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# *In Vitro* Antimicrobial Activity of Curcumin-Copper Complex

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## Abstract

The curcumin-copper complex was characterized by a bluish green color when dissolved in dimethylsulfoxide (DMSO), and 20000 µg/ ml were prepared by their dissolving in DMSO. Through using agar well diffusion method, 100 µL of 20000 µg/ml revealed the inhibitory zones (IZs) around the growth of five clinical pathogenic isolates were *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli* and *Klebsiella* sp. compared with tetracycline disc (30 µg/ml) besides *Candida albicans* was tested. These IZs were 12, 12, 13,16, and 40 mm respectively. Relatedly, *E. coli*, *Klebsiella* sp. and *C. albicans* were inhibited by 2000-10000 µg/ ml while no effect of 10000 µg/ml on *S. aureus* and *S mutans.* Red blood cells had affected by 100 µL obtained from 20000 µg/ml of the complex during 1 hour but 10000 µg/ ml resulted in very slightly effect against the cells. Analysis of gas chromatography-mass spectrometry detected that the complex of curcumin copper contained S-methyl methanethiosulfinate; benzenediazonium, 2-hydroxy-, hydroxide, inner salt; methane, (methylsulfinyl)(methylthio)-; 2,4-dithiapentane; silanol, dimethyl-; thiodiglycol; 2-aminoimidazole-5-propionic caid; 1,2,4,5-tetrathiane; 1,2,4-trithiolane; sulfurous acid, diethyl ester; ethanol, 2-[2-(4-pyridyl)ethylamino]-; and methanesulfinothioic acid, S-1-propyl ester and dimethyl sulfone. It was concluded that the tested complex has antimicrobial activity against some species of bacteria and *C.albicans* as well. The complex is needed to be studying *in vivo* and clinically in future

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work so that it may be used as a drug for treating the microbial pathogens which cause the diseases.

**Keywords**: Curcumin- Copper Complex, Antimicrobial Activity, GC-MS Analysis.

## Introduction

Chemotherapy of antimicrobial compounds is known as an important medical science that is a modern aspect for humanity. During many decades, substances were discovered and designed as antimicrobial agents are drugs for the treatment of the infections (Okonko *et al*., 2008). Curcumin represents a natural product which is given by perennial herb *Curcuma longa* Linn. It has antimicrobial activity, antifungal, antioxidant, anti-inflammatory and anticancer effects (Anand *et al*., 2008; Patel and Majumdar, 2009; Sharma *et al*., 2010; Garcia-Gomes *et al*., 2012; Boztas *et al*., 2013 ).

The studies showed that the complexing curcumin with transition metals had interesting. The metals lead to increasing curcumin bioavailability and bioactivity. Examples of these metals that used in complexing curcumin are copper, manganese, iron, and palladium (Chattopadhyay *et al*., 2004; David *et al*., 2005; Anand *et al*., 2008; Tajbakhsh *et al*., 2008; Zebib *et al*., 2010; Kanhathaisong *et al*., 2011; Joseph *et al*., 2012; Rodrigues *et al*., 2012 ).

Extracting curcumin is obtained by turmeric through using solvents such as ethanol and then purification by techniques of chromatography (Li *et al*., 2014; Priyadarsini, 2014).

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Previous studies showed the complex of metal-curcumin exhibited the antimicrobial activity against microorganisms. The mixture of curcumin with cobalt (II) led to the antimicrobial effects on bacteria including *S.aureus, E. coli* and *K. pneumonia* (Girish *et al*., 2019). The curcumin had the inhibition against different microorganisms including fungi, examples were *Candida albicans*, *Cryptococcus neoformans,* and *Rhizoctonia solani*. Also, synthesis of curcumin with zinc acetate salts and copper resulted in antibacterial activity ( Moghadamtousi *et al*., 2014; Mishra and Velingkar, 2015).

The complex of curcumin–silver(I) was tested and revealed inhibition zones (IZs) against some bacteria by which the IZ was recorded against *S. aureus* to be most sensitive compared with other tested bacteria (Syed *et al*., 2015). Relating to the complex of metal- curcumin, Gubendran *et al*., (2016) found that the complex gave the inhibitory effects on some of the bacteria were *S. aureus, E. coli, K. pneumonia,* and *P. fluorescence*. The present study was designed to evaluate the antimicrobial activity of the curcumin-copper complex, and detection of the compounds in the complex by using GC-MS technique.

## Materials and methods

### Preparation of curcumin-copper complex

The curcumin-copper complex was prepared according to (Peacock, 1971; Shen *et al*., 2005; Barik *et al*., 2007; Kanhathaisong *et al*., 2011; Refat, 2013).

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## First step:

## Synthesis of copper II bis acetyl acetone complex (compound I)

Through using clean beaker (250 ml), dissolving 20 gm of dehydrated copper chloride in 130 ml of distilled water for obtaining solution of dehydrated copper chloride while 25 ml of acetyl acetone were mixed with 50 ml of 95% ethanol by using clean beaker ( 500 ml) to form solution of acetyl acetone–ethanol. By helping clean glass rode, magnetic bar and stirrer hot plate (100 ºC) for 15 minutes, both solutions were mixed until they changed into a deep blue solution. In third beaker (250 ml), dissolving 34 gm of anhydrous sodium acetate in a 100 ml of distilled water then the sodium acetate solution was added and left for 10 minutes until forming blue mixture solution that led to giving the blue (sky like color ) precipitate (very thick slurry). The solution was then heated by using a water bath up to 15 minutes that smell of acetic acid had felt while the thickness of the slurry increased. The slurry is cooled at room temperature then filtered by filter paper and kept for 3 days to be dry where the drying was completely done through using the oven at 100 $C^0$. The product was recrystallized from a small volume of absolute methanol (1 gm of the complex: 100 ml of methanol) to produce deep blue needle shape crystals.

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## Second step:

## Synthesis of curcumin-copper complex (compound II)

Through using pestle mortar, 1 gm of copper II bis acetyl acetone complex (compound I) was mixed with 1.83 gm of vanillin as well as 5 ml of both KOH and absolute ethanol as a cofactor. The mixture was placed in pestle mortar to be pulverized and deep bright in color, then 20% citric acid cold solution, 2 ml of 95% ethanol and 1 ml of acetone were placed on the mixture to give a greenish-brown precipitate. The precipitate was left at the fridge for overnight until the precipitated complex was given. The complex was recrystallized by addition of methanol and acetone ( 1:1).

## Evaluation of antimicrobial activity

Amount of curcumin-copper complex was dissolved in DMSO for getting 20000 µg/ml of bluish green solution, then sterilized by syringe Millipore (0.22 µM). Petri dishes of nutrient agar (NA) were prepared, and 100 µL of 1.5 x $10^9$ cell/ml obtained from each one of bacterial suspension (*S. aureus*, *S. mutans*, *E. coli* and *Klebsiella* sp.) was spread on each dish by using a sterile cotton swab. Aseptically and by using cork borer, well (7 mm in size) was done in each dish and loaded with 100 µL of 20000 µg/ml. Except for NA, the complex was tested against *C. albicans* by using Petri dishes of potato dextrose agar (PDA). All dishes were incubated at 37 ºC for 48-72 hours until the inhibitory zones were observed and measured by millimeter (mm). The test was done in comparison with pure tetracycline disc contained 30 µg/ml. Additionally, in preliminary screening, the different

concentrations (2000, 5000, 10000 and 15000 µg/ml) of the complex were tested against the mentioned microorganisms.

## Effect of the curcumin-copper complex on RBCs

According to (Nair *et al*., 1989) with some modifications, human blood ($O^+$) was placed in the anticoagulant tube, and 1 ml mixed with 20 ml of normal saline (sodium chloride) for getting the blood solution. A number of the plastic tubes were used by which each tube contained 2 ml of the solution. One of tube contained mixing 2 ml of the blood solution with 100 µL of DMSO that was considered as positive control while other tube had 2 ml of the blood solution only as a negative control. Other four tubes were used where each tube had 2 ml of the blood solution. First of four tubes was mixed with 100 µL of 20000 µg/ml, the second tube contained 100 µL of 15000 µg/ml, the third tube possessed 100 µL of 10000 µg/ml, and the fourth tube mixed with 100 µL of 5000 µg/ml. Relatedly, 4 ml of blood solution were placed in a tube mixed with 100 µL of 20000 µg/ml in comparison with two control tubes were positive tube contained 4 ml of the blood solution mixed with 100 µL of DMSO but negative tube contained 4 ml of the blood solution only. All tubes were left at room temperature for 1 hour until the changing color of the blood solution was observed by the naked eye.

## Gas chromatography-mass spectrometry (GC-MS) analysis

The volume of 15000 µg/ml that obtained from the curcumin-copper complex which was dissolved in DMSO, filtered by syringe Millipore ( µM

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0.22) then by Whatman filter paper. The filtrate was subjected to MassHunter/ GC-MS. The GC-MS device was programed by contents of (Table 1).

**Table 1:** Parameters of GC-MS by which solution of curcumin copper-complex was analyzed

| **Properties** | **Parameter** |
|---|---|
| Ion source temperature | 230 ºC |
| Quad temperature | 150 ºC |
| Interface temperature | ( MSD transfer line ) 290 ºC |
| Solvent cut time | 4.00 min. |
| Start time | 4.00 min. |
| End time | 35.00-40 min. |
| ACQ mode | Scan |
| Scan speed | 1562 (N2) |
| Start m/z | 35 |
| End m/z | 650 |
| **Gas Chromatography** | |
| Column oven temperature | initial 40c hold 5 mint |
| | Rate 1 10 c/min |
| | Final temperature: 280 to end run |
| Injection temperature | 290 ºC |
| Injection mode | pulsed splitless |
| Flow control mode | Constant flow |
| Pressure | 7.0699 psi |
| Total flow | 19 ml/min. |
| Colum flow | 1 ml/min. |
| Purge flow | 3 ml/min. |
| Injection volume | 1Ul |
| | |
| **Column Type** | |
| HP-5MS | 5% phenyl methyl siloxen type |
| HP-5MS | 5% phenyl methyl siloxane 30m x250 Um x0.25 mm |

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## Statistical analysis

The statistical analysis was carried out by using a program of SPSS (Chi-Square).

## Results

## Evaluation of antimicrobial activity

The final product of the curcumin- copper complex was a greenish-brown precipitate ( Fig.1) by which 20000 µg/ml exhibited the inhibition against five clinical microbial isolates. The highest inhibitory zone (IZ) was measured against *C.albicans* followed by *Klebsiella* sp., *E.coli*, *S. aureus*, and *S.mutans*. These IZs of bacteria were compared with the effects of pure tetracycline disc which contained 30 µg/ml. (Table 2) and (Fig.2). In primary antimicrobial screening, the concentrations (15000, 10000, 5000 and 2000 µg/ml) of the complex were used. *E.coli* and *Klebsiella* sp. were inhibied by 10000-15000 µg/ml but *C.albicans* was inhibited by 2000 µg/ml. Relatedly, 15000-20000 µg/ ml revealed an effect on the growth of *S.aureus* and *S.mutans*. All tested bacteria were not affected by 2000-5000 µg/ ml. Statistical analysis appeared significant differences between the antibacterial effect of curcumin-copper complex and tetracycline ( Fig.3).

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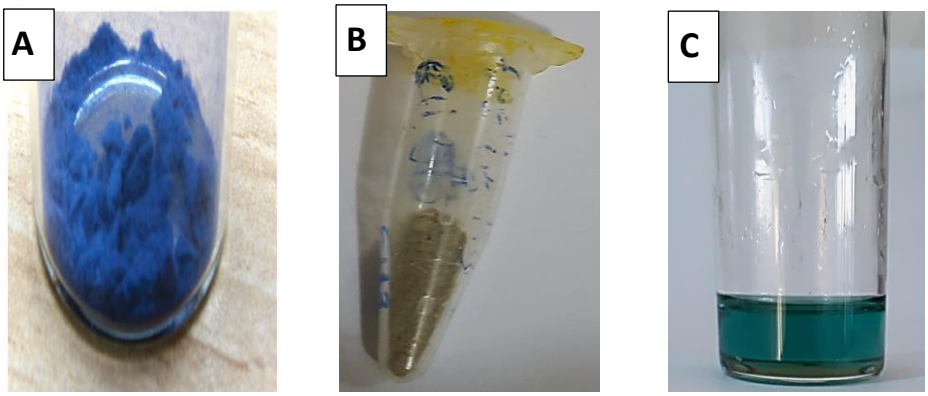


Table 2: Antimicrobial activity of curcumin copper-complex against five isolates of the clinical microbial pathogens. The test was performed by agar well diffusion method through using 20000 µg/ml.

| **Inhibition zones (IZs) measured by millimeter (mm)** | | | | | |
|---|---|---|---|---|---|
| ***S.aureus*** | | ***S.mutans*** | ***E.coli*** | ***Klebsiella* sp.** | ***C.albicans*** |
| CC. | 12 | 12 | 13 | 16 | 40 |
| Tet. | 20 | 25 | 16 | No effect | ------ |

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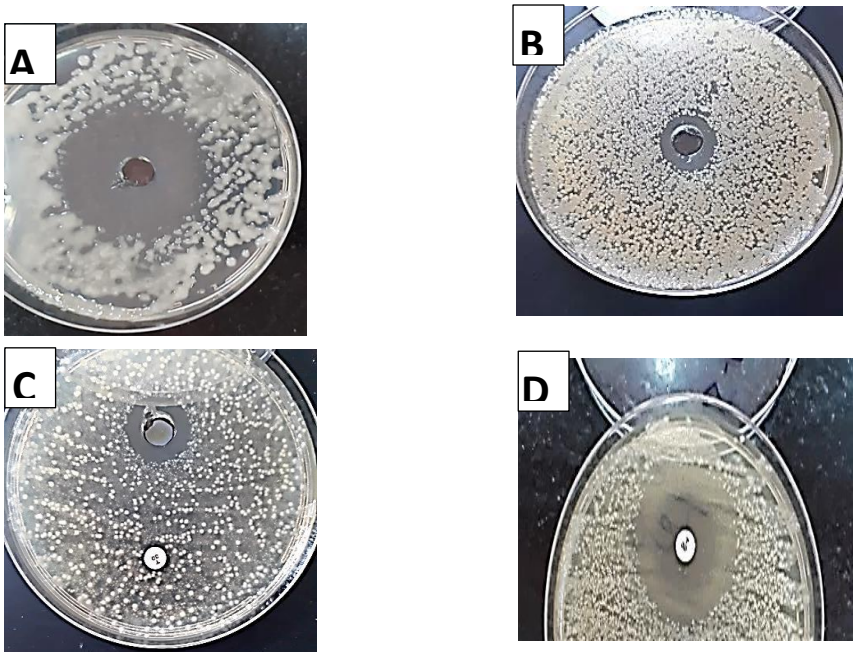


**Fig.2:** Antimicrobial activity of curcumin-copper complex against four isolates of the clinical microbial pathogens. The test was performed by agar well diffusion method by using 20000 µg/ml. **A**: Against *C. albicans*. **B**: Against *S.mutans*. **C**.

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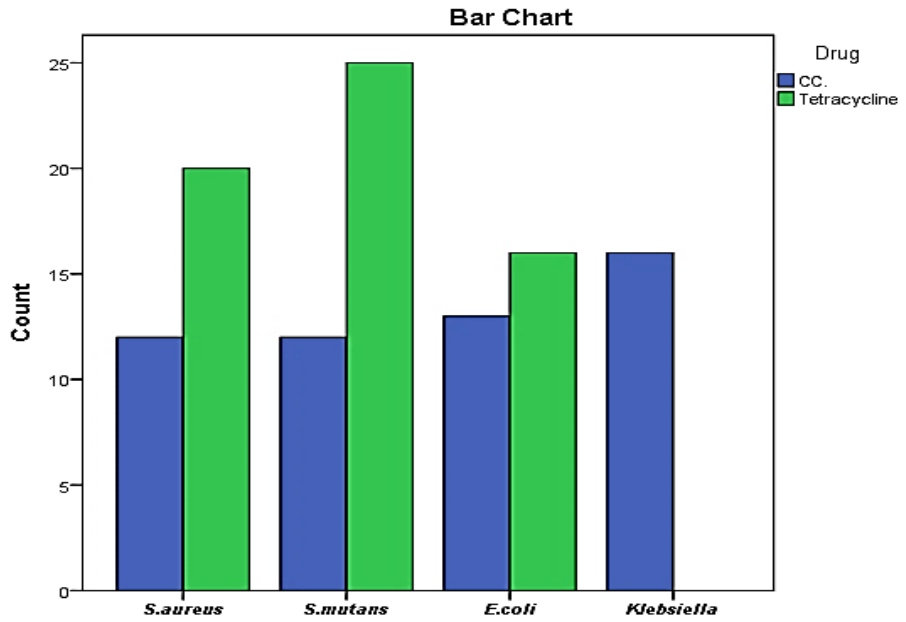


**Fig. 3:** Statistical analysis appeared significant differences between the antibacterial effect of curcumin- copper complex and tetracycline. CC.: Effect of curcumin copper complex. $X^2$: 22.42 df:3 P-value:0.01 Sig:0.02 Tab.$X^2$ :11.34.

## Effect of curcumin-copper complex against human RBCs

The color of blood solution was changed by 20000 µg/ml of the curcumin-copper complex when placed on the tube which contained the solution (2 ml) directly. In addition, the reddish black precipitate was formed at the bottom of the tube after 10 minutes of the test time period (1 hr.). The color of the blood solution that mixed with 15000 µg/ml changed but less than 20000 µg/ml, and also precipitate was observed before 30 minutes of the test time period . Relating to the test, the color of the blood solution was very slightly

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changed by using 10000 µg/ml while 5000 µg/ml had no change in the blood color . The results of the changing blood color were recorded by the naked eye, and the tested tubes were compared with negative and positive control ones. (Table 3) and ( Fig.4). Relatedly, 20000 µg/ml slightly changed in the blood color when the 100µL of the concentration mixed with 4 ml of the blood solution besides less precipitate (Fig.5).

**Table 3:** Effect of four concentrations produced by curcumin-copper complex against human RBCs at room temperature during 1 hr.

| **Effects of curcumin-copper complex on the human RBCs** | | | | |
|---|---|---|---|---|
| **Test time** | **Used concentrations** | | | |
| | 20000 µg/ml | 15000 µg/ml | 10000 µg/ml | 5000 µg/ml |
| After 10 min. | ++ | + | NT | NT |
| After 30 min. | +++ | ++ | ± | NT |
| After 60 min. | ++++ | +++ | ± | NT |

**NT**: No change in the color of blood solution. **±:** Very slightly change in the blood color. +: Slightly change in the blood color. **++:** Change in the blood color. **+++:** Continuous change in the blood color. **++++:** Increasing change in the blood color

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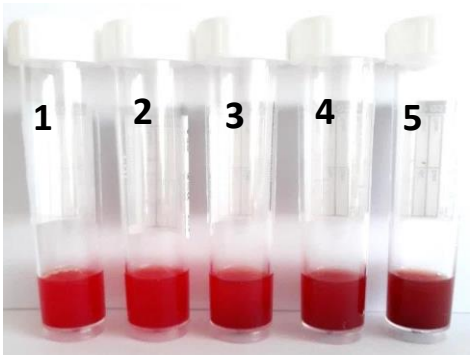


**Fig.4:** Effect of curcumin-copper complex against human RBCs.: 1: Negative control tube. 2: Positive control tube. 3: Mixing 100 µL of 10000 µg/ml with blood solution. 4: Mixing 100 µL of 15000 µg/ml with blood solution. 5:


**Fig.5:** Effect of curcumin-copper complex against human RBCs. 1: Negative control tube. 2: Positive control tube. 3: Mixing 4 ml of blood solution with 100 µL of 20000 µg/ml. 4: Mixing 2 ml of blood solution with 100 µL of 20000 µg/ml. Both control tubes contained 4 ml of the blood solution

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## GC-MS analysis

GC-MS analysis appeared six peaks which indicated to the compounds in the sample (15000 µg/ml) of curcumin-copper complex and dimethyl sulfone as well as DMSO (sample solvent). The compounds were detected by retention times were 6.19-16.60 minutes (Table 4) and (Fig.6).

**Table 4:** The compounds of the sample (15000 µg/ml) of curcumin-copper complex as well as DMSO ( sample solvent) detected by GC-MS.

| Peak | Retention time (min.) | Compounds |
|---|---|---|
| 1 | 6.19 | Dimethyl sulfoxide ( DMSO) |
| 2 | 14.69 | Dimethyl sulfone |
| 3 | 14.77 | {S-Methyl methanethiosulfinate} and {Benzenediazonium, 2-hydroxy-, hydroxide, inner salt} |
| 4 | 15.27 | {Methane, (methylsulfinyl)(methylthio)-} and {2,4-Dithiapentane} |
| 5 | 16.37 | {Silanol, dimethyl-} and {Thiodiglycol} |
| 6 | 16.43 | {2-Aminoimidazole-5-propionic acid},{1,2,4,5-Tetrathiane} and {1,2,4-Trithiolane} |
| 7 | 16.60 | {Sulfurous acid, diethyl ester}, {Ethanol, 2-[2-(4-pyridyl)ethylamino]-} and {Methanesulfinothioic acid, S-1-propyl ester} |

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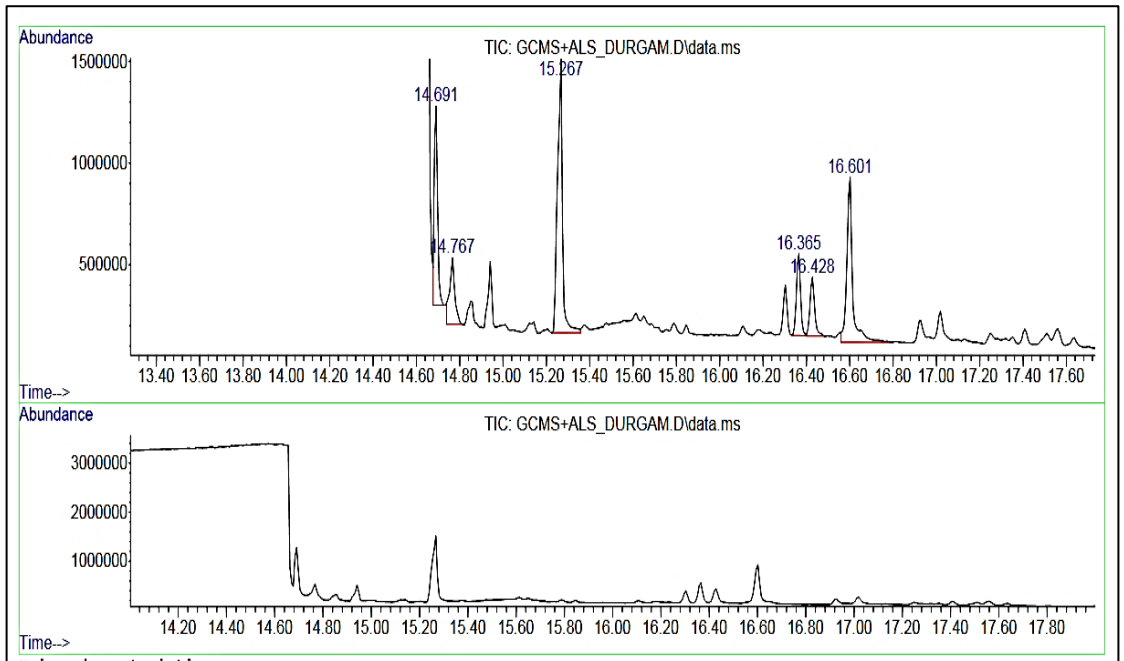


Noise characteristics:
Noise start : 14 min.
Noise end : 18 min.
Number of points: 576
Maximum noise: 3.39487e+006 abundances
Minimum noise: 77934 abundances
Peak-to-Peak noise: 3.31694e+006 abundances
Root-mean-square noise: 1.1704e+006 abundances

The table below is based on an integrated chromatogram.
Tailing calculated at 10% height.

| # | RetTime | Area | BslPH | PWidth | Resol | Tailing | PtP S/N | RMS S/N | Plates |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.19 | 13964379 | 574966 | 0.827 | 0.00 | 0.50 | 0.17 | 0.49 | 310 |
| 2 | 14.69 | 1028312 | 978267 | 0.049 | 11.46 | 1.50 | 0.29 | 0.84 | 505501 |
| 3 | 14.77 | 530759 | 327352 | 0.076 | 0.72 | 1.00 | 0.10 | 0.28 | 206791 |
| 4 | 15.27 | 2126282 | 1347119 | 0.132 | 2.83 | 0.75 | 0.41 | 1.15 | 74075 |
| 5 | 16.37 | 538410 | 402850 | 0.070 | 6.43 | 1.25 | 0.12 | 0.34 | 307174 |
| 6 | 16.43 | 431282 | 288968 | 0.090 | 0.46 | 1.25 | 0.09 | 0.25 | 183219 |
| 7 | 16.60 | 1487063 | 809885 | 0.229 | 0.64 | 1.00 | 0.24 | 0.69 | 29040 |

**Fig. 6:** GC-MS analysis of the compounds in the curcumin-copper complex. Also, DMSO ( sample solvent) was detected.

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## Discussion

Antimicrobial agents of chemotherapy drugs possess the advantages of the different affinity due to the various biochemical properties between the target (microorganisms) and humans (host). These agents are used to treat the infections due to the selective toxic effects of the agents which lead to destroy the microorganisms which cause the infections but no harm of host cells.Therefore, the concentrations of the agents are scientifically measured to be effective on the microbial pathogens and they do not affect on the host (Whalen *et al*., 2015). Researchers showed that curcumin exhibited the inhibition effects on the growth of pathogenic bacteria such as *S.aureus* has methicillin-resistance as well as *E. coli* (Mun *et al*., 2013; Tyagi *et al*., 2015). *S. mutans* was inhibited by curcumin as well as synergism of the antibiotics by curcumin example, tetracycline and cefixime led to increasing the potentiality against *S. aureus* (Moghaddam *et al*., 2009; Song *et al*., 2012; Mun *et al*., 2013; Betts and Wareham, 2014). According to the researchers, curcumin has the ability to inhibit microbial pathogens including antibiotic-resistant bacteria. The results of the current study showed that the tested microorganisms were inhibited ( Table 2) and ( Figures: 2 and 3). These results may be attributed to the antimicrobial activity of the curcumin in addition to its complexing with copper as a transition metal that has an ability for increasing antimicrobial activity.

The coordinating complexes which formed by metals of transition were interested in many researchers to study the biological activities including antibacterial effects against microorganisms. One of the complexation aims is

to modify the chemical structure of the complex by which it may increase the antimicrobial activity ( Johari *et al*., 2009). The complexation of copper with cobalt and amino acids resulted in the antimicrobial potentiality against bacteria such as *E.coli* which was sensitive to the complex of CoCl2, copper, and phenylalanine (Stanila *et al*., 2011). Also, other studies showed that there are bioactive drugs have a modification of their pharmacological and toxicological effects when given by the forms of compounds mixed with metals. Therefore, various ions of metals were used such as copper due to its ability to form complex has low molecular which can give the beneficial results for treating the several diseases (Joseph and Janaki, 2014). Copper has importance in the bio-metal aspect where it is necessary for normal metabolism of human, and diseases of nutrition deficiency. Because of its ligand bio-relevance, the copper is a good agent for pharmacological studies including the compounds have antimicrobial activity (Gonzalez-Vilchez and Vilaplana, 2005; Tisato *et al*., 2010; El-Sherif,2012). Based on the previous studies, the present study found that complexation of the curcumin with copper, a bio-relevant ligand, gave the antimicrobial activity of the curcumin. Therefore, the study was agreed with these studies. In comparison, Alhasan (2015) found that the MIC of ketoconazole ( tablet) was 2000 µg/ml against *C.albiacns*. The current study showed that *C.albiacns* was inhibited by 2000 µg/ ml of curcumin-copper complex. This indicated that the complex had inhibition like MIC of ketoconazole.

Specifically, previous studies showed that s-methyl methanethiosulfinate had antimicrobial activity against *E. coli* O157:H7. Additionally,

benzenediazonium, 2-hydroxy-, hydroxide, inner salt in the garlic clove oil extract which gave the antimicrobial effects. Relatedly, silanols had antimicrobial activity by which they were compared with alcohols and phenols. Also, chemical compounds belong esters possessed the activities against microorganisms (Moon *et al*., 2001; Kim *et al*., 2007; Johnson *et al.*, 2013; Lubenets *et al*., 2017 ). The GC-MS analysis of this study appeared that the curcumin- copper complex contains these compounds which had antimicrobial effects. It may be said the ability of the complex to inhibit the tested microorganism due to s-methyl methanethiosulfinate, benzenediazonium, 2-hydroxy-, hydroxide, inner salt, silanols, and esters as well as sulfurous acid and 2-aminoimidazole-5-propionic acid (Table 4) and ( Fig.6).

The properties of cytotoxic complexity were not increased by copper ion which revealed to be a high antioxidant agent (Morales *et al*., 2019). Three concentration (1, 10, 100 µg/ml) of free curcumin were used by which 10 µg/ml led to change in morphology of human RBCs after 30 minutes of the incubation. Also, the curcumin had effect on mean cellular volume (MCV). Depending on the dose, the toxic effects of curcumin on RBCs were recorded (Storka *et al*., 2013). Results of the current study showed that no effect on human RBCs when 5000-10000 µg/ ml of the curcumin-copper complex were used. This may indicate that copper chloride which was mixed with curcumin decreased the RBCs cytotoxic effect in spite of 15000-20000 µg/ ml had effects (Table 3 ) and ( Figures: 4 and 5).

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## Conclusions and recommondations

The curcumin-copper complex has antimicrobial activity against some microbial pathogens including gram-positive and gram-negative bacteria as well as *C.albicans*. The complex very needs to separate its compounds by using techniques of the chromatography, and they are separately tested against the microbial pathogens. After testing, we can determine the bioactive compound (s) by which it ( they) can be studying *in vivo* and clinically to evaluate it (them) as the drug (s) that will be used in the hospitals for treating microbial diseases.

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