

**Special Issue:**

Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

The Potential of Cinnamon (*Cinnamomum verum*) Bark Extract Oil as an Alternative Antibacterial Effect Against *Staphylococcus aureus*

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Abstract | The aim of this study was to investigate and compare the antimicrobial effect of cinnamon bark extract essential oil (EO) against *Staphylococcus aureus* isolated from Middle ear infection in dogs. Cinnamon EO was chemically characterized by gas chromatography/mass spectrometry (GC-MS). The minimum inhibitory concentration (MIC), diffusion performance and the minimum bactericidal concentration (MBC) of cinnamon EO were determined. Analysis showed that *Staphylococcus aureus* was phenotypically identified by growth characteristics on the special culture media. Also, the biochemical properties were identified via the Vitek 2 Compact system. On hydro-distillation of bark of *C. verum* (essential oil) was obtained and stem bark yielded 0.9% (w/w) yellowish transparent oil. These extracts were characterized by GC-mass spectroscopy which indicated that cinnamaldehyde and eugenol were the major components of the oxygenated fraction (43.15% and 11.75% relative to oil, respectively). The MIC results showed that the concentration of 12.5 mg/ml of cinnamon oil and 25 mg/ml of cinnamon oil extract the MBC. In the well diffusion assay the inhibition zones directly proportion with the cinnamon oil extract concentration. Taken together, the results demonstrate the antibacterial effect of cinnamon EO and may offer alternative to traditional antibiotics.

Keywords | Cinnamon, *Staphylococcus aureus*, Bark extract**Received** | August 15, 2025; **Accepted** | September 24, 2025; **Published** | October 14, 2025***Correspondence** | Ahmed Saad, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; Email: hadadahmed234@gmail.com**Citation** | Saad A, Yahya NZ (2025). The potential of cinnamon (*Cinnamomum verum*) bark extract oil as an alternative antibacterial effect against *Staphylococcus aureus*. J. Anim. Health Prod. 13(s1): 550-557.**DOI** | <https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.550.557>**ISSN (Online)** | 2308-2801**Copyright**: 2025 by the authors. Licensee ResearchersLinks Ltd, England, UK.This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium, and it is a major pathogen in humans and animals, causing a wide variety of illnesses ranging from skin and soft tissue infections to life-threatening invasive diseases (Chessa *et al.*, 2015). *Staphylococcus aureus* is a major opportunistic pathogen and is one of the most important pathogenic *Staphylococcus* species in veterinary medicine. *S. aureus* is dangerous because of its deleterious effects on animal health and its potential for transmission from animals to humans and vice-versa (Peton and Loir, 2014). The history of *S. aureus* treatment is marked

by the development of resistance to each new class of anti-staphylococcal antimicrobial drugs, including the penicillins, sulfonamides, tetracyclines, glycopeptides, and others (David and Daum, 2017). Because of a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance, the bacterium is a versatile pathogen capable of causing a wide variety of human diseases (Sadiq and Yahya, 2021; Abed *et al.*, 2024). The use of herbs and medicinal plants as the first medicines is a universal phenomenon (Al-Khafaji, 2013).

Medicinal plants may be defined as any plant that can be put to culinary or medicinal use such as garlic and

Cinnamon. Cinnamon is one of the most important herbal drugs and has been widely used in Asia for more than 4000 years. It belongs to the laurel family (Nafia, 2012), as per the National Center for Complementary and Integrative Health (NCCIH). Cinnamon mainly contains essential oils and important compounds like cinnamaldehyde, eugenol, cinnamic acid and cinnamate (Hameed *et al.*, 2016). Cinnamon has been traditionally applied to the treatment of inflammatory disorders and gastric diseases. After chemical profiling of cinnamon's components, their biological activities including antimicrobial, antiviral, antioxidant, antidiabetes, gastroprotective and immunomodulatory were reported by many investigators (Khudair *et al.*, 2024; Mustafa and Wasman, 2020).

Therefore, the purpose of this study was to assess the antibacterial activity of the *in vitro* extract of cinnamon bark to use against *Staphylococcus aureus* infection as an alternative therapy.

MATERIALS AND METHODS

PLANT MATERIAL (BARK OF CINNAMON)

The classification of the plant was carried out in Abu Graib, Baghdad by the Ministry of Agriculture, State Board for Seed Testing and Certification S.B.S.T.C.

COLLECTION OF PLANT

Cinnamon barks (*Cinnamomum*) were purchased from an open market in Baghdad (Al-Rashed Street) and were identified by The National Herbarium in Baghdad province. The dried barks were ground into powder using a mechanical grinder.

PLANT EXTRACTION

On hydro-distillation of bark of *C. verum*, essential oil was obtained while stem bark yielded (w/w) yellowish transparent oil which turned red after the storage as described by Yu *et al.* (2020).

EXTRACTION PERCENTAGE YIELD OF ESSENTIAL OIL

The collected *C. verum* sample was used to extract the essential oil by hydro distillation method. The extraction yield has been accomplished by the equation below (Tambe and Gotmare, 2020; Aboktifa *et al.*, 2025):

$$\text{Oil yield \%} = \frac{\text{Volume of oil extracted} \times 100}{\text{weight of the sample}}$$

GC-MASS ANALYSIS

The GC-MS analysis was conducted via an Agilent 7820A GC system connected to a mass spectrometer (Agilent, USA). The analytical column employed was an Agilent HP-5 MS Ultra Inert (30 mm × 250 μm × 0.25 μm).

BACTERIAL SAMPLE COLLECTION

Staphylococcus aureus was isolated from Middle ear infection in dogs. The swabs were inoculated in mannitol, and blood agar were streaked from broth culture and incubated as mentioned above. Microorganisms were isolated in pure culture and identified by conventional methods for bacteria which showed growth of typical colonies (Barrow and Feltham, 2004; Al-Nassry, 2011).

PREPARATION OF DIFFERENT CONCENTRATIONS OF CINNAMON OIL EXTRACT

By mixing 1 ml, stock solutions were prepared. Cinnamon bark oil extract with a dimethyl sulphoxide DMSO to achieve a sample concentration 10%. Concentrations of 10 to 0.1562% were then prepared by combining the known amount with Dimethyl sulphoxide (DMSO) from the stock solution (Hovijitra *et al.*, 2016; Flayyih and Majeed, 2012).

DETERMINATION OF (MIC) OF THE CINNAMON OIL

For MIC for cinnamon oil, two-fold serial dilution of the cinnamon oil was prepared by first reconstituting it in DMSO. It was then diluted in sterile DMSO to achieve a decreasing concentration range of 10 to 0.1562% (v/v) was prepared. The microdilution studies were carried out in sterile 96-well microplates with a U-shaped bottom. All of the wells of the plates were filled with 100 μL of broth (Mueller Hinton broth) (Abdelatti *et al.*, 2023).

A total of 100 μL of diluted of the cinnamon oil were added to the first well of each column (columns 1 to 7), (serial dilutions were performed by passing 100 μL of cinnamon oil through wells 1 to 7 of the lines (10, 5, 2.5, 1.25, 0.625, 0.3125, 0.1562 %). Then, 10 μL of the relevant standardized inoculum (10⁶cfu/ml of *S. aureus*) was put to each test hole. For the negative control (column 8), 200 μL of Mueller Hinton was added to the blank wells that were left without microorganisms. The plates were incubated for 24 h at 37 degrees Celsius. After incubation, 20 ml of 0.125 percent (w/v) TTC solution was added to each well, and the plates were incubated for another 2 h (Veiga *et al.*, 2019).

DETERMINATION OF ANTIBACTERIAL ACTIVITY OF CINNAMON OIL

The antibacterial activities of cinnamon oil were determined by the agar well diffusion method (Lafta and Sadeq, 2024). *S. aureus* was first sub-cultured in nutrient broth at 37°C for 24 h (Sreeshma *et al.*, 2022). Standardized inoculum (10⁶ CFU/ml; 0.5 MacFarland) of *S. aureus* was mixed sterile Muller-Hinton Agar so as to achieve confluent growth. The plates were allowed to dry, and a sterile cork borer (6 mm diameter) was used to bore wells in the agar. Subsequently, a 50 μl volume of the different concentration of oil (5, 2.5, 1.25, 0.625, 0.3125%) was introduced in

triplicate wells of the agar lates. Sterile DMSO served as negative control. The plates were allowed to stand for at least 1 h for diffusion to take place and then incubated at 37°C for 24 h. The zone of inhibition was recorded to the nearest size in mm (Boorn *et al.*, 2010).

TIME KILL-ASSAY

The time-kill curve assay of cinnamon extract against *S. aureus* were based on the National Committee for Clinical Laboratory Standards (NCCLS). Briefly, bacterial suspension equivalent to 0.5 McFarland (1.5 x 10⁸ CFU/ml) was prepared from overnight bacterial culture. 0.1 ml of the prepared bacterial suspension was diluted in 14.9 ml of Mueller-Hinton broth and incubated at 37°C for 1 h to obtain 10⁶ CFU/ml bacterial suspensions, after that, cinnamon extract and concentrations from 4x MIC to 0.25x MIC were prepared. Bacterial colonies were calculated at 0, 1, 2, 4, 6, and 24 h through the incubation time by making serial dilutions and spreading of 20 µl of each dilution on Mueller-Hinton agar plate (triplicate); colonies range 30-300 CFU/plate was accepted (Miles *et al.*, 1938; Zykov *et al.*, 2018). The trapezoidal method was used to estimate the area under the time kill curve of concentration of extract (Chiou, 1978):

$$AUC_{kill} = \sum \left(\frac{\log(C_n) + \log(C_{n+1})}{2} \cdot \Delta t \right)$$

Where the AUC kill is the area under the killing curve of cinnamon extract, log (C_n) is the logarithm of a specific concentration at a specific time, log (C_{n+1}) is the logarithm of the next concentration while (Δ t) resemble the difference between their times.

RESULTS AND DISCUSSION

BIOCHEMICAL CHARACTERIZATION OF STAPHYLOCOCCUS AUREUS

Identification of *Staphylococcus* isolate as illustrated in Figure 1 were performed by using the conventional culture methods on enrichment and selective media, revealing the colonies of *Staphylococcus* spp. Blood agar, colonies of *Staphylococcus aureus* are frequently surrounded by a clear zone of hemolysis i.e., beta-hemolysis (Hatem, 2017). On the other hand, the appearance of colonies on mannitol agar were converted to yellow (Subhi *et al.*, 2017) (Figure 1).

All isolates were streaked on mannitol salt agar which is considered as selective and differential medium for the isolation, purification and identification of *Staphylococci*, and for detecting the ability of each isolate to ferment mannitol. The isolates appear Gram positive cocci mostly arranged in grape-like irregular clusters. These findings were in agreement with previous studies (Hatem, 2017).

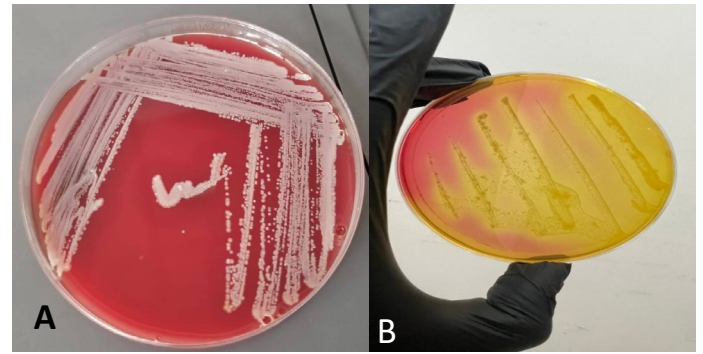


Figure 1: Colonies of *Staphylococcus* isolate (A) blood agar, (B) Mannitol agar.

Table 1: Distribution of bacterial isolates from ear swabs (n = 75) confirmed by VITEK 2 system.

Bacteria	Number of samples	Percentage (%)
<i>S. aureus</i>	38	50.6
<i>E. coli</i>	21	28
<i>Streptococcus</i>	9	12
<i>Klebsiella spp</i>	4	5.3
<i>Pseudomonas spp</i>	2	2.6
<i>S. grimesii</i>	1	1.32
Total	75	100

DIAGNOSIS OF STAPHYLOCOCCUS AUREUS BY VITEK-2 SYSTEM

All positive isolates of *Staphylococcus aureus* that isolated from human swabs were confirmed with VITEK 2 systems, which have high sensitivity and specificity (Table 1). VITEK 2 systems are characterized by the ability to diagnose bacterial isolates faster and more efficiently. Using (64) biochemical tests for diagnosis with special kits and away from the contamination that may cause confusion in the detection of the pathogen (Mohamed *et al.*, 2023).

CINNAMON (CINNAMOMUM VERUM) BARK OIL EXTRACTION

On hydro-distillation of bark of *C. verum*, essential oil was obtained while stem bark yielded 0.9% (w/w) yellowish transparent oil which turned red after the storage as show in Figure 2. The essential oil has a sharp fragrant odor. The morphological characters were almost like earlier studies, however, oil composition varied substantially (Jayaprakasha *et al.*, 2002; Malsawmtluangi *et al.*, 2016).

GC-MASS ANALYSIS OF CINNAMON (CINNAMOMUM VERUM) BARK EXTRACT

Byusing the National Institute of Standards and Technology (NIST) database, the compound's name, chemical formula, peak area, and biological activity were determined (Figure 3). The relative proportion was determined by comparing its average peak area to the total area. Analysis of cinnamon

oil indicated that cinnamaldehyde (Bicyclo[4.2.0]octa-2,4-diene-7-carbonitrile) and eugenol (1H-Purin-6-amine, N-(3-methyl-2-butenyl) were the major components of the oxygenated fraction (43.15% and 11.75% relative to oil, respectively) as listed in Figure 3. Camphene was the major component of the hydrocarbon fraction. Generally, cinnamon oil is characterized by high levels of oxygen-containing monoterpenes, including cinnamaldehyde, which is the main component of cinnamon essential oil.

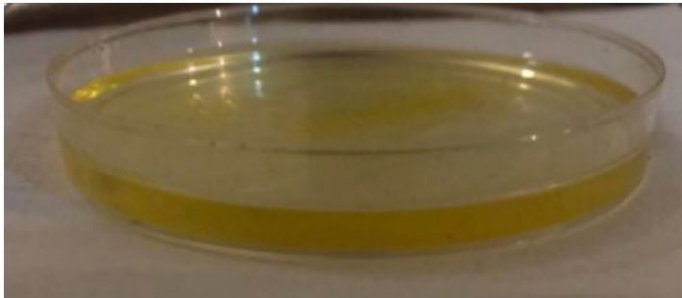


Figure 2: Cinnamon oil.

As a result, the chemical components and antioxidant impact, this study looked at the antibacterial properties of *C. verum* essential oil. As shown in Figure 3, GC-MS analysis revealed the presence of 17 chemical components in *C. verum* essential oil. The primary components of the essential oil were (E)-cinnamaldehyde (43.15%), linalool (11.57%), -caryophyllene (6.45%), eucalyptol (5.42%), and eugenol (4.61%). p-cymene (1.91%), - humulene (1.71 %), -cadinene (1.42%), - pinene (1.33%) and limonene (1.23%). Several investigations have revealed that cinnamaldehyde is the predominant chemical constituent in essential oil from the bark of *C. verum* (Jantan *et al.*, 2008; Unlu *et al.*, 2010), which supports our findings (Meena *et al.*, 2012, Al-Timimi, 2024).

MINIMUM INHIBITORY CONCENTRATION OF CINAMMON EXTRACT

The results showed that the concentration 1.25% of cinnamon oil was effective to prevent *S. aureus* from growing,

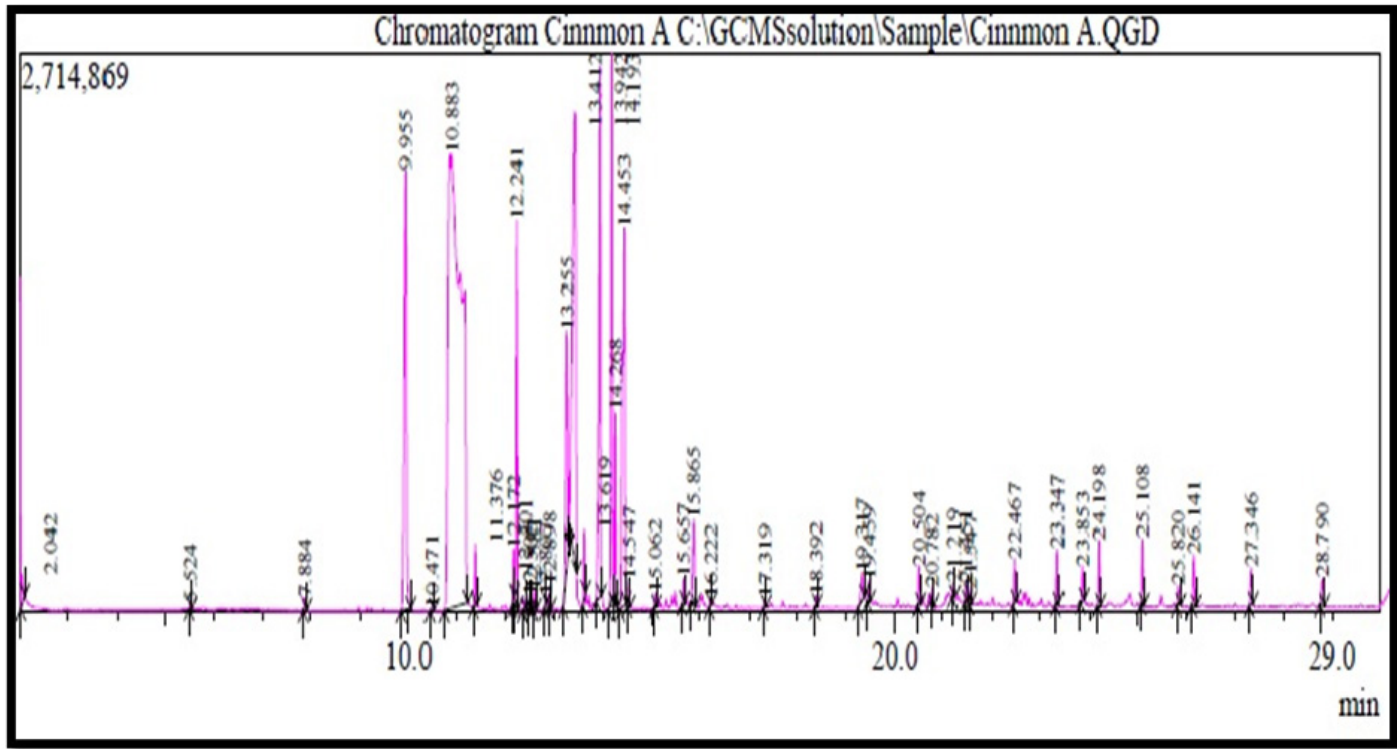


Figure 3: GC-mass analysis of cinnamon (*Cinnamomum verum*) bark extract.

Table 2: Antibacterial activity of cinnamon oil.

Conc.	10	5	2.5	1.25	0.652	0.3125
Zone of inhibition	23.0±0.52A a	19.0±0.21A a	16.0±0.14B a	14.0±0.27C a	12.0±0.17D a	10.0±0.23E a
DMSO 50%	0.0±0.0A b	0.0±0.0A b	0.0±0.0A b	0.0±0.0A b	0.0±0.0A b	0.0±0.0A b

Values represent mean ±S. E. Different capital letters mean significant (P<0.05) results between different concentrations. Different small letters mean significant (P< 0.05) results between solvent and oil.

Table 3: Area under the time-kill curve of cinnamomum verum bark extract against *S. aureus*.

Groups	Control	0.25 MIC	0.5 MIC	1 MIC	2 MIC	4 MIC
MIC:1.25	522.38±	487.21±	245.25±	88.42±	64.17±	48.92±

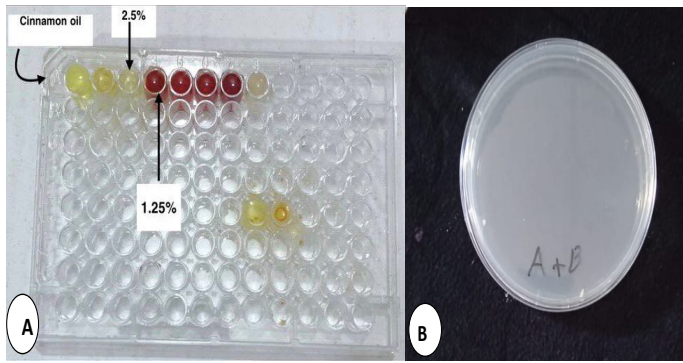


Figure 4: (A) Minimum inhibitory concentration (MIC) and (B) Minimum bacteriocidal concentration (MBC) of cinnamon oil.

these concentrations were shown to have a positive value that inhibit the growth was consider (MIC) and 2.5% of cinnamon oil extract killed *S. aureus* so they was considered as the (MBC) that tested in micro-dilution assay as shown in the. Pink color indicates (growth) and transparence to yellow indicates (No growth). Based on visual readings that obtained using TTC as cellular indicator by observing whether or not the red color results from the reductions of TTC (colorless) to formazan (red) develops (Veiga *et al.*, 2019). The MIC and MBC were calculated using the lowest concentration, and color development was used to confirm the test results (Sakkas *et al.*, 2016).

ANTIBACTERIAL ACTIVITY OF CINNAMON OIL EXTRACT

In the agar well diffusion assay, different concentrations of cinnamon oil extract (10, 5, 2.5, 1.25, 0.652 and 0.325) were used, triggering various degrees of inhibition zones against *S. aureus* as in Table 2 The size of the inhibition zones differed according to the cinnamon oil extract concentration; the size of the inhibition zones increased proportionally with an increase in the cinnamon oil extract concentration. The results showed that *S. aureus* was sensitive significantly ($p < 0.05$) to cinnamon oil extract. DMSO was used as a control, providing no visible zone of inhibition, DMSO was used in *in-vitro* studies as a solvent for cinnamon oil, it is considered to be one of the solvents that can be used to screen the anti-microbial behavior of cinnamon oil extract due to its 100% biologically inert substances. Our result was similar to the results of (Raeisi *et al.*, 2015; Parisa *et al.*, 2019) who detected oil extract of cinnamon bark powder have good antibacterial activity against the test isolate (*S. aureus*). This antibacterial activity of the extract of cinnamon oil can be attributed to its composition of bioactive metabolites causing physiological and pharmacological action, including alkaloids, tannins, saponins, flavonoids, terpenoids and steroids that have antibacterial activity with different mechanisms of action (Compean and Ynalvez, 2014).

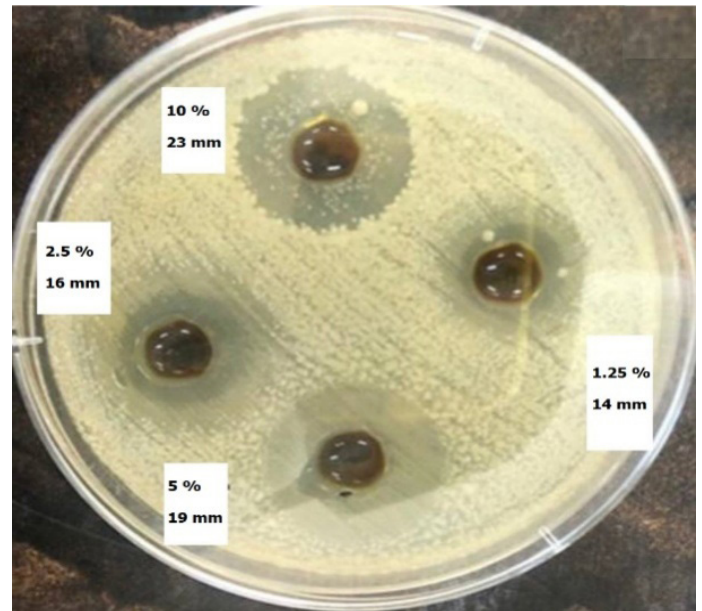


Figure 5: Sensitivity of *S. aureus* to different concentrations of cinnamon oil extract.

TIME KILL-ASSAY

TIME KILLING ASSAY

Results of time killing revealed that the area under the time of killing curve kinetics was calculated highest MIC recorded from the micro dilution MIC assay which was 2000 $\mu\text{g/ml}$ for the *S. aureus*. The *in vitro* concentrations that involved in the study were 0.25x MIC, 0.5x MIC, 1x MIC, 2x MICs and 4x MICs. All of concentrations from 4x MIC through 2x MICs achieved obvious bactericidal effect by reducing of ≥ 3 log₁₀ of the total number of cfu/ml of *S. aureus* in comparison to control, 0.25x MIC and 0.5x MIC; while 1x MIC showed a drop in growth curve at 1st six hours, then after, there was continued inhibition of growth as reported at the 24th hour as mention Table 3.

$$AUC_{kill} = \sum \frac{\log(cn) + \log(cn + 1)}{2} \cdot \Delta t$$

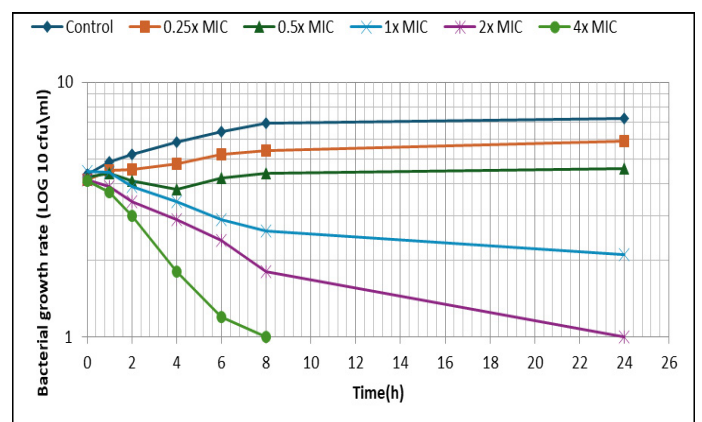


Figure 6: Time kill curve of cinnamon (*Cinnamomum verum*) bark extract against *S. aureus*.

Our results of MIC study revealed that isolate of *S. aureus*

is highest MIC value was 12.5 mg/ml. These results were consistent with time-kill curve is combined and extensive tool to assess both bacteriostatic and bactericidal effects of the antibiotics; it depends on the change in the logarithmic number of bacterial colonies through defined chronological pattern (Mouton *et al.*, 2005).

According to the obtained curves and the calculated areas under each one of them, both 0.25x MIC and 0.5x MIC concentrations showed no significant antibacterial effect in comparison to the control curve in contrast to 1x MIC that showed a significant bacteriostatic effect against *S. aureus* throughout 24 hrs. such bacteriostatic effect is expected since the 1x MIC of cinammon has located within the determined range of *S. aureus* sensitivity toward Fosfomycin which determined from 32 to 2048 µg/ml (Abo El-Wafa and El-Dsoky, 2019). Each of 2x MICs and 4x MICs concentrations showed a distinguished bactericidal effect at the 4th and 6th hrs. of the experiment respectively as same as what reported by Fransen *et al.* (2017).

CONCLUSION

The *C. verum* oil extract at different concentrations had antimicrobial activity against *S. aureus* bacteria isolated from otitis infection in dogs. The GC-MS analysis of the *C. verum* oil showed the presence of 17 volatile components previously reported to possess antibacterial effects. Hence the antibacterial properties demonstrated by the cinnamon oil extract can be attributed to the compounds identified caryophyllen, humulene, and eugenol. Therefore, *C. verum* oils may be used in the medicinal formulation of antimicrobial drugs.

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NOVELTY STATEMENT

this study was to investigate extract plant by Clevenger apparatus, these extracts were characterized by GC-mass spectroscopy and effective antibacterial activity against *Staphylococcal* and could represent a potential alternative to treat common.

AUTHOR'S CONTRIBUTION

AS: Conceptualization, methodology, validation, writing original draft, writing review and editing.

NZY: Conceptualization, methodology, review and editing,

supervisor.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

we don't use AI programmes or sites in this research.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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