Phytochemical Screening of Rhizome Extract of *Curcuma longa* Linn. Grown in Bangladesh through GC-MS and its Bioactivities

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ABSTRACT: The methanolic extract of *Curcuma longa* L. rhizome was subjected to Gas Chromatography-Mass Spectrometric (GC-MS) analysis. The chromatographic investigation of the extract showed 27 constituents of which 19 compounds were newly found from *C. longa*. Here, the peaks obtained in the total ion chromatogram were compared with the National Institute of Standards and Technology (NIST) database for identification of the constituents. The most plentiful component was 9-octadecenamide (Z) (5.93%). The component present in the lowest amount was 4-hydroxy-3-methoxy benzoic acid methyl ester (0.08%). The rhizome extract of *C. longa* and its six fractions (F1-F6) obtained through vacuum liquid chromatography (VLC) were also investigated for antioxidant, cytotoxic and antimicrobial activities.The crude extract and its fractions (F4-F6) exhibited strong antioxidant activity having IC_{50} values of 19.85, 18.41, 26.72 and 16.79 µg/ml, respectively compared to the standards butylated hydroxyl anisole (IC_{50} = 11.42 µg/ml) and ascorbic acid (IC_{50} = 9.01 µg/ml). Strong cytotoxicity was found in crude extract (LC_{50} = 1.81 µg/ml) and fractions F4 (LC_{50} = 2.4 µg/ml), F5 (LC_{50} = 2.5 µg/ml) and F6 (LC_{50} = 2.3 µg/ml) when compared to the standard, tamoxifen (LC_{50} = 0.13 µg/ml). Fractions F1-F3 displayed mild to moderate antibacterial activity against *Bacillus megaterium*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* in comparison with the standard, Kanamycin. The compounds obtained through GC-MS may explain the above activities. The present results showed the prospect of this medicinal plant to explore further to determine the bioactive components.

Key words: Gas chromatography-mass spectrometry (GC-MS), Vacuum liquid chromatography (VLC), Methanolic rhizome extract, Bioassay.

INTRODUCTION

The use of medicinal plants with healing properties have been widened in recent years for their presence of bioactive compounds with cures for diverse ailments.[1-4] Conservative medicinal practices form a fundamental part of substituted medicine. Because of their active chemical constituents, these single or polyherbal provision habitually create positive responses.[5,6]

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Curcuma longa L. (Family: Zingiberaceae), commonly known as turmeric, is used in conventional medication as a domestic cure for various diseases. It is dispersed throughout the humid regions of the world. The rhizome part of turmeric usually contains volatile and non-volatile chemical constituents. The chemical components of volatile oil are usually recognized by using GC and GC-MS. The volatile essential oils contribute in the aroma of turmeric whereas the phenolic compounds, curcumin and its analogues are accountable for its vivid yellow colour. The rhizome oil of *C. longa* from Lahore,

Pakistan was reported to have turmerone (25.3%), α-tumerone (18.3%) and curlone (12.5%).[8] From another study, the leading chemical compounds of the volatile oil were recognized as ar-turmerone, zingiberene, turmerone and curlone.[9] Essential oil from the rhizomes of two types of *C. longa*, yellow and red cultivated in Bangladesh has been reported. The major compounds from the yellow type were tumerone (17.16%) and culone (13.82%) whereas the red type contained carvacrol (21.14%) and citral (13.91%) as the chief constituents.[10] Though huge research has been conducted on the chemical composition of the oil elsewhere, no work has been done in Bangladesh on the constituents of methanolic extract of rhizome of turmeric through GC-MS. Hence, the goal of current study was to explore the phytochemical content of *C. longa* grown in Bangladeshi tropical weather by GC-MS analysis and assess the antioxidant, cytotoxic and antimicrobial activities of the crude methanolic extract for probable isolation of lead compounds for drug development.

MATERIALS AND METHODS

Plant extract and fraction preparation. The plant was identified by a taxonomist at the Bangladesh National Herbarium (BNH), Dhaka, Bangladesh (voucher specimen no: DACB 45102). After gathering of plant fragments (rhizome part, ~1500 g) from local market, Dhaka, Bangladesh in July, 2017 it was dried at room temperature and powdered by a grinder. The powdered part was soaked in methanol (MeOH) twice consecutively for 7-8 days each time, with intermittent stirring. The methanolic extract was filtered after each soaking period and the filtrate was concentrated with a rotary evaporator to obtain the final crude extract (~15.69 g). This extract was subjected to VLC over silica gel 60 H. Initially the VLC was eluted with *n*-hexane: DCM solvent system in order of increasing polarities (9:1, 5:5, 0.5:9.5) to get three fractions (F1-F3) followed by DCM: MeOH (9.85:0.15, 9:8:0.2, 0:10) to get another three fractions (F4-F6).

Gas chromatography-mass spectrometry (GC-MS). GC-MS analysis was done with a Clarus® 690 gas chromatograph (Perkin Elmer, CA, USA) using a column (Elite-35, 30 m × 0.25 mm; Perkin Elmer, CA, USA) with 0.25 µm film which was equipped with a Clarus® SQ 8 C mass spectrophotometer (Perkin Elmer, CA, USA). As a transporter gas pure helium (99.999 %) was used. The sample (1 µl) was examined in electron ionization (EI) mode. The scan time and mass range were 1 s and 50–600 m/z, respectively. [11]

Antioxidant activity. The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method was used to estimate the antioxidant potential.[12] The experimental samples (crude extract and VLC fractions) were prepared in test tubes by serial dilution. Each sample solution (2 mL) of different concentration was mixed with 2 mL of DPPH (20 µg/ml) to obtain diverse concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.562 µg/ml). The absorbance of each sample was measured at 517 nm in a spectrophotometer. Ascorbic acid (ASA) and butylated hydroxyl anisole (BHA) were used as positive controls. The scavenging capacity was calculated by the following equation:

Scavenging ability (I%) = [(Absorbance of control − Absorbance of sample)/ Absorbance of control] × 100.

A curve was constructed by plotting the obtained percent inhibition (I%) versus the concentration of the test sample. Thus, the fifty percent inhibitory concentration (IC_{50}) value for each sample was obtained.

Cytotoxic activity. The general toxicity of different extracts was measured by brine shrimp lethality bioassay test.[13] In this experiment, 10 brine shrimp naupli were taken in each pre-marked glass test tube which was filled with 5 ml of simulated sea water. The trial samples were dissolved in dimethyl sulfoxide (DMSO). The median lethal concentration (LC_{50}) of the test samples were calculated by plotting percentage of the shrimp mortality against the logarithm of the sample concentrations. Tamoxifen was considered as the positive control whereas DMSO was used as the negative control.

Antimicrobial activity. The disc diffusion method was used for screening of antibacterial and antifungal activities.[14-16] Here the tested samples (crude extract and VLC fractions) were assessed against two Gram-positive bacteria [*Staphylococcus aureus* (ATCC 25923) and *Bacillus megaterium* (ATCC 28318)], two Gram-negative bacteria [*Pseudomonus aeruginosa* (ATCC 27833) and *Escherichia coli* (ATCC 28739)] and two fungal strains [*Aspergillus niger* and *Aspergillus flavus*]. The tested samples were dissolved individually in DCM or methanol. The sample-treated discs were placed in nutrient agar (NA) medium inoculated with the bacteria and potato dextrose agar (PDA) medium inoculated with the fungi. The plates were incubated for 24 hrs at 37°C and 48 hrs at 28°C for bacteria and fungi, respectively. The diameter of inhibitory zones was measured in millimeter (mm) around the sample treated disc. By this, the sensitivities of the microorganism to the samples (100 µg/disc) were determined. Kanamycin (30 µg/disc) was used as the antibacterial standard whereas ketoconazole (30 µg/disc) was used as the antifungal standard.

Statistical analysis. Antioxidant and cytotoxic activities were tested three times and standard deviation (SD) was calculated with Microsoft Excel.

RESULTS AND DISCUSSION

GC-MS analysis. The mass spectra of methanolic extract of *C. longa* rhizome revealed 27 peaks which represented the presence of 27 major compounds by library search and spectral matching with the National Institute of Standards and Technology (NIST) database. The components were identified by their retention time (RT), molecular weight, molecular formulae, percent peak area etc. Nineteen compounds have been newly identified from this plant and to the best of our knowledge firstly reported from *C. longa*. The results of phytochemical compounds via GC-MS of the methanolic rhizome extract of *C. longa* are presented in Figure 1 and in Table 1.

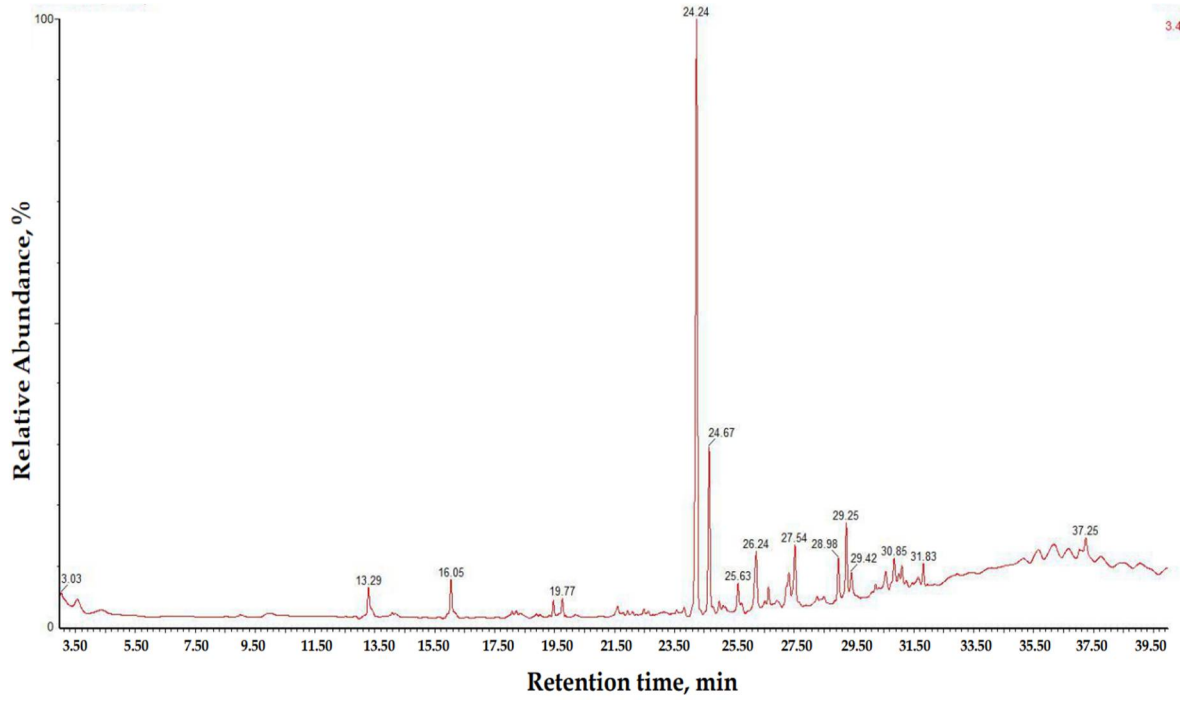
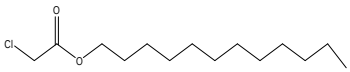
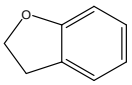
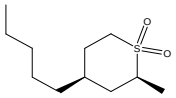
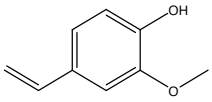
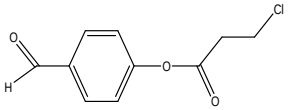
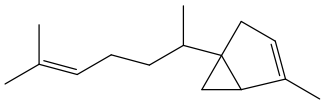
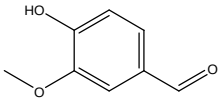
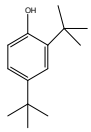
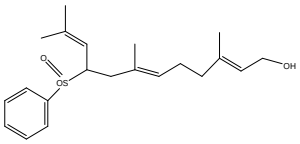
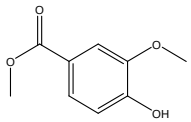
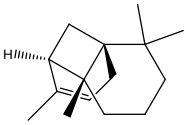
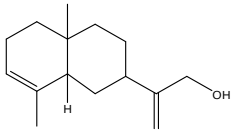
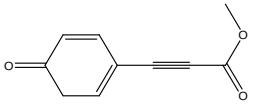
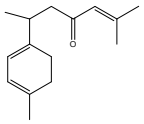
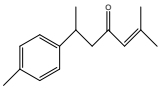
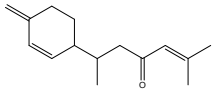
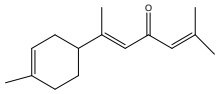
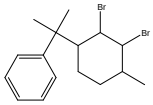
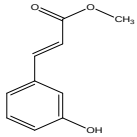
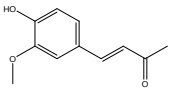
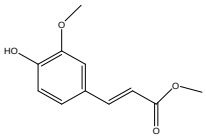
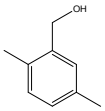
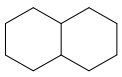
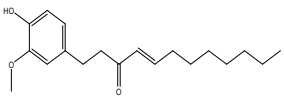
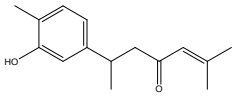
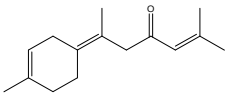
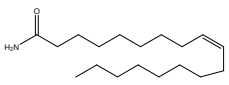


Figure 1. GC-MS Chromatogram of methanolic extract of *C. longa* rhizomes.

Table 1. Phytochemical constituents of methanolic extract of *C. longa* rhizomes determined by GC-MS analysis.

Peak	RT (min)	Peak area %	Molecular weight	Molecular formula	Name of compound	Structure	Compound type and remarks
1	8.998	0.22	262	$C_{14}H_{27}O_2Cl$	Chloroacetic acid, dodecyl ester		Fatty acid (Newly identified)
2	13.293	1.61	120	C_8H_8O	Bezofuran, 2,3-dihydro		Heterocyclic (Newly identified)
3	14.09	0.83	218	$C_{11}H_{22}O_2S$	Trans-2-methyl-4-*N*-pentylthiane,S,S,dioxide		Heterocyclic (Newly identified)
4	16.047	1.57	150	$C_9H_{10}O_2$	2-Methoxy-4-vinyl phenol		Phenolic (Newly identified)
5	18.09	0.33	212	$C_{10}H_9O_3Cl$	4-Formylphenyl-3-chloropropanoate		Phenolic (Newly identified)
6	18.224	0.59	204	$C_{15}H_{24}$	2-Methyl-5-((*R*)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene		Sesquiterpene
7	19.471	0.37	152	$C_8H_8O_3$	Vanillin		Phenolic
8	19.759	0.54	206	$C_{14}H_{22}O$	2,4-di-tert-butyl phenol		Phenolic (Newly identified)
9	20.202	0.18	362	$C_{21}H_{30}O_{3S}$	(2*E*,6*E*) 3,7,11-trimethyl-9-(phenyl sulfinyl) dodeca-2,6,10-trien-1-ol		Sesquiterpene (Newly identified)
10	21.945	0.08	182	$C_9H_{10}O_4$	Benzoic acid, 4-hydroxy-3-methoxy-methyl ester		Phenolic
11	22.106	0.08	204	$C_{15}H_{24}$	(1*R*,4A*R*,8A*R*)-2,5,5,8a-tetramethyl-4,5,6,7,8,8a-hexahydro-1H-1,4a-Methanonaphthalene		Selinane (Newly identified)
12	22.481	0.19	220	$C_{15}H_{24}O$	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalen-2-yl) prop-2-en-1-ol		Selinane (Newly identified)
13	23.581	0.37	180	$C_{10}H_{12}O_3$	Cyclohexadien-4-one-1-propiolic acid, methyl ester		Phenolic (Newly identified)

Peak	RT (min)	Peak area %	Molecular weight	Molecular formula	Name of compound	Structure	Compound type and remarks
14	23.829	0.36	218	$C_{15}H_{22}O$	Tumerone		Bisabolane
15	24.245	4.71	216	$C_{15}H_{20}O$	ar- Turmerone		Bisabolane
16	24.667	0.31	218	$C_{15}H_{22}O$	2-Methyl-6-(4-methylene cyclohex-2-en-1-yl) hept-2-en-4-one		Bisabolane (Newly identified)
17	26.229	2.31	218	$C_{15}H_{22}O$	*E*-Atlantone		Bisabolane
18	26.651	0.78	372	$C_{16}H_{22}Br_2$	p-Menthane,2,3-dibromo-8-phenyl-		Monoterpenoid (Newly identified)
19	27.529	2.04	178	$C_{10}H_{10}O_3$	(*E*)-methyl 3-(3-hydroxyphenyl) acrylate		Phenylpropene (Newly identified)
20	28.99	1.26	192	$C_{11}H_{12}O_3$	(*E*)-4-(4-hydroxy-3-methoxyphenyl) but-3-en-2-one		Phenylpropene
21	29.252	2.46	208	$C_{11}H_{12}O_4$	(*E*)-methyl 3-(4-hydroxy-3-methoxyphenyl) acrylate		Phenylpropene (Newly identified)
22	29.419	1.09	136	$C_9H_{12}O$	2,5 Dimethylphenyl methanol		Phenolic (Newly identified)
23	30.23	0.43	138	$C_{10}H_{18}$	Napthalene, decahydro-		Selinane (Newly identified)
24	30.565	1.17	304	$C_{19}H_{28}O_3$	1-(4-Hydroxy-3-methoxy phenyl) dodec-4-en-3-one		Bisabolane (Newly identified)
25	30.854	1.30	232	$C_{15}H_{20}O_2$	6-(3-Hydroxy-4-methyl phenyl)-2-methyl hept-2-en-4-one		Bisabolane (Newly identified)
26	31.826	0.53	218	$C_{15}H_{22}O$	(*E*)-Gamma-atlantone		Bisabolane
27	37.248	5.93	281	$C_{18}H_{35}ON$	9-Octadecenamide, (Z)-		Fatty acid (Newly identified)

Antioxidant activity. The crude extract and column fractions (F4-F6) showed significant antioxidant activity with IC_{50} values of 19.85, 18.41, 26.72, 16.79 µg/mL, respectively. These values were compared with that of the positive control BHA (11.42 µg/mL) and ASA (9.01 µg/mL). The other column fractions (F1-F3) did not show any antioxidant activity and not shown in Figure 2.

Cytotoxic activity. The crude extract showed strong cytotoxicity with LC_{50} value of 1.81 µg/ml in comparison with Tamoxifen (0.13 µg/ml) as positive control. LC_{50} values for other fractions F4 (2.4 µg/ml), F5 (2.5 µg/ml), F6 (2.3 µg/ml) were found to be significant also. The remaining fractions (F1-F3) showed no cytotoxic effect.

Antimicrobial activity. The crude extract and VLC fractions revealed mild antibacterial activity. Among all fractions, F1, F2 and F3 displayed moderate activities against *B. megaterium*, *S. aureus* and *P. aeruginosa*. F3 and F4 exhibited mild activity against *E. coli* whereas F5 and F6 showed no activity against the tested bacteria. The fungal strains were also found to be resistant to the test samples (Table 2).

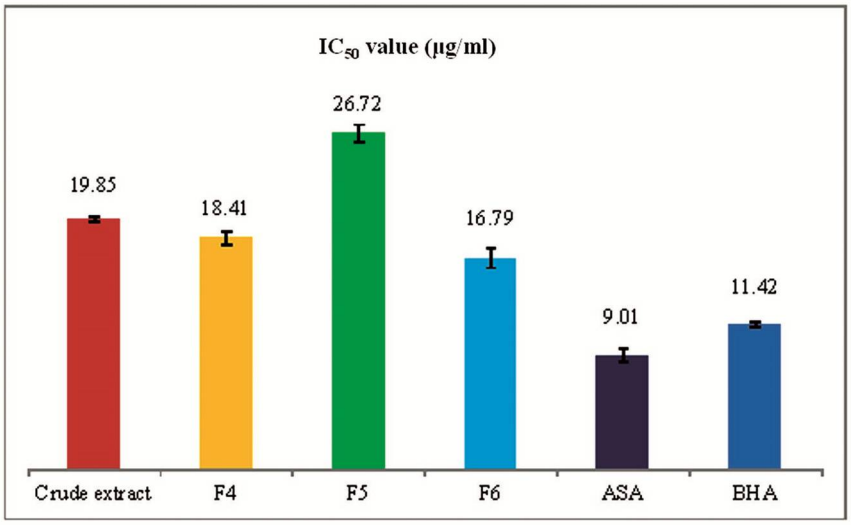


Figure 2. Antioxidant activity of the test samples.

Table 2. Antimicrobial screening of crude extract and VLC fractions.

		Diameter of zone of inhibition (mm)					
		Bacterial Strain				Fungal Strain	
		Gram positive		Gram negative			
		B. megaterium	*S. aureus*	*P. aeruginosa*	*E. coli*	*A. niger*	*A. flavus*
Crude extract		7	7	7	7	------	------
F1		9	10	7	------	------	------
F2		9	------	8	------	------	------
F3		9	8	7	8	------	------
F4		------	------	------	8	------	------
F5		------	------	------	------	------	------
F6		------	------	------	------	------	------
Positive control (30 µg/disc)	Kanamycin	50	16	16	17	NA	NA
	Ketoconazole	NA	NA	NA	NA	30	48

"-" indicates no sensitivity; "NA" indicates not applicable.

A total of 235 compounds with various activities were isolated and identified from different parts (flower, leaf, root and rhizome) of *C. longa* which had been reviewed elsewhere.[17] Among 27 phytochemical compounds identified in the current study from rhizome of *C. longa* through GC-MS, 7 are phenolic, 7 are bisabolane, 3 are selinane, 3 are phenylpropene, 2 are heteroyclic, 2 are sesquiterpene, 2 are fatty acid and 1 is monoterpenoid type. Many reports are available relating to the chemical constituents of *C. longa* rhizome extracts worldwide. In the rhizome extract of *C. longa* from India (Madhya Pradesh) the major components were ar-tumerone (18.41%), curlone (10.17%), tumerone (9.52%).[18] In Vellore district, Tamil Nadu, India, bicyclo [4.1.0]-3-heptene, xylopropamine, α-curcumene, β-curcumene, curdione were the main constituents.[19] Sometimes the construction of plant secondary metabolites is controlled by ecological and edaphic factors such as rainfall, temperature, humidity and soil nutrients.[20] Variation in environmental, soil nutrients and agro-climatic conditions in different zones results in varied extract yield and content.[21]

In this study, the crude extract of *C. longa* and the VLC fractions (F4-F6) showed significant antioxidant activity. Dietary intake of antioxidant compounds such as phenolic acids and flavonoids may be considered as primary resistance to encounter the likely hazards of oxidative damage.[22] Phenolic compounds in spices and herbs extensively added to their antioxidant properties that are admitted by several studies.[23-25] In this experiment, 7 phenolic compounds have been obtained through GC-MS. The acquired results point out that the antioxidant activity may be ascribed to the phenolic compounds that resulted in elevated reducing capability.

From the pharmacological viewpoint, a good connection has been found with the brine shrimp lethality assay to identify cytotoxic components in terrestrial plant extracts which supports its use in the ethno medicine for anti-cancer activity.[26] The LC_{50} value of the crude extract (1.81 µg/ml) demonstrates that the plant has significant cytotoxic activity. The results of this bioassay confirmed the previous uses and findings.[27,28] From the literature review, it is shown that 4-hydroxy-3-methoxy benzoic acid methyl ester has potential to reduce the expansion of various prostate cancer (PCA) cells.[29] Vanillin was reported to be anti-mutagenic in both bacterial and mammalian cells.[30] It repressed cytogenetic wound in cultivated Chinese hamster ovary cells and fibroblasts.[31,32] It also restrained chemically-induced hepato carcinogenesis.[33] 2,4-Di-tert-butyl phenol is a lipophilic phenol which showed noteworthy cytotoxicity against HeLa cells.[34] The cytotoxic compounds present in the extract may act individually or synergistically and were liable for the experimental hatchability inhibition and mortality. Further investigation should be needed to isolate and purify the bioactive constituents with cytotoxic activity for additional assessment in human cell line cultures.

The disc diffusion method generally proves the existence of antimicrobial substances. In this assay, the crude methanolic extact of *C. longa* rhizomes displayed mild activity against all the tested bacteria. But some VLC fractions (F1-F3) also revealed mild activities against *B. megaterium*, *S. aureus* and *P. aeruginosa*. F4 exhibited mild activity against *E. coli*. The mild activity of crude extract may be due to the very low concentration (100 µg/disc) of the extract. Some research reports indicated that ethanol extract of *C. longa* exhibited strong antimicrobial activity.[35,36] Some reports also mentioned that alkaloid and flavonoid type compounds are accountable for the antibacterial actions in higher plants.[37] 2-Methoxy-4-vinyl phenol is reported to exhibit strong *in silico* activity against LpxC, an essential enzyme in the lipid A biosynthetic pathway in gram negative bacteria.[38] There are reports on antimicrobial activity of the p-coumaric acid by both increasing bacterial cell membrane permeability and DNA intercalation.[39,40] Two coumaric acid derivatives [(*E*)-methyl 3-(3-hydroxyphenyl) acrylate and (*E*)-methyl 3-(4-hydroxy-3-methoxyphenyl) acrylate] were obtained in the GC-MS analysis.

These coumaric acid derivatives can be further investigated for their specific antibacterial property.

Among other studies, the aromatic substance 2-methoxy-4-vinyl phenol is used as a flavoring agent, whereas ar-turmerone is found to be more powerful platelet inhibitor than aspirin.[41] Also ar-turmerone is predicted to develop insulin resistance and improve type 2 diabetes.[42,43] The cytotoxicity and antioxidant activity were found to be more prominent in our study with the methanolic rhizome extract of *C. longa*, whereas the essential oil extract has been vastly reported for their antimicrobial activity. Different extraction procedure can be used for isolation of desired bioactive compounds from *C. longa.*

CONCLUSION

In conclusion, it is revealed that 19 (nineteen) compounds have been newly identified from the turmeric rhizome extract through GC-MS analysis. The crude extract of the plant and its fractions have significant antioxidant and cytotoxic activity as well as mild to moderate antimicrobial activity. These attributed features may be due to the presence of such kinds of bioactive compounds. Particular fractions with these biological properties can pave the way for further research to isolate lead compounds which could be useful predecessors for scheming valuable beneficial drugs.

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