Extraction, determination and evaluation the anti-virulence compounds of *Citrus aurantium* against multi-drug resistant *Staphylococcus aureus*

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ABSTRACT

Citrus aurantium L., sometimes referred to as the bitter or sour orange, is a member of the *Rutaceae* family (order *Sapindales*) and is indigenous to a large region of Asia (from northern China to India) and Oceania (Queensland, Australia). The present study aimed to investigate the anti-virulence compounds of *C. aurantium* extracts against *S. aureus*. Ethanol extract showed good activity against *S. aureus* at concentration (20 mg/ml), with inhibition zone diameter (24.5 mm) and lowest MIC value (2.5 mg/ml), and the least activity was recorded by acetone extract giving 12 mm inhibition zone diameter with the highest MIC value (5 mg/ml). While propanol extract showed mild antibacterial activity. The ethanol extract showed stronger effect than acetone and propanol extracts on hemolytic activity of *S. aureus* at different concentrations (0.625, 125, 250, 500, 1000 and 2000 µg/ml). The best fractionation of ethanol extract was dichloromethane that effect on hemolytic activity and protease production of *S. aureus* at concentration (1000 µl/mg). Dichloromethane fraction was utilized for gas chromatography-mass spectrometry (GCMS) analysis.

Keywords: *Staphylococcus aureus; Citrus aurantium*; Anti-virulence compounds; Hemolytic activity; Protease production.

INTRODUCTION

The World health organization (WHO) priority programmes state that microbial resistance poses a risk to the public health since it makes it harder to treat diseases brought on by resistant germs, mostly because there aren't enough conventional antibiotics available. [1]. One of the most common and ubiquitous infectious pathogens that cause sickness and death is *S. aureus*. There has recently been a steady and rising interest in the extraordinarily large quantity of toxins and other virulence agents generated by *S. aureus,* as well as how they impact illness. This bacterium may cause a diverse range of illnesses, from minor skin lesions to potentially fatal pneumonia and sepsis [2]. Methicillin-resistant MRSA (Methicillin-resistant *Staphylococcus aureus*) has just lately emerged as a common source of hospital- or community-based infections. MRSA currently accounts for ten times as many infections as all MDR Gram-negative bacteria combined. The World Health Organization (WHO) identified MRSA as one of 12 priority infections that pose a risk to human health [3]. Hospital staff members act as carriers of *S. aureus*, which is one of the most common causes of hospital-acquired infections. This contributes significantly to the spread of the illness among patients [3]. Numerous potential virulence factors are present in *S. aureus*, including inhibitors of phagocytosis like the immunoglobulin binding protein A and the capsule. Other virulence factors include extracellular proteins that encourage host tissue colonization and poisons that can destroy host tissue and subsequently induce illness symptoms. [4]. There is several virulence factors present in *S. aureus*. This is how the pharmaceutical industry might adopt a strategic approach by creating new entities that specifically target the virulence elements that cause resistance. More than 60 percent of persistent and chronic microbial illnesses are brought on by bacterial biofilms [5]. One of the most significant virulence factors in *Staphylococcus aureus* bacteria is the haemolysin protein. This substance is an extracellular toxin that is discharged into the environment and damages red blood cells, leading to hemoglobin loss in the body of the host. The Quorum sensing (QS) system controls the production of this toxin as well as other virulence features in *S. aureus* [6].

The Quorum sensing system is a method of cell-to-cell communication that regulates a variety of physiological processes, including as pathogenicity, the formation of biofilms, and bioluminescence. Bacteria use QS to communicate by synthesizing distinct signal molecules, some of which are exclusive to Gram-positive or Gram-negative bacteria while others are produced by both. These signal molecules are used for cross-talk between bacteria and their environment and

for communication between kingdoms. [7]. As a result, a unique antipathogenic technique recently used combinations of substances that may interfere with the bacteria's QS. This is why using crude plant extracts, with their distinctive structural diversities, is viewed as a potentially effective alternative method of addressing the problem of rising antibiotic resistance [8]. Current research has demonstrated that a variety of plants with various botanical origins provide excellent candidates for use as antibiotic adjuvants [9] [10]. The sour or bitter orange, *Citrus aurantium L. (Rutaceae)*, is widely consumed around the world as marmalade as an addition [11]. The plant's oils have a wide range of applications as antibacterial, antifungal, anti-inflammatory, antioxidant, and analgesic agents, and are usually regarded as safe [12], because of the different bioactive chemicals it contains, including polyphenols, essential oils, minerals, and flavonoids, *C. aurantium L.* is one of the species that has been used for therapeutic reasons. The majority of significant classes of antibiotics, such as tetracycline, cephalosporin, aminoglycosides, and macrolides, had a setback and lost their viability throughout the 19th century as a result of the increase in microbial resistance. [13]. Currently, its impact is significant due to treatment failure linked to antibiotic resistant bacteria, and this has turned into a global concern for overall health [14]. Thus, it becomes crucial to find novel antibacterial agents. In modern research, natural products continue to be one of the primary sources of novel medications. They come from eukaryotic microorganisms, prokaryotic bacteria, plants, and other animals. The majority of the antimicrobial chemicals that have been found so far come from microbial and plant sources [15].The present study describes The investigation of anti-virulence compounds of *Citrus aurantium* extracts against *S. aureus* .

MATERIALS AND METHODS

Bacterial strain

A clinical isolate *S. aureus* ACL51 was kindly obtained from Dr. Said El. Desouky, Professor of Microbiology at Faculty of Science, Al-Azhar University, 11884 Nasr City-Cairo, Egypt. The isolate has been identified to the species level consistent with the manufacturer's guidelines and verified by VITEK-2 system. The strain was stored at - 4 °C in nutrient broth (NB) with 12% (v/v) glycerol. The culture was maintained on the respective agar slant and sub-cultured at regular intervals of 14 days.

The antibiotic susceptibility test of *S. aureus*

According to guidelines established forwarded by the Clinical and Laboratory Standards Institute [16], disc diffusion was used to test overall susceptibility of antibiotics. Thirteen filter paper discs were saturated with the following antibiotics; Amoxicillin (AMC 25), Amoxicillin/clavulanic acid, Ceftriaxone (CRO30), Ampicillin-sulbactam (SAM20), Tigecycline (TGC15), Cephalosporin (CE30), Gentamycin (CN10), Ceftazidime (CAZ30), Cefotaxime (CTX30), amikacin (AK30), Cefadroxil (CFR30), Vancomycin (VA30) and Clarithromycin (CLR) (15µg or unit/disc) have been placed on the Mueller-Hinton agar plate surfaces. The agar plates with the antibiotic discs were incubated for 24 hours at 37 °C. The microbial growth response to the antibiotic discs has been calculated as the inhibition zone diameter surrounding the discs, measured in millimeters.

Plant extraction

Fresh *C. aurantium* fruits were collected and identified from the Ministry of Agriculture medicinal plants sale outlets in Giza, Egypt, [17]. For extraction, plant parts were dried at room temperature in shadow, then powdered by using a mechanical grinder (KM30) and then powder was stored in a sterile bottle at room temperature. The powder (500 g) was mixed with 3,000 ml of methanol (70%) for 7 days at room temperature. The supernatant of the mixture was filtered and then collected through filter paper. The filtrate was concentrated using rotary evaporator (Heidolh. Germany). The extracted powder was kept at -20°C until used. Successive extraction of plant extracts were performed using ethanol, 95% acetone and 95% propanol at a concentration of 1:10 (w/v) for 2 days, followed by the filtration. To get a dry crude *C. aurantiu*m extract, the solvent was removed using a vacuum rotary evaporator operating at low pressure. The finished dry crude was then stored for later use at 4 °C. The provided dissolved extract was first evaporated to obtain an orange sticky residue. The collected extract was suspended in a solution composed of 50% water and 50% methanol. The mixture was partitioned successively with n-hexane (300mL X 3), dichloromethane (300mL X 3) and ethyl acetate (300mL X 3) producing 4.3g, 2 g and 1.3g residues, respectively after evaporation under vacuum at 40°C.

Antibacterial screening by agar well diffusion method for plant extracts

The antibacterial activity of extracts was assessed using the agar well diffusion technique. *Staphylococcus aureus* ACL51 seed in the Muller Hinton agar media individually. Using a cup-borer, a well was being created in the plates after the material had hardened (6 mm in diameter). The plates were incubated overnight at 37°C with 100 µl of extract at a concentration of 20 mg/ml in each well. The diameter of the zone of inhibition was used to calculate the rate of bacterial growth. Three separate runs of the tests were completed and the mean data are shown. A negative control was done using 1% DMSO. To measure the MIC of the plant extracts, 96-well microliter plates were used in a micro broth dilution procedure [18]. *Staphylococcus aureus* ACL51 suspension was prepared in Mueller–Hinton broth to yield a cell concentration of 10^8 CFU/mL. A final volume of 200 µl was achieved in each well (180 µl bacterial suspension and 20 µl plant extract). Two control wells were maintained for each test batch. These included test control (well containing extract and the growth medium without inoculum) and organism control (the well containing the growth medium and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control wells were regarded as MIC.

Quantitative hemolytic assay

In brief, overnight culture of *S. aureus* cells were diluted to 1:100 in TSB and then cultured with or without *C.aurantiu*m peel extract and purified fractions at different concentration under MIC values at 37°C for 16 h with shaking at 250 rpm [19] . The cell cultures (100 µl including cells and culture supernatant) were added into diluted human red blood cells that had previously been separated by centrifugation at 900xg for 5 min, washed with PBS buffer three times and diluted at 3 % of red blood cells in PBS buffer. For hemolytic activity, 5 µl of bacterial culture spot inoculated on to blood agar plates, the sub-MIC of the potent *C.aurantium* extracts (1000 µl/ml) was added to bacterial culture spot and the other Spot without extract was left as positive control. The blood agar plates incubated at 37°C for 24 h. plates were visually inspected for clear zone (hemolysis) around the colony. The diameter of the clear zone is a qualitative method used as an indicator of hemolysis production [20].

Protease enzyme:

The effect of *C.aurantiu*m peel extract and purified fractions of the selected strain *S. aureus* on skim milk agar plates was screened according to the method **[21]** with little modifications . Briefly, *S. aureus* was cultured over night at 37°C and diluted 1:100 in fresh LB broth. 5 µl of bacterial culture spot inoculated on to skim agar plates, the sub-MIC of *C.aurantiu*m peel extract and purified fractions was added to bacterial culture spot and the other spot without fraction was left as positive control. The skim agar plates incubated at 37°C for 24 h. Photolytic activity results were displayed as mm diameter of clearing zone around the colonies.

Gas chromatography–mass spectrometry (GC-MS) analysis for dichloromethane of Ethanol extract:

The chemical composition were performed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The temperature of the column oven was originally maintained at 50 °C before being raised by 5 °C/min to 250 °C and maintained for 2 min. by 30°C/min, the temperature was raised to 300°C, where it was held for two minutes. temperatures were maintained for the MS transfer line and injector at 270 and 260 degrees, respectively; [22].

RESULTS AND DISCUSSION

The examination of antibiotic susceptibility of *S. aureus*

This study found that from tested *S. aureus* there was exhibited antibiotic susceptibility test of *S. aureus*, thirteen different antibiotics have been used in this experiment. The results show that six antibiotics exerted inhibitory activity against *S. aureus* growth. The inhibition zone diameters were arranged in descending order as following; 10 mm (Cefadroxil CFR30), 14 mm (Tigecycline TGC15), 20.0 mm (Vancomycin VA30), 29 mm (clarithromycin CLR15), 18 mm (amikacin AK30), and 12 mm (gentamycin CN10),Whwile *S. aureu*s was resistant to the effect of the other used antibiotics (Fig. 1). The antibiotic susceptibility profile of the bacterial isolates showed that tested *S. aureus* were multidrug-resistant to seven antibiotics, an indication that infections caused by these isolates may be difficult to treat. The World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) have

come out with reactive and preventive approaches to solving challenges of infections caused by MDR pathogens [23, 24]. The major preventive approach is to stop the spread of antibiotics resistance via Antibiotics Stewardship Programs (ASP) and the discovery of new antimicrobial drugs and/or new ways to treat MDR infection cases [24].

Antibacterial efficacy, Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Citrus aurantium* extracts (ethyl alcohol, acetone and propanol) on *S. aureus*

Bacterial susceptibility to *Citrus aurantium* extracts was shown to differ depending on the concentration and extracted methods used in the study. Ethyl alcohol extract inhibited *S. aureus* with a maximum inhibition zone of 24 ± 0.77 which demonstrate exhibited highest antibacterial activity compared with other extracted substance. Acetone and propanol extract of *Citrus aurantium* showed the lowest inhibition zone value 12± 0.13 & 13± 0.47 as compared with Ethyl alcohol extract. Using microliter plate test; we discovered that significant antibacterial activity in bacterial strains was reflected by the larger diameter of inhibition seen with lower MIC values. Ethyl alcohol extract from *Citrus aurantium* displayed highest antibacterial activity against *S. aureus* while the minimum bactericidal concentrations of ethanol, acetone and propanol extract against *S. aureus* were 5, 10 and 10 mg/ml, respectively indicating the strongest effect of extracts as demonstrated in Table 1. In general, the antimicrobial activity of the tested extracts is comparable with the standard drugs, Vancomycin. The results indicated that *Citrus aurantium* extracts showed very good inhibition activity against *S. aureus.* Similar studies [25, 26] confirmed the antibacterial properties of essential oils (EOs) and crude extracts from various parts of *Citrus reticulata* and *Citrus aurantifolia*, including their flowers, leaves, roots, stems, and peels. These substances showed broad-spectrum antibacterial activity against clinically significant pathogens, with a focus on Gram-positive bacteria, particularly *S. aureus*. The most effective component of *Citrus aurantium* that affected the antibacterial capability of EOs was limonene.

Effect of the crude extracts (n-hexane, dichloromethane and ethyl acetate) on haemolysin production:

Tested *S. aureus* which causes the complete lysis of red blood cells beta haemolysin. The selected strain was tested for ability to produce enzymes important as virulence factor haemolysin. Bacterial Haemolysin inhibition of *Citrus aurantium* crude extract showed significant hemolysis inhibiting activity ($p< 0.05$) against tested *S.auerus.* In Haemolysin quantification assay, a concentration-dependent reduction in haemolysin formation was observed in test bacterial pathogens (Fig. 1). The hemolytic activity of the *Citrus aurantium* extracts were tested on *S. aureus* under various concentrations (0.625, 125, 250, 500, 1000 and 2000 µg/ml) at sub-lethal dose of each extract. According to clinical and Laboratory Standards institute (CLSI) guidelines (CLSI, 2015), in order to detect the lethal dose of each extract and then sub lethal concentrations under MIC values of whole extracts of *Citrus aurantium* showed reducing effect to hemolytic activity of *S. aureus* strain, the hemolytic activity on *S. aureus* strain when used ethyl alcohol extract was reduced to 29.3% when supplemented with this extract at concentration of 2000 µg/ml with slightly affecting the planktonic growth when compared with the positive control. On other hand, the result exhibits that no significant different between the treated and untreated on hemolytic activity on *S. aureus* when useingAcetone and Propanol extract supplemented with gradient concentration under minimum inhibitory concentration (Table 2 & figure 2). Erythrocytes are very susceptible to oxidative stress-induced stress. This sensitivity is linked to their plasma lipid structure, which is rich in polyunsaturated fatty acids, constant exposure to oxygen, and the production of ROS by activated inflammatory cells [27]. Furthermore, the existence of significant amounts of hemoglobin red blood cells to high rates of oxidation on it [28]. As a result, hemolysis is commonly used as an essential indication of free radical damage to erythrocyte membranes [29].

Effect of the three fractions (n-hexane, dichloromethane and ethyl acetate) of ethanol extracts on Hemolytic activity:

The hemolytic activity of three fractions (n-hexane, dichloromethane and ethyl acetate) of ethanol extracts on tested *S.auerus* strain at the concentration (1000µg/ml) of this dichloromethane extract put the high reduction level was 72.8% with concentration of 1000µg/ml when compared with the positive control without affecting the planktonic growth. Also, lowering efficacy on hemolytic activity was n-hexane and ethyl acetate extract was 28.2 and 30% respectively

when compared with the positive control without affecting the planktonic growth as shown in figure (3).

Protease production:

The selected strain was tested for ability to produce protease enzymes as virulence factor before and after treatment with the sub MIC from (1000µl / ml) of the three fractions. The obtained results demonstrated that dichloromethane only the fraction that had the ability to affect the tested bacteria lost its ability to produce extracellular protease in skim milk agar plate compared with the positive control (Fig. 4). Our result correlates with Nwankwo *et al* (2023) who said that the greatest level (1.0 mg/mL) at which *Sida linifolia* ethanol leaf fraction exhibited its greatest inhibition was found [30]. The leaf fraction ability to suppress protease activity may be due to the abundance of phytochemicals like kaempferol, catechin and other flavonoids that are known to do so. Many inflammatory illnesses have been linked in studies to lysosomal leakage or immune cells excessive release of proteases [31]

Identification of Bioactive Compounds by GC-MS chromatogram

Total 42 compounds have been identified by GC–MS analysis of dichloromethane fraction of ethyl alcohol extract of *citrus uranium* (Fig.4). GC-MS analysis showed the presence of organic acids, phenols, ketones, steroids and esters. The high-level chemicals in the dichloromethane fraction were 3',5'-Dimethoxyacetophenone (11.67%) at Retention Time 14.64 , 1,2-Cyclohexanediol,1-methyl-4-(1-methylethenyl) (10.69%) at Retention Time 10.35 , á-Sitosterol (9.09%) at Retention Time 35.67, 1,2-15,16-Diepoxyhexadecane (7.40%) at Retention Time 15.40 , n-Hexadecanoic acid (7.33%) at Retention Time 22.02 and 9,12-Octadecadienoyl chloride,(Z,Z) (6.26%) at Retention Time 24.57 (table 5) .

Many pharmacological effects of essential oils, including fatty acids and organic acids, have been reported, including anti-microbial, antioxidant, insecticide, antinociceptive activity and anti-helminthic properties [32]. Similarly, Hexadecanoic acid, 1-(hydroxymethyl)-2-hydroxyethyl ester is a palmitic acid ester used in medicines that is similar to n-Hexadecanoic acid. According to Lalitha et al., *Psychotria nilgiriensis*, it had a 3.01% composition [33]. Nonetheless, the GC-MS analysis of *citrus uranium* in the current investigation revealed a 3.27% level as shown in figure (5). The ester derivative n-Hexadecanoic acid's 5-reductase activity was suppressed, and it also possesses antimicrobial, anti-cancer, anti-anemic, anti-diabetic and pesticide characteristics [34]. Many studies have examined the effects of various fatty acids and their methyl esters on biofilms [35, 36].

Guzzo et al. showed the effectiveness of caffeine and oleanolic acid derivatives against clinical isolates of *P. aeruginosa* in terms of both anti-swarming and anti-biofilm properties. Another investigation, however, demonstrated the Hexadecanoic acid Methyl Ester anti-*Pseudomonas aeruginosa* activity[37]. Benzaid et al. isolated the essential oil from *C. L. aurantium* and investigated its biological characteristics. Linalool was present in less amounts than limonene in the isolated EO. These goods exhibit a number of qualities, including anti-stress, anti-inflammatory ,antioxidant and perhaps disease-preventing characteristics [38, 39]. The antibacterial qualities of linalool and limonene were demonstrated [40], supporting the potential application of *C. aurantium* L. EO to regulate the development of different bacterial strains. Growth inhibition may lessen the development and disruption of biofilms [41].

CONCLUSIONS

This study revealed that *C. aurantium* extracts have inhibitory effect on growth and blood hemolysis of *S. aureus*. Ethanol extract has the most effects on blood hemolysis than propanol extract, but acetone extract does not have effect on blood hemolysis of *S. aureus* at the concentration used. Dichloromethane fraction showed a stronger inhibition on hemolytic activity and photolytic activity of *S. aureus* than n-hexane fraction and ethyl acetate fraction at the same concentration. The major constituents of dichloromethane fraction of ethyl alcohol extract of *C. aurantium* were 3',5'-Dimethoxyacetophenone , 1,2-Cyclohexanediol,1-methyl-4-(1-methylethenyl), á-Sitosterol , 1,2-15,16-Diepoxyhexadecane , n-Hexadecanoic acid and 9,12-Octadecadienoyl chloride.

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Table 1: The antibacterial efficacy and minimum inhibitory concentration (MIC) of *Citrus aurantium* extracts against *S. aureus*

solvents	*S. aureus*			
	ZI(mm)	MIC(mg /ml)	MBC (mg/ml)	Positive control (vancomycin30)(mm)
Ethanol extract	24 ± 0.77	2.5±0.8	5±0.41	20±1.9
Acetone extract	12± 0.13	5±0.62	10±1.2	20±2.1
Propanol extract	13± 0.47	5±0.36	10±0.87	20±0.98

Table 2: Anti-hemolytic effect *C. aurantium* extracts (ethyl alcohol, acetone and propanol) against *S. aureus*

C. aurantium extracts	Concentration µg/ml	Growth O.D $_{600}$	Hemolysis %
Ethyl alcohol extract	C_{+ve}	1.201±0.017	100
	Blank	0.016±0.023	10.07
	2000	0.75±0.015	29.3
	1000	1.28±0.013	53.2
	500	1.39±0.006	43.4
	250	1.39±0.051	22.9
	125	1.31±0.014	21.1
	0.625	1.36±0.034	15.3
Acetone extract	C_{+ve}	0.702±0.002	100
	Blank	0.056±0.024	11.5
	2000	0.625±0.014	16.3
	1000	0.833±0.012	14.2
	500	0.800±0.023	15.3
	250	0.800±0.023	10.2
	125	0.747±0.014	9.3
	0.625	0.800±0.023	11.2
Propanol extract	C_{+ve}	0.799±0.060	100
	Blank	0.058±0.003	14.36
	2000	0.89±0.019	15.05
	1000	0.939±0.005	13.27
	500	0.865±0.022	16.4
	250	0.845±0.005	17.7
	125	0.845±0.005	12.2
	0.625	0.846±0.012	14.5

Table 3: Effect of three fractions (n-hexane, dichloromethane and ethyl acetate) on Hemolysis test for *S. aureus*

Fractions	Treatment	Growth O.D $_{600}$	Hemolysis	
			O.D $_{546}$	%
n-hexane	C_{+ve}	1.206±0.018	0.571±0.032	100
	Blank	0.026±0.015	0.0575±0.009	10.07
	1000 µg/ml	0.616±0.035	0.161±0.021	28.2
dichloromethane	C_{+ve}	0.712±0.002	0.702±0.029	100
	Blank	0.056±0.024	0.081±0.002	11.5
	1000 µg/ml	0.513±0.021	0.51±0.021	72.8
ethyl acetate	C+ve	0.81±0.060	1.01±0.001	100
	Blank	0.058±0.003	0.11±0.011	14.36
	1000 µg/ml	0.99±0.019	0.31±0.005	30.05

Table 4: Effect of three fractions (n-hexane, dichloromethane and ethyl acetate) on protease production for *S. aureus*

Treatment	Growth	Protease		
		24 h	48 h	72h
0 C+ve	+	+	++	+++
0 Blank	-	-	-	-
n-hexane	+	+	++	+++
Dichloromethane	+	-	-	-
Ethyl acetate	+	+	++	+++

+: produced, ++: increased, +++: more increasing and -: absent

Table 5: Phytoconstituents identified in the dichloromethane fraction of ethyl alcohol extract of *citrus uranium*

Peak No.	Components identified	Retention Time (min)	Peak Area (%)	Molecular Formula	Molecular Weight
1	l-Menthone	5.87	1.03	C10H18O	154
2	Levomenthol	6.37	3.21	C10H20O	156
3	4-Amino-1,5-pentandioic acid	8.68	2.89	C7H13NO4	175
4	2-Methoxy-4-vinylphenol	9.46	5.40	C9H10O2	150
5	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)	10.35	10.69	C12H20O3	212
6	2,7-Octadiene-1,6-diol, 2,6-dimethyl	10.87	0.99	C10H18O2	170
7	Caryophyllene	11.11	0.51	C15H24	204
8	cis-5,8,11,14,17-Eicosapentaenoic acid	11.32	0.30	C20H30O2	302
9	Nerolidol-epoxyacetate	11.82	0.37	C17H28O4	296
10	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3 -yl ester	11.97	0.27	C16H26O2	250
11	Limonen-6-ol, pivalate	12.31	1.05	C15H24O2	236
12	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dime thyl-7-(1methylethenyl)-,[1S-(1à,7à,8aà)]	12.63	1.73	C15H24	204
13	trans-Sesquisabinene hydrate	13.26	0.83	C15H24	204
14	Cholestan-3-ol, 2-methylene-,(3á,5à)	13.61	0.45	C28H48O	400
15	Cholestan-3-ol, 2-methylene-,(3á,5à)	13.93	0.30	C28H48O	400
16	3,4,5,6-Tetramethoxyphenol	14.01	0.55	C16H24	216
17	3',5'-Dimethoxyacetophenone	14.64	11.67	C10H12O3	180
18	1,2-15,16-Diepoxyhexadecane	15.40	7.40	C16H30O2	254
19	n-Decylsuccinic anhydride	15.49	0.31	C14H24O3	240
20	Cholestan-3-ol, 2-methylene-,(3á,5à)	15.77	0.16	C28H48O	400
21	Lactaropallidin	16.94	0.65	C15H24O3	252
22	2-[4-methyl-6-(2,6,6-trimethylcycloh ex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	17.07	0.32	C23H32O	324
23	2,5,5,8a-Tetramethyl-4-methylene-6,7 ,8,8a-tetrahydro-4H,5H-chromen-4a-y l hydroperoxide	18.84	0.94	C14H22O3	238
24	6,9,12,15-Docosatetraenoic acid, methyl ester	19.01	0.34	C23H38O2	346
25	Cholestan-3-ol, 2-methylene-,(3á,5à)	19.29	0.41	C28H48O	400
26	Hexadecanoic acid, methyl ester	20.57	1.15	C17H34O2	270
27	Hexadecanoic acid, ethyl ester	21.67	3.27	C18H36O2	284
28	n-Hexadecanoic acid	22.02	7.33	C16H32O2	256
29	methyl ester of 9,12-octadecadienoic acid, (E,E)	23.20	2.10	C19H34O2	294

30	methyl ester of 11-octadecenoic acid	23.30	1.43	C19H36O2	296
31	Linoleic acid ethyl ester	24.23	5.45	C20H36O2	308
32	Ethyl Oleate	24.31	2.80	C20H38O2	310
33	Chloride of 9,12-octadecadienoyl, (Z,Z)	24.57	6.26	C18H34O2	282
34	Octadecanoic acid	24.82	0.74	C18H36O2	284
35	Imazalil	24.92	0.39	C14H14Cl2N2O	296
36	Di-n-octyl phthalate	29.47	0.27	C24H38O4	390
37	2,2,4-Trimethyl-3-(3,8,12,16-tetrame thyl-heptadeca-3,7,11,15-tetraenyl)-c yclohexanol	32.73	0.23	C30H52O	428
38	Stigmast-5-en-3-ol, (3á,24S)	34.51	0.18	C29H50O	414
39	Vitamin E	34.77	3.57	C29H50O2	430
40	Ethyl iso-allocholate	35.18	0.19	C26H44O5	436
41	Pregnan-20-one, cyclic 20-(1,2-ethanediyl acetal), 3-(acetyloxy)-5,6-epoxy-16-methyl (3á,5à,6à,16à)	35.36	2.78	C26H40O5	432
42	á-Sitosterol	35.67	9.09	C29H50O	414

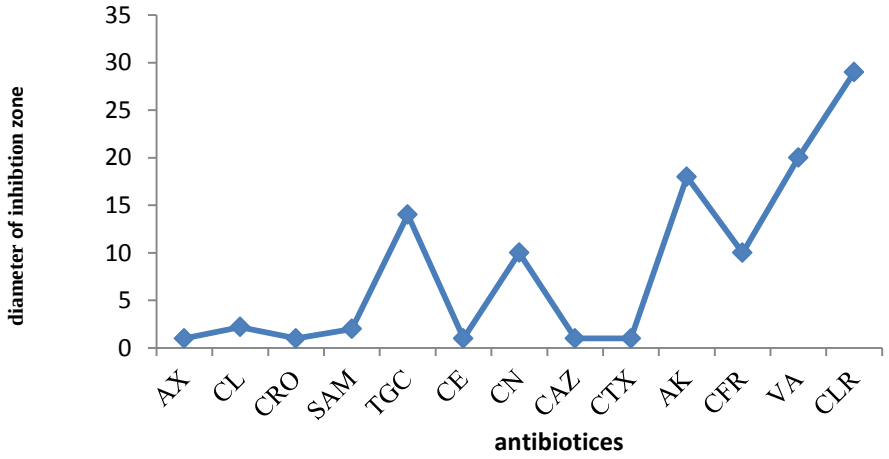


Figure 1: The *S. aureus* antibiotic sensitivity test displaying the inhibition zone diameter (mm) created by: Tigecycline TGC15, 14 mm; CFR30, 10 mm; VA30, 20 mm; CLR15, 29 mm; AK30 ,18 mm; and CN10, 12 mm.

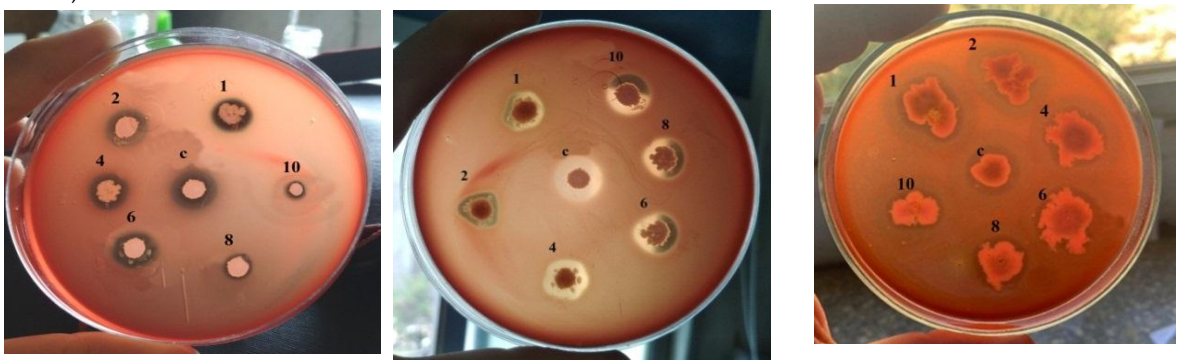


Figure 2: Effect of sub inhibitory concentrations of *Citrus aurantium* extracts on hemolytic activity of *S. aureus* 1) 0.625 µg/ml; 2) 125µg/ml; 4) 250µg/ml; 6) 500µg/ml; 8) 1000µg/ml; 10) 2000µg/ml; c) 0 µg/ml

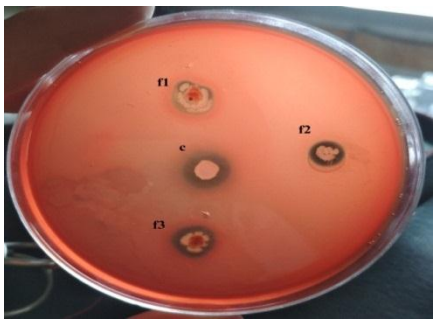


Figure 3: Effect of three fractions (n-hexane, dichloromethane and ethyl acetate) on hemolysis test for *S. aureus* c) positive control; f1) n-hexane; f2) dichloromethane; f3) ethyl acetate)

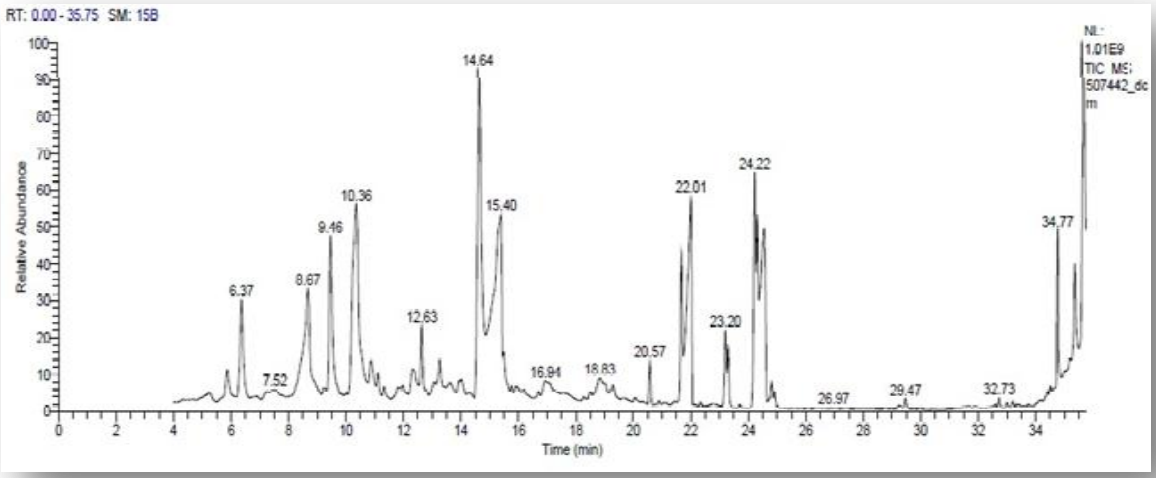
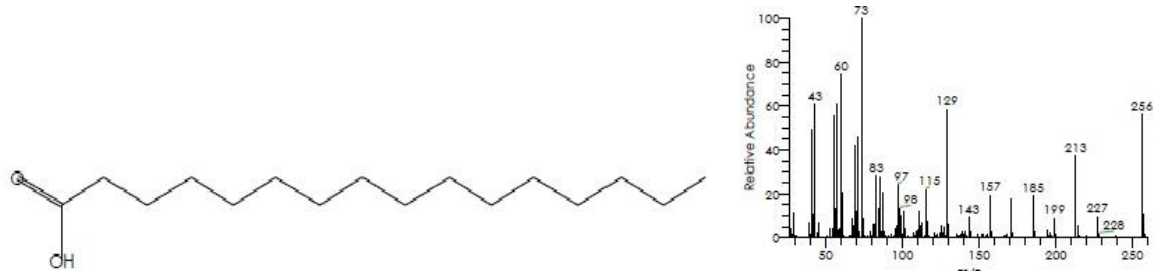
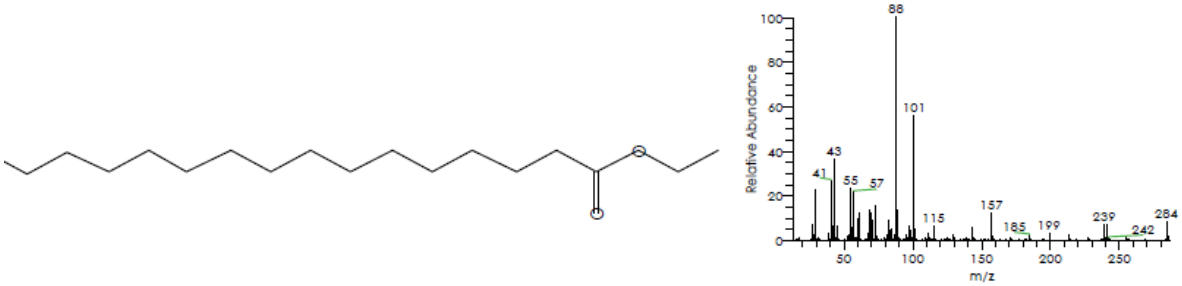


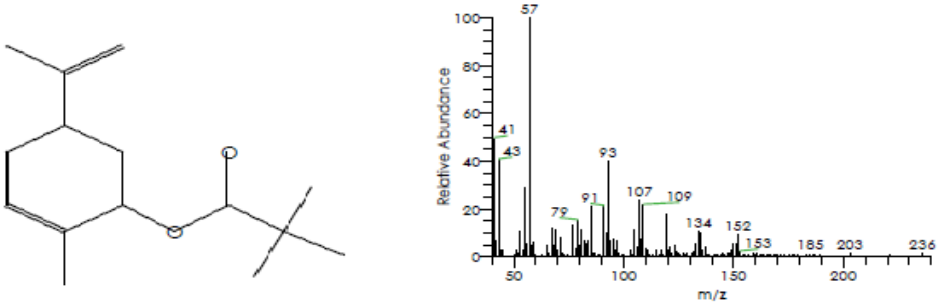
Figure 4: GC-MS chromatogram of dichloromethane fraction.



n-Hexadecanoic acid



Hexadecanoic acid, ethyl ester



Limonen-6-ol, pivalate

Figure 5: The chemical structure for both Hexadecanoic acid, ethyl ester and n-Hexadecanoic acid and Limonen-6-ol, pivalate

استخلاص وتحديد وتقييم المركبات المضادة لعوامل الضراوة للنارنج ضد المكورات العنقودية الذهبية المقاومة للعديد من المضادات الحيوية

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الملخص العربى:

النارنج يشار إليه أحيانًا باسم البرتقال المر أو الحامض، وهو عضو في عائلة الجذور (رتبة سباينداليس) وهو موطن لمنطقة كبيرة في آسيا (من شمال الصين إلى الهند) وأوقيانوسيا (كوينزلاند، أستراليا). هدفت الدراسة الحالية إلى فحص المركبات المضادة للفوعة لمستخلصات النارنج ضد المكورات العنقودية أظهر مستخلص الإيثانول فعالية جيدة ضد بكتريا S. aureus بتركيز (20 مجم / مل)، بقطر منطقة التثبيط (24.5 مم) وأدنى قيمة MIC (2.5 مجم / مل)، وأقل فعالية سجلت بواسطة مستخلص الأسيتون بإعطاء تثبيط 12 مم. قطر المنطقة بأعلى قيمة MIC (5 مجم / مل). بينما أظهر مستخلص البروبانول نشاطًا خفيفًا مضادًا للبكتيريا. أظهر مستخلص الإيثانول تأثيراً أقوى من مستخلصي الأسيتون والبروبانول على النشاط الانحلالي للمكورات العنقودية الذهبية بتركيزات مختلفة (0.625 ، 125 ، 250 ، 500 ، 1000 و 2000 ميكروغرام / مل). كان أفضل تجزئة لمستخلص الإيثانول هو ثنائي كلورو ميثان الذي يؤثر على النشاط الانحلالي وإنتاج الأنزيم البروتيني من المكورات العنقودية بتركيز (1000 ميكرولتر / مجم)، كما تم استخدام جزء ثنائي كلورو ميثان لتحليل كروماتوجرافيا الغاز - قياس الطيف الكتلي .

الكلمات الاسترشادية: المكورات العنقودية الذهبية, النارنج, المركبات المضادة لعوامل الضراوة, النشاط الانحلالى, انتاج الانزيم البروتينى.