic acid (3). Amorphous white powder, 2.0 mg (0.04% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (1H, *d*, *J* = 16.0 Hz, H-7), 7.08 (1H, br.*d*, *J*=8.0 Hz, H-6), 7.02 (1H, br.*s,* H-2), 6.90 (1H, *d*, *J*=8.0 Hz, H-5), 6.28 (1H, *d, J* = 16.0 Hz, H-8), 3.92 (3H, *s*, OCH_3-3), 3.77 (3H, *s*, OCH_3-4).

4-Hydroxy-3-methoxycinnamic acid (4): Yellow gum, 4.0 mg (0.08% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (1H, *d*, *J*=15.7 Hz, H-7), 7.42 (1H, *d*, *J* = 8.1 Hz, H-6), 7.04 (1H, br.*s*, H-2), 6.92 (1H, *d*, *J* = 8.1 Hz, H-5), 6.47 (1H, *d*, *J*=15.7 Hz, H-8), 5.87 (1H, br.*s*, OH-4), 3.94 (3H, *s*, OCH_3-3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 127.7 (C-1), 147.9 (C-3), 146.8 (C-4), 140.5 (C-7), 183.3 (C-9), 55.9 (C-10).

4-Hydroxybenzaldehyde (5). Pale brown powder, 3.5 mg (0.07% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.96 (1H, *s*, CHO-1), 7.80 (2H, *d*, *J*=8.0 Hz, H-2), 7.80 (2H, *d*, *J*=8.0 Hz, H-6),7.38 (2H, *d*, *J*=8.0 Hz, H-3), 7.38 (2H, *d*, *J*=8.0 Hz, H-5), 6.00 (1H, br.*s*, OH-4).

2,3,5,6-Tetrahydroxyarturmerone (6). Amorphous white powder, 3.0 mg (0.06% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.17 (1H, br.*s*, H-12), 3.44 (1H, m, H-8), 2.85 (1H, *dd*, *J*=15.7 Hz, 6.0 Hz, H_a-10), 2.75 (1H, *dd*, *J*=15.7 Hz, 8.3 Hz, H_b-10), 2.45 (3H, *s*, H_3-7), 2.25 (3H, *s*, H_3-14), 2.00 (3H, *s*, H_3-15), 1.40 (3H, *d*, *J*=6.90 Hz, H_3-9).

4-Hydroxybisabola-2,10-dien-9-one (7). Colorless gum, 2.8 mg (0.056% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.81 (1H, *d*, *J*=10.5 Hz, H-2), 6.06 (1H, *s*, H-10), 6.02 (1H, *d*, *J*=10.5 Hz, H-3), 2.48 (2H, *s*, H_2-8), 2.32 (2H, *s*, H_2-5), 2.32 (2H, *s*, H_2-6), 2.15 (3H, *s*, H_3-12), 1.92 (1H, *s*, H-1), 1.89 (3H, *s*, H_3-13), 1.60 (1H, *s*, H-7), 1.24 (3H, *s*, H_3-15), 0.90 (3H, *d*, *J*=6.5 Hz, H_3-14).

Bioassays. The preliminary antimicrobial activity of the extractives was determined at 400 µg/disc by the disc diffusion method[7]against a number of Gram positive and Gram negative bacteria and fungi (Table 1). The bacterial and fungal strains used in this experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Here, standard Kanamycin (30 µg) disc was used as the reference. For cytotoxicity screening, DMSO solutions of all the extractives, *n*-hexane (HSF), carbon tetrachloride (CTSF), chloroform (CFSF) fraction and methanol extract (MEWP)) were applied against *Artemia salina* in a one-day in *vitro* assay.[8,9] For the experiment, 4 mg of each of the Kupchan fractions was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.78125 µg/ml were obtained by serial dilution technique. Vincristine sulphate and DMSO were used as the positive and negative control, respectively. Table 2 shows the results of the brine shrimp lethality bioassay after 24 hr exposure of the shrimps to all the samples and the positive control, vincristine sulfate.

Both the bioassays were performed in triplicate. The zone of inhibition and LC_{50} were calculated as mean ± SD (n = 3) for the antimicrobial screening and brine shrimp lethality bioassay, respectively.

RESULTS AND DISCUSSION

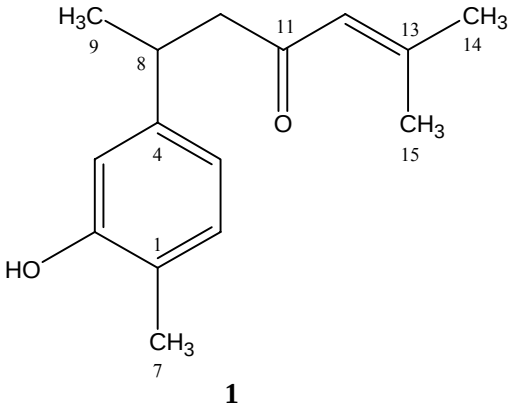
Repeated chromatographic separation and purification of carbon tetrachloride soluble fraction of a methanolic extract of the rhizomes of *C. longa* over silica gel provided seven compounds (**1**-**7**). The structures of the isolated compounds were deduced by extensive NMR data analyses as well as by comparison with previously reported values. Among the compounds, turmeronol-A (**1**), turmeronol-B (**2**), and 4-hydroxybisabola-2,10-diene-9-one (**7**) have previously been isolated from the same plant, while 3,4-dimethoxycinnamic acid (**3**), 4-hydroxy-3-methoxycinnamic acid (**4**) and 4-hydroxybenzaldehyde (**5**) and 2,3,5,6-tetrahydroxyarturmerone (**6**) are reported here from *Curcuma* species for the first time.

The ^{13}C NMR spectrum of compound **1** displayed 15 carbon resonances including a carbonyl carbon at δ 200.0 and an oxygenated quaternary carbon at δ 155.2. The DEPT experiment revealed that only 10 out of these 15 carbons had attached protons and indicated that compound **1** consisted of 4 methyls, 1 methylene, 5 methine and 5 quaternary carbons. These data were consistent with those observed for turmeronol-A (**1**), previously reported from *C. longa*.[10]

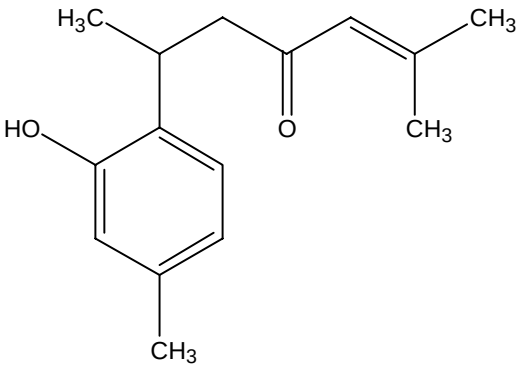
The ^{1}H-NMR spectrum of compound **1** displayed two doublets centered at δ 6.64 (1H, *J*=1.8 Hz) and 7.01 (1H, *J*=8.0 Hz) and a double doublet at δ 6.69 (1H, *J*=8.0 Hz, 1.8 Hz), which indicated the presence of a 1,2,4-trisubstituted benzene ring. It also revealed two double doublets centered at δ 2.58 (1H, *J*=15.7 Hz, 6.0 Hz) and δ 2.69 (1H, *J*=15.7 Hz, 8.0 Hz) which could be assigned to the *geminal* methylene protons at C-10. The downfield shift of the protons indicated the presence of a keto group at the adjacent position (C-11). A singlet for an olefinic proton was observed at δ 6.01 which could be assigned to H-12. The multiplet of one proton intensity at δ 3.23 could be attributed to the methine proton, H-8. The spectrum also displayed methyl resonances at δ 2.09 (br. *s*), 1.84 (br. *s*), 2.19 (br. *s*) in addition to a three proton doublet (*J*=6.9 Hz) at δ 1.22. These signals were assigned to the vinylic methyls at C-13,

aromatic methyl at C-1 (δ 2.19) and C-8, respectively. On the basis of above spectral data, compound **1** was identified as turmeronol-A. Comparison of these spectroscopic features with previously reported values confirmed its identity as turmeronol-A (**1**).[10]

The ^{1}H NMR spectrum of compound **2** displayed a broad singlet at δ 6.73 and two doublets (J=8.0 Hz) centered at δ 6.70 (1H) and δ 7.01 (1H). The absence of any other aromatic proton indicated the presence of a 1,3,4-trisubstituted benzene ring. It also revealed a broad doublet at δ 2.29 (2H, *J*=6.7 Hz) which could be assigned for the *geminal* methylene proton at C-10. The remaining signals in the ^{1}H NMR spectrum were almost identical to those observed for turmeronol-A (**1**), suggests a close structural similarity between these two compounds. On this basis, the compound was characterized as turmeronol-B (**2**), the identity of which was further substantiated by comparison of its spectral data with previously reported values.[10]

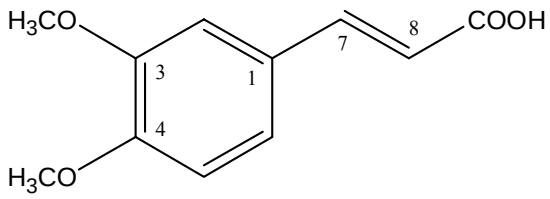


1



2

The ^{1}H-NMR spectrum of compound **3** displayed two singlets centered at δ 3.92 and δ 3.77 for methoxy protons. It also showed a broad singlet at δ 7.02 and two doublets (*J*=8.0 Hz) centered at δ 6.90 (1H) and δ 7.08(1H), typical for a 1,3,4-trisubstituted benzene ring. The *trans* olefinic protons appeared as doublets at δ 7.60 (1H, *J*=16.0 Hz) and δ 6.28 (1H, *J*=16.0 Hz) and were assigned to H-7 and H-8, respectively. The relatively low field shift of H-7 could be explained by its beta (β) position to the carbonyl group, probably in the form of a carboxylic acid. The spectrum also displayed two singlets of three proton intensity each at δ 3.77 and δ 3.92, indicative of methoxyl groups attached to an aromatic ring. The above spectral features are in close agreement with those observed for 3,4-dimethoxycinnamic acid (**3**).[11] Thus, compound **3** was characterized as 3,4-dimethoxycinnamic acid (**3**).



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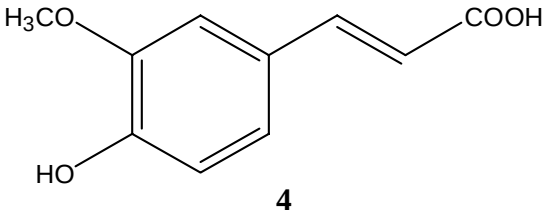

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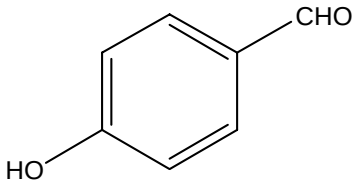

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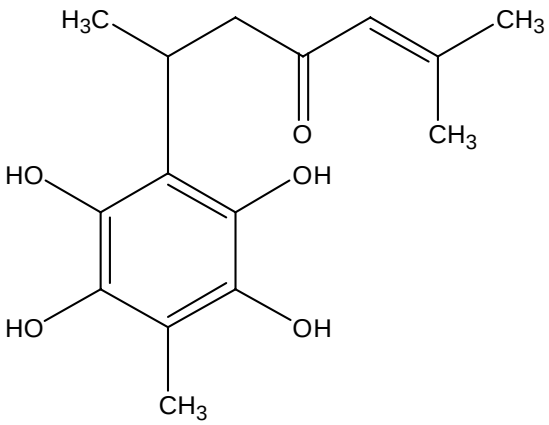

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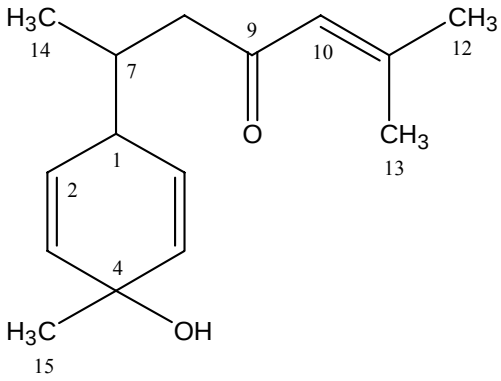

7

The ^{1}H-NMR spectral data (400 MHz, $CDCl_3$) of compound **4** was almost identical to that of 3,4-dimethoxycinnamic acid (**3**). However, the ^{1}H-NMR spectrum of compound **4** displayed a three proton singlet at δ 3.94, instead of two three proton resonances at δ 3.94 and 3.77 observed for compound **4**. This demonstrated that compound **4** was a demethoxy analog of 3,4-dimethoxycinnamic acid **(3)**. This was substantiated by the presence of a broad singlet of one proton intensity at δ 5.87, which could be ascribed to a hydroxyl group proton. Thus, compound **4** was identified as 4-hydroxy-3-methoxycinnamic acid.[10] The ^{13}C NMR spectral data (see experimental) was in support of the proposed structure. Thus, compound **4** was characterized as 4-hydroxy-3-methoxycinnamic acid.[12]

The ^{1}H NMR spectrum of compound **5** showed a sharp downfield singlet at δ 9.96 for an aldehyde group proton. It also showed two doublets (J = 8.0 Hz) centered at δ 7.80 and 7.38 (each 2H) which could be assigned to the *ortho* coupled *para*-disubstituted aromatic protons. The spectrum also showed a broad singlet at δ 6.00 demonstrative of a hydroxyl group. These ^{1}H NMR data suggested that the compound **5** must be a 1,4-disubstituted benzene, where one of the substituents was an aldehyde function and the remaining one was a hydroxyl functionality. Thus, it was characterized as 4-hydroxybenzaldehyde (**5**), the identity of which was confirmed by comparison with previously reported values.[13]

The ^{1}H NMR spectrum of compound **6**, was in part, identical to that observed for turmeronol A (**1**). Thus, it showed 3 three proton singlets at δ 2.00, 2.25, 2.45, a three proton doublet at δ 1.40, an olefinic proton at δ 6.17, a methine proton at δ 3.44 and resonances at δ 2.75 (1H, *dd*, *J*=15.7 Hz, 8.3 Hz) and 2.85 (1H, *dd*, *J*=15.7 Hz, 6.0 Hz) for a methylene group. However, the ^{1}H NMR spectrum of compound **6** did not show any downfield proton resonances, except that at δ 6.17. This revealed that compound **6** had a fully substituted benzene ring. On this basis, compound **6** was tentatively identified as 2,3,5,6-tetrahydroxyarturmerone, which appears to be new. However, additional spectral data such as ^{13}C NMR, HSQC, HMBC and MS would be required to confirm its structure.

The ^{1}H NMR spectrum of compound **7** showed the presence of an isopropylidene group with two vinylic methyls at δ 1.89 (3H, *s*) and δ 2.15 (3H, *s*) and an olefinic proton at δ 6.06 (1H, *s*) conjugated with a carbonyl group. The spectrum also showed a secondary methyl group (δ 0.90, 3H, *d*, *J*=6.0 Hz), a tertiary methyl group (δ 1.24, 3H, *s*) on an oxygenated carbon and two olefinic proton doublets (*J*=10.5 Hz) centered at δ 6.02 and 6.81. In addition, the ^{1}H NMR spectrum also displayed two methines at δ 1.60 and δ 1.92 and three methylene group proton resonances at δ 2.32 (4H) and δ 2.48 (2H), which could be assigned to H-7, H-1, H_2-5 and H_2-6 and H_2-8, respectively. On this basis, compound **7** was identified as 4-hydroxybisabola-2,10-dien-9-one (**7**),

the identity of which was substantiated by comparison with literature values for 4-hydroxybisabola-2,10-dien-9-one[14] previously known to occur in *Curcuma* species.

The methanolic crude extract, the *n*-hexane, carbon tetrachloride and chloroform soluble fractions of the methanolic extract exhibited mild to moderate amtimicrobial activity against most of the test organisms. The zone of inhibition produced by the extractives was found to be 08-10 mm at a concentration of 300 µg/disc (Table 1).

The lethality of the methanolic crude extract, *n*-hexane, carbon tetrachloride and chloroform soluble fractions of the methanolic extract to brine shrimp was evaluated on *A. salina*.[8] The LC_{50} values of *n*-hexane and carbon tetrachloride and chloroform soluble partitionates and the crude methanol extract were found to be 24.06 µg/ml, 1.56 µg/ml, 17.17 µg/ml, and 13.63 µg/ml, respectively (Table 2). Comparison with positive control vincristine revealed that cytotoxicity exhibited by the extractives were promising and further bioactivity guided investigation can provide antiproliferative, antitumor, pesticidal and other bioactive agents.

Table 1. Antimicrobial activity of *C. longa* extractives

Test microorganisms	Diameter of zone of inhibition (mm)				
	HE	CTE	CE	ME	KAN
Bacillus cereus	10.17 ± 0.29	8.23 ± 0.25	10.08 ± 0.38	8.2 ± 0.13	15.025 ± 0.25
B. megaterium	10.33 ± 0.58	10.08 ± 0.14	10.17 ± 0.15	10.3 ± 0.15	18.10 ± 0.10
B. subtilis	9.00 ± 0.50	9.00 ± 0.25	9.15 ± 0.13	9.2 ± 0.10	17.18 ± 0.16
Staphylococcus aureus	8.17 ± 0.29	8.23 ± 0.25	8.12 ± 0.10	9.5 ± 0.25	15.10 ± 0.10
Sarcina lutea	7.83 ± 0.29	8.15 ± 0.13	9.15 ± 0.15	8.2 ± 0.10	15.13 ± 0.13
Escherichia coli	10.00 ± 0.50	8.10 ± 0.17	9.23 ± 0.25	10.2 ± 0.15	16.18 ± 0.16
Pseudomonas aeruginosa	-	8.12 ± 0.20	-	8.1 ± 0.15	12.03 ± 0.06
Salmonella paratyphi	8.50 ± 0.50	7.92 ± 0.14	8.12 ± 0.10	9.4 ± 0.26	16.13 ± 0.13
S. typhi	8.17 ± 0.29	8.00 ± 0.50	9.10 ± 0.10	9.0 ± 0.06	15.10 ± 0.10
Shigella boydii	8.67 ± 0.29	9.15 ± 0.13	9.13 ± 0.12	9.2 ± 0.013	15.15 ± 0.18
S. dysenteriae	8.00 ± 00	8.17 ± 0.15	8.10 ± 0.10	9.1 ± 0.10	16.12 ± 0.10
Vibrio mimicus	-	8.10 ± 0.10	9.13 ± 0.15	9.35 ± 0.18	16.17 ± 0.15
V. parahemolyticus	8.00 ± 0.50	8.17 ± 0.15	9.17 ± 0.21	9.2 ± 0.12	15.12 ± 0.13
Candida albicans	-	8.20 ±0.20	9.17 ± 0.15	8.1 ± 0.18	15.21 ± 0.26
Aspergillus niger	-	8.23 ± 0.21	8.30 ± 0.30	-	15.13 ± 0.15
Sacharomyces cerevacae	-	-	9.17 ± 0.21	-	10.23 ± 0.25

The diameter of zone of inhibition are expressed as mean ± SD (n=3); a diameter less than 8 mm was considered inactive; HE: *n*-hexane extract; CTE: carbon tetrachloride extract; CE: chloroform extract; ME: methanolic extract; KAN: kanamycin.

Table 2. Brine shrimp lethality bioassay of *C. longa* extractives

Sample	LC_{50} (µg/ml)	95% Confidence Limit	Regression equation	K^2	
				Calculated	Tabular
Vincristine sulphate (positive control)	0.44	0.20 - 0.98	Y = 0.5805X +1.502	1.125	15.507
n-hexane extract	24.06	14.85 - 38.96	Y = 0.897X - 1.0647	4.557	15.507
CCl_4 extract	1.56	0.54 - 4.52	Y = 0.3362X + 4.338	1.355	15.507
$CHCl_3$ extract	17.17	8.59 - 34.34	Y = 3.1507 + 0.3828X	1.943	15.507
Methanol extract	13.63	8.04 - 23.12	Y = 1.6987 + 0.5892X	2.703	15.507

The values of LC50 are expressed as mean ± SD (n=3); VS: vincristine sulphate (Std.); HE: *n*-hexane partitionate; CTE: carbon tetrachloride partitionate; CE: chloroform partitionate; ME: methanolic extract.

The crude methanolic extract along with *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions of *C. longa* showed significant antimicrobial and cytotoxic activities, which supports the traditional use of this plant in various diseases.

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REFERENCES

1. Hatcher, H., Planalp, R., Cho, J., Torti, F.M. and Torti, S.V. 2008. Curcumin: From ancient medicine to current clinical trials. *Cell Mol. Life Sci.* **65**, 1631-1652.
2. Chethankumar, M., Srinivas, L. 2008. New biological activity against phospholipase A_2 by Turmerin, a protein from *Curcuma longa L*. *Biol. Chem*. **389**, 299-303.
3. Wang, S.S., Zhang, J.M., Guo, X.H., Song, Q.L. and Zhao, W.J. 2007. A new eudesmane sesquiterpene lactone from *Curcuma wenyujin*. *Yao Xue Xue Bao*. **42**, 1062-1065.
4. Ohtsuki, T., Tamaki, M., Toume, K. and Ishibashi, M. 2008. A novel sesquiterpenoid dimer parviflorene F induces apoptosis by up-regulating the expression of TRAIL-R2 and a caspase-dependent mechanism. *Bioorg. Med. Chem.* **16**, 1756-1763.
5. Pozharitskaya, O.N., Ivanova, S.A., Shikov, A.N. and Makarov, V.G. 2008. Separation and free radical-scavenging activity of major curcuminoids of *Curcuma longa* using HPTLC-DPPH method. *Phytochem. Anal.* **19**, 236-243.
6. Vanwagenen, B.C., Larsen, R., Cardellina, J.H.I.I., Randazzo, D., Lidert Z.C. and Swithenbank, C. 1993. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri. J. Org. Chem*. **58**, 335-337.
7. Bauer, A.W., Kirby, W.M.M., Sherries, J.C. and Truck, M. 1966. Antibiotic susceptibility testing by standard single disc diffusion method. *Am. J. Clini. Pathol.* **45**, 426-493.
8. Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, J.B., Nicholsand, D.E. and Mclaughlin, J.L. 1982. Brine shrimp; a convenient general bioassay for active plant constituents. *Planta Med*. **45**, 31-34.
9. McLaughlin, J.L and Rogers, L.L. 1998. The use of biological assays to evaluate botanicals. *Drug Infor. J*. **32**, 513-524.
10. Imai, S., Morikiyo, M., Furihata, K., Hayakawa, Y. and Seto, H. 1990. Turmeronol A and Turmeronol B, New Inhibitors of Soybean Lipoxygenase. *Agric. Biol. Chem.* **54**, 2367-2371.
11. Arruda, M.S.P., Fernandes, J.B.M., da Silva, F.D.G.F., Vieira, P.C. and Pirani, J.R. 1992. Quinolone alkaloids from *Zanthoxylum acutifolium*. *Phytochemistry* **31**, 3617-3619.
12. Quackenbush, R.C., Bunn, D. and Lingren, W. 1986. HPLC determination of phenolic acids in the water-soluble extract of *Zostera marina* L. (eelgrass). *Aqua*. *Bot*. **24**, 83-89.
13. Jin-yan, C., Lin, Z., and De-zhi, Z. 2007. Chemical Constituents from *Bletilla ochracea* Schltr. *Chem. Res. Chin. Univ.* **23**, 705-707.
14. Ohshiro, M., Kuroyanagi, M. and Ueno, A. 1990. Structures of sesquiterpenes from *Curcuma longa*. *Phytochemistry* **29**, 2201-2205.