



Evaluation of the Combined Antibacterial Effect of Ciprofloxacin and Staphyloxanthin from *Staphylococcus aureus* Against Pathogenic Bacteria.

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Abstract:

Staphyloxanthin is golden yellow-orange carotenoid pigment produced by most *Staphylococcus aureus* bacteria, which plays an important role in virulence factors protecting bacteria from ROS produced by immune system. The aim of this study was to evaluate antibacterial activity of Staphyloxanthin and its combination with Ciprofloxacin antibiotic against some pathogenic bacteria. Twenty-five *S.aureus* isolates were obtained from various clinical sources in Baghdad hospital and identified. Antibiotics susceptibility tests revealed the highest resistance to (Penicillin G 100%, cefoxitin and oxacillin 96%), while lowest resistance to Amikacin and gentamicin, Clindamycin 20%, 16%, 12% respectively, Staphyloxanthin pigment production was detected on skim milk agar, 84% of the isolates were producers and the quantitative estimation using Spectrophotometer at O.D450nm revealed S4 isolate was highest production (Means O.D $\pm$ S.D=1.248). Staphyloxanthin pigment was extracted using methanol and purified by Column chromatography. Antibacterial activity of purified staphyloxanthin was evaluated by agar well diffusion method against (*P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *E. cloacae*, *E. coli*, *E. faecalis*) at different concentrations (100- 12.5 ug/ml). The maximum inhibition zones were observed against *E. cloacae* (22 ± 1 mm), followed by *K. pneumoniae* (20.5 ± 0.5 mm) and then *E. faecalis* (16.5 ± 2.1 mm), while weak activity was observed against

E.coli (10 ± 1), statistically analysis showed significant differences ($p \leq 0.05$), and no activity was recorded against (*P. aeruginosa*, *S. aureus*). Combination effect of Staphyloxanthin with Ciprofloxacin showed high Antibacterial efficacy as indicated by the increase in diameter of inhibition zone compared to ciprofloxacin alone as bacterial follows *K.pneumoniae*, *E.clocaea*, *E.faecalis* ($p \leq 0.05$), while no enhancement was observed in *E. coli*.

Keywords: Staphyloxanthin , Antibacterial Activity, Ciprofloxacin.

Note: The research is based on an M.A thesis: yes.

Introduction:

Staphylococcus aureus is Gram-positive coccus, a facultative anaerobic bacterium that is both a commensal organism and an opportunistic pathogen, capable of causing a wide spectrum of human diseases (Tigabu & Getaneh, 2021) It is one of the most clinically relevant bacterial pathogens, causing.

Infections range from superficial skin infections (SSTIs) to life-threatening disorders such as pneumonia, endocarditis, osteomyelitis, septic arthritis, and bacteremia (Touaitia et al., 2025) The production of several extracellular virulence factors, including hemolysin, coagulase, enterotoxin, protein A, and staphyloxanthin (STX), as well as the formation of biofilms, allow *S.aureus* to survive in adverse environmental conditions (Selvaraj et al., 2020), STX an essential virulence factor in more than 90% of *S.aureus* strains, it gives the colonies their distinctive golden coloration and helps in the detoxification of reactive oxygen species (ROS) produced by the host immune system (Fiedor & Burda, 2014) and stabilizes the cell membrane by reducing membrane fluidity (Selvaraj et al., 2020) Furthermore, staphyloxanthin has been suggested to exhibit potential in vitro Antimicrobial activity (Barretto & Vootla, 2018a). Ciprofloxacin is an antibiotic that belongs to the fluoroquinolone (FQ) class and is used to treat both Gram-positive and Gram-negative bacteria that impacts its target by inhibiting the DNA gyrase known as topoisomerase II and topoisomerase IV. (Shariati et al., 2022). In addition (STX) its use has been proposed as a potential adjunctive therapy based on comparative studies with traditional antibiotics. (Hamzaa & Alwanb, 2025).

Methodology:

1- Isolation and Identification *Staphylococcus aureus*

total of twenty-five *Staphylococcus aureus* clinical isolates was collected from different clinical sources (wound, blood, urine, ear, nasal, Aspiration sample) over period of two months (from September 2025 to November 2025). The isolates were obtained from Baghdad hospitals, presumptively identified based on culture characteristics and biochemical tests, and confirmed identification by using VITEK-2 system (M. Kim *et al.*, 2008)

2- Antibiotics Susceptibility Test

S.aureus isolates on Mueller-Hinton agar plates were tested for antibiotic susceptibility using the Kirby-Bauer disc diffusion method, recommended by CLSI 2025 to the following antibiotics:

(penicillin, oxacillin, cefoxitin, ciprofloxacin, moxifloxacin, levofloxacin, Amikacin, gentamycin, Erythromycin, clindamycin) the inhibition zones surrounding the discs were measured in millimeters (mm) with a metric ruler According to guideline CLSI (2025).

3- Indicator Pathogenic Bacteria

Staphylococcus aureus, *Pseudomonas aeruginosa*, *E.coli*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Klebsiella pneumonia*, all pathogenic isolates were obtained from Baghdad hospitals and were confirmed by Vitek-2 compact system.

4- Detection of Staphyloxanthin Pigment Production in

Staphylococcus aureus isolates

With some modification Twenty-five *S.aureus* isolates inoculation into 10ml of Trypticase soya broth and incubated at 37 °C for 18h. after incubation and all isolates streaking on Mannitol salt agar to obtained pure colonies. subsequently subculture on Skime milk agar and incubated at 37 °C for two days and two days at room temperature. The appearance of golden- yellow or orange pigmentation colonies considered positive outcome for staphyloxanthin production (Grinsted & Lacey, 1973).

5- Quantification Estimation of Staphyloxanthin Production

STX quantitative evaluated by measuring the absorbance of methanolic extracts from tested isolates by UV-visible spectrophotometer at OD450 nm. Isolates were classified by their optical densities (OD) as intensely

pigmented ($OD_{450} > 0.2$), faintly pigmented ($OD_{450} = 0.1-0.2$), or non-pigmented ($OD_{450} < 0.1$) (Elmesseri *et al.*, 2022).

6- Extraction OF Staphyloxanthin Pigment

The pigment of *S.aureus* (STX) was extracted from isolate (S4) by using methanol with some modifications.

Bacterial cells were collected after 48 hours of growth on skim milk agar plates at 37°C and then 48 hours at room temperature. Agar surfaces were washed twice with sterile double distilled water (each with 3 mL). The bacteria were then centrifuged at 6000 rpm for 15 minutes. After discarding the supernatant, the pellet was re-suspended in double-distilled water and centrifuged at 6000 rpm for 15 minutes. The extraction of staphyloxanthin from bacterial cell pellets was mixed with 6ml of 99.8% methanol and coated in aluminum foil to prevent light exposure, heated in water bath at 55 °C for 5 minutes while being stirred gently, until all the visible pigments of bacteria were extracted and dissolved in methanol. The Yellow golden -orange colored supernatant liquid was cooled and ,centrifuged at 10000 rpm for 15 minutes. The extraction process was done twice until no additional pigment could be recovered. Methanolic extract measured absorption at 450nm (Marshall & Wilmoth, 1981a).

7-Purification of Staphyloxanthin by column chromatography

crude extract of Staphyloxanthin purified using column chromatography. The crude extract was dissolved in ethyl acetate and subjected to column chromatography using silica gel (60-120 mesh size, 20 g; Merck), packed in a glass column (1.5 × 60 cm) Elution was performed with ethyl acetate, and the colored fractions were collected. Due to the pigment's light sensitivity, all purification operations were carried out in the dark (Pelz *et al.*, 2005).

8- Detection and Confirm Presence of purified Staphyloxanthin

TLC and Ultraviolet (UV) analysis with a UV–vis spectrophotometer was employed to confirm the presence of the purified pigment.

8-1Thin-layer Chromatography (TLC)

Staphyloxanthin detects and identity using thin layer chromatography on silica-gel). The TLC plates were spotted with staphyloxanthin extract. The plates were then placed in a jar containing benzene-methanol-acetic

acid 87:11:2 (vol/vol/vol) as a mobile phase. Staphyloxanthin typically appears as colored spot with golden yellow -orange or bands.

The R_f value was determined using the following equation.
R_f = Distance of spot sample movement /Distance of spot solvent movement (Marshall & Wilmoth, 1981a).

8-2UV-Visible Spectrophotometric Scanning (300-800nm)

The pigment extract was evaluated by scanning the absorbance between 300-800nm. Using a UV-Vis spectrophotometer, the maximum absorption spectra (λ max) were determined using 95% methanol as a blank (Chaturwedi *et al.*, 2024).

9-Antibacterial Activity of Staphyloxanthin purified by Agar Well Diffusion

Purified staphyloxanthin was tested for antibacterial activity using Agar well diffusion. Against some bacterial pathogenic isolates (*Pseudomonas aeruginosa*, *E.coli*, *Klebsiella pneumonia*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Enterococcus faecalis*). indicator bacteria were inoculated on an MHA plate, Wells were prepared with a 6mm diameter sterile cork borer and filled with 100 μ l of purified extracts at different concentrations (100, 50, 25, 12.5,) μ g/ml. After the plates were incubated for 24 hours at 37°C The antibacterial of pigment by applying rules to determine the zones of inhibition in millimeters and Experimental was performed in duplicate, and result expressed as mean $\pm$ SD. (Barretto & Vootla, 2018).

2.10. Antibacterial Activity of Ciprofloxacin

The sensitivity of ciprofloxacin to clinical isolates was determined using agar well diffusion method at different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 μ g/ml) according to previous studies by (Bodunrinde *et al.*, 2020) . The lowest concentration that produced measurable inhibition zones was selected for subsequent combinations experiments.

2.11. Screening For Synergistic Effect of Ciprofloxacin and Staphyloxanthin

To determine the combination effect of staphyloxanthin purified and Ciprofloxacin by agar well diffusion as described by (Dhairh and Al-Azawi 2022) with some modification, Each Mueller-Hinton agar plate was inoculated with a standardized bacterial isolated suspension (0.5

McFarland) by equally swabbing its whole surface. For the well diffusion procedure, Three wells prepared of 6 mm diameter were created using a sterile cork bore in each agar plate as follows , each well was properly labeled and filled with 100 μ L of Staphyloxanthin (STX) alone ,Ciprofloxacin(CIP) alone, and combination of CIP and STX alone (50ul STX and 50ul CIP). lowest effective concentration of ciprofloxacin was selected for each bacterial isolate and combined with lowest concentration of pigment. All these plates were incubated in the dark for 24 hours at 37°C. followed by the measurement of zones of inhibition (ZOI) around the wells.by ruler and recording the results, All Experiments were performed in duplicate and result express as mean $\pm$ SD.

2.12 Statistical Analyses:

Statistical analysis was performed using a one-way analysis of variance (ANOVA) with LSD test at $p \leq 0.05$ and Results were expressed as mean $\pm$ SD (SPSS v 20).

Result and discussion

1-Isolation and Identification of *Staphylococcus aureus*.

twenty-five clinical isolates of *S.aureus* were obtained from different clinical specimens in Baghdad hospital ,all isolate grew on Mannitol salt agar and ferment mannitol ,produce yellow colonies ,on blood agar most isolated exhibited B-hemolysis and formed smooth,round,grayish to creamy colonies while 84% produced golden yellow -orange pigmentation on skim milk agar ,figure(1)

Biochemical tests revealed that all isolates (100%) were catalase-positive, tube-coagulase-positive, and oxidase-negative, and identification was confirmed vitek2 compact system.



Figure 1: *S.aureus* colonies on Mannitol salt agar

2-Antibiotics Susceptibility Test

Antibiotics susceptibility test was performed to ten different Antibiotics for all isolates *S.aureus* figure(2) result revealed resistance percentage as follows: penicillin G(100%),cefoxitin, oxacillin (96%),ciprofloxacin, moxifloxacin, levofloxacin (24%) , Amikacin(20%) , Gentamicin(16%), Erythromycin(80%), clindamycin (12%). previous local study conducted in Iraq by (Mohammed *et al.*,2017) *S.aureus* isolates exhibited percentage Resistance (100%) cefoxitin ,(100%)

penicillin G , (87%) oxacillin (Hoshyar *et.al*,2025) resistance to gentamicin , Amikacin (16%), (Al-Marjani *et al.*, 2017) resistance to levofloxacin and ciprofloxacin exhibited as 20% (Gade & Qazi, 2013) 21% resistant to Moxifloxacin ,(Aziz & Hassan, 2019) resistance to erythromycin 85% ,(Bushra *et al.*, 2018) resistant to clindamycin 17%.

High resistance of *Staphylococcus aureus* to penicillin ,oxacillin, cefoxitin may be attributed to production beta lactamase enzyme which hydrolyzed beta lactam ring and presence of the *mecA* gene, which encodes PBP2a with low affinity for β -lactam antibiotics (C. Kim *et al.*, 2012) while Gentamicin , Amikacin antibiotics remain effective treatment option of *S.aureus* because aminoglycoside (Gentamicin, Amikacin) may require for mechanisms inactivation Antibiotics by Aminoglycosides-modifying-enzymes (Ma *et al.*, 2025)

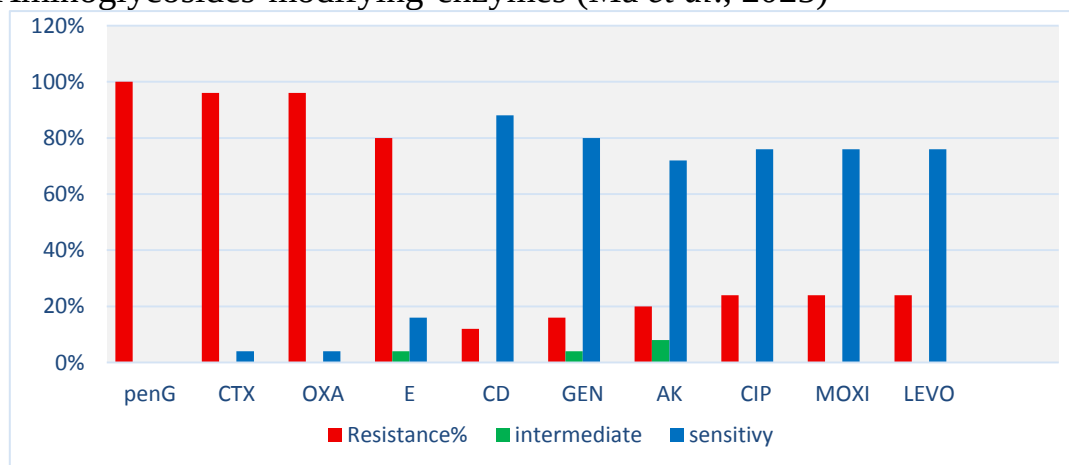


Figure 2 : pattern Antibiotics susceptibility of *Staphylococcus aureus*

3- Detection of Staphyloxanthin Pigment production Among *S.aureus* Isolates

all *S.aureus* isolates were subculture on Skim milk agar result revealed Out of 25 isolate ,21(84%) of isolates produces staphyloxanthin pigment indicate as golden yellow -orange color on media, while 4 (16%) non produced staphyloxanthin indicate as absence golden yellow-orange color figure(3).

these finding consistent with study by (Judi & Naser, 2025) Revealed 75% of *S.aureus* isolates produce STX, (Mishra *et al.*, 2011) has reported Staphyloxanthin (STX), which is produced by over 90% of *S.aureus* isolates.

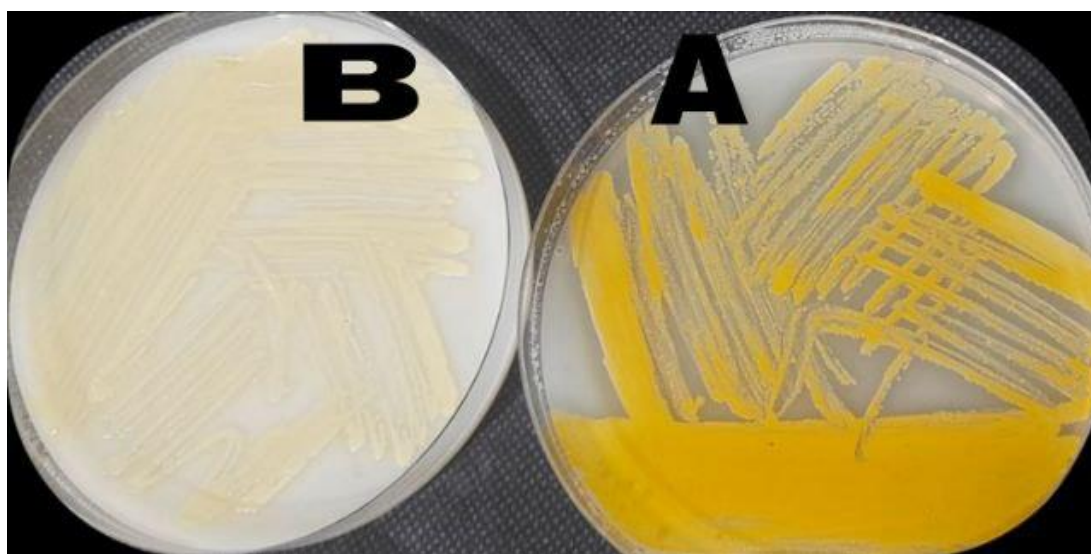


Figure 3: A- isolate production of STX on skim milk B- isolate non produce STX

4-Quantification Estimation of STX production

quantitative estimated of Staphyloxanthin by UV-Visible spectrophotometry measurement for methanolic extract O.D at 450nm ,result demonstrated statically significant different among highly ,weakly , non-produce STX group ($P < 0.001$), and S4 isolate showed high absorbance at 450nm (Means O.D $\pm$ S.D=1.25),indicated of highly production for STX were selected further for extraction and purification. Overall, 60% of the high produce, 20% weakly ,20% non-product were

shown in Figure(4), study by (Elmesseri *et al.*, 2023) exhibited Variability in STX production. This variability may be attributed to differences in expression of ctrOPQMN operon which is responsible for Staphyloxanthin biosynthesis and non-producer harbor gene mutant (Liu *et al.*,2005).

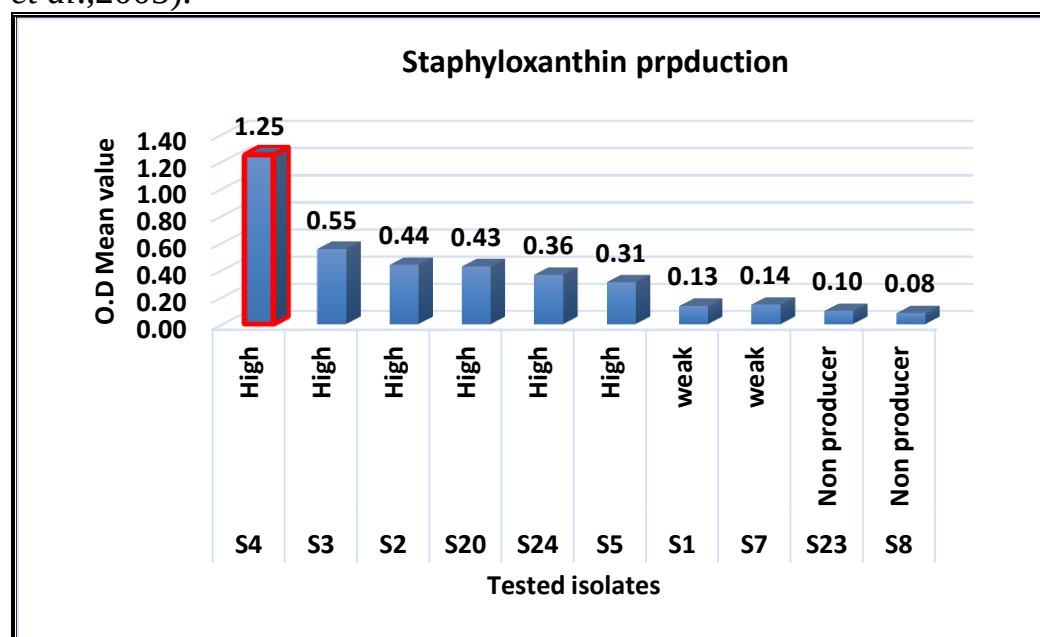


Figure 4: Variability in Staphyloxanthin production among isolates

5- Extraction of Staphyloxanthin pigment:

In the present study staphyloxanthin pigment extracted from S4 isolate by using solvent methanol (99.8%) , yielding 0.4g crude extract. Then methanol extract was scanning using UV-visible spectrophotometer (200-800nm), where observed three Peaks at (320,444,468nm) with maximum Peake around 444nm-468nm show in Figure (5). These finding consistent with local study in Iraq by (Nafe'a, 2014) that revealed of crude staphyloxanthin exhibited three Peaks at (315,450,463nm),while (Dong *et al.* , 2019) has reported that crude staphyloxanthin has Absorption spectrum between 400-500nm

Therefore, The presence of absorption peaks within this range suggested of Successfully Staphyloxanthin extraction.

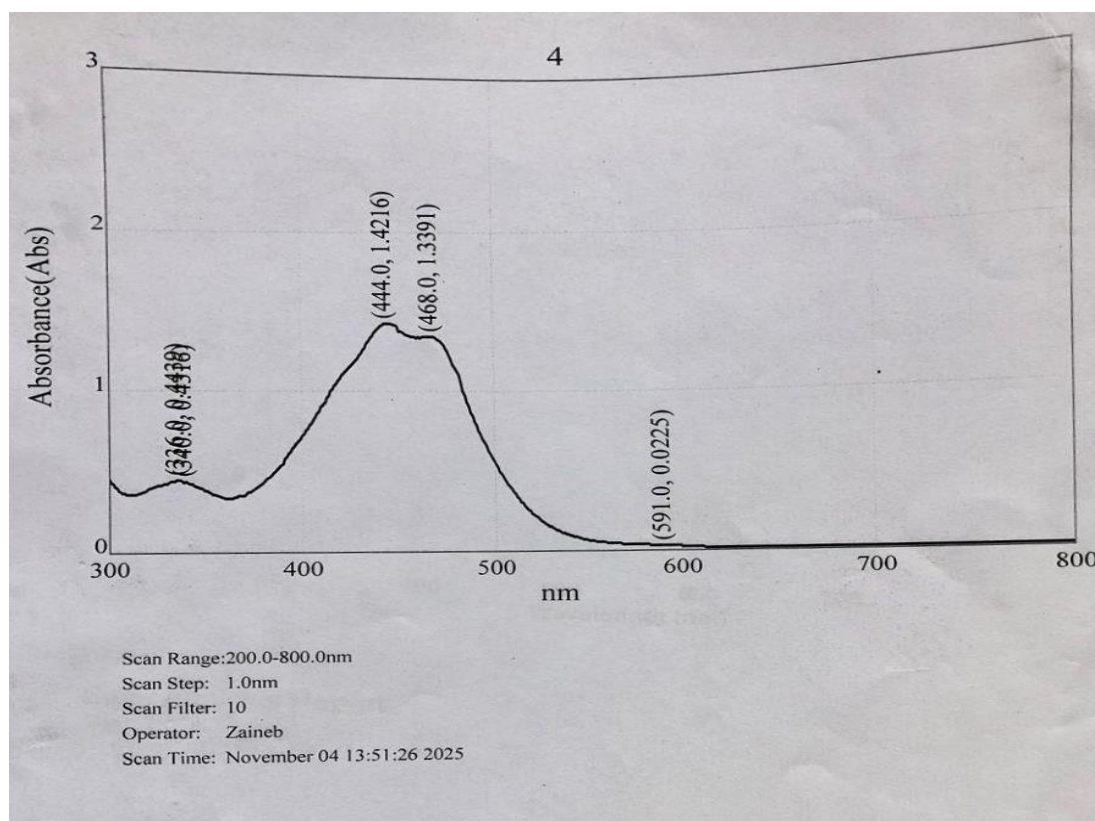


Figure 5 : UV-visible spectrum for methanolic extract from S4 isolated were demonstrated peak around 444nm-468nm

6-Purification of Crud Staphyloxanthin

Purification of crude STX by silica gel chromatography with ethyl acetate as mobile phase, a total of 19 fractions were collected, absorbance was measured at 460nm , showing statically significant difference among fraction ($p < 0.001$).

fractions from (7-15tube) exhibited intense golden yellow-orange coloration with high absorbance at 460nm particularly fraction 13 showing high absorbance (2.289) Figure(6), these fractions were pooled and dried at 40 °C in oven, and store at 4 °C in dark, Previous studies demonstrated column chromatography was used to purify the bacterial pigments. to produce as much pigment as possible(Poddar *et al.*, 2021)

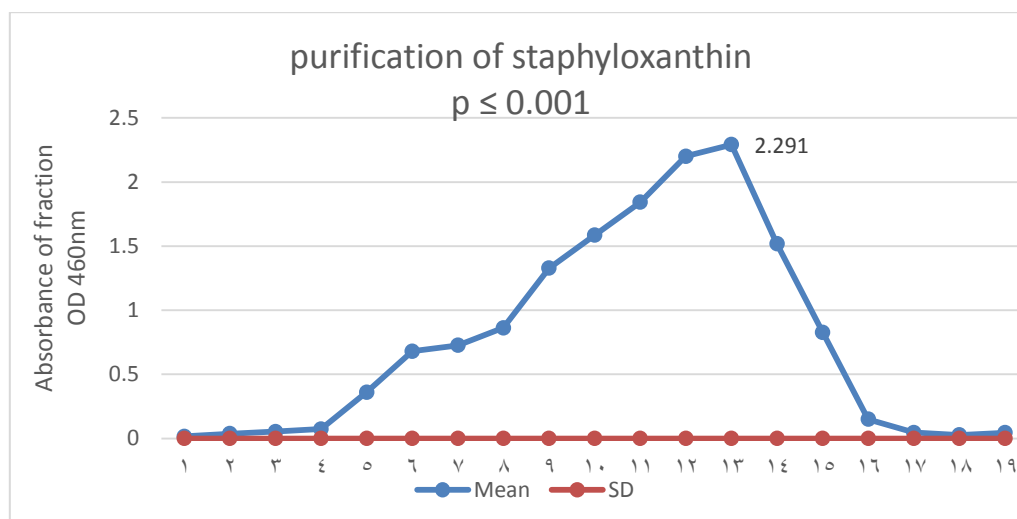


Figure 6: purification of Staphyloxanthin by Column chromatography

7- Detection of Purified Staphyloxanthin

7-1 Thin layer chromatography

the result showed two distinct band or spot on TLC plate ,were low orange band represent Staphyloxanthin and upper yellow band derivatives of Staphyloxanthin which consistent with study by (Múniera-Jaramillo et al., 2024), RF value was =0.58

7-2 visible spectrophotometry

UV-visible spectrum of 3ml of STX purified in 10mm cuvette quartz was scanned from (300-800nm), the spectrum showed single broad absorption peak around 460nm showed in Figure (7),these consistent with local previous study conducted in Iraq by (Raja & Alattar, 2024a) demonstrates staphyloxanthin pigment exhibited absorption peak at approximately 460nm.

Internationally study by Marshall & Wilmoth, (1981b) , reported broad single peak around 460nm is characteristic of purified staphyloxanthin pigment.

These confirm presence of purified Staphyloxanthin and successful purification.

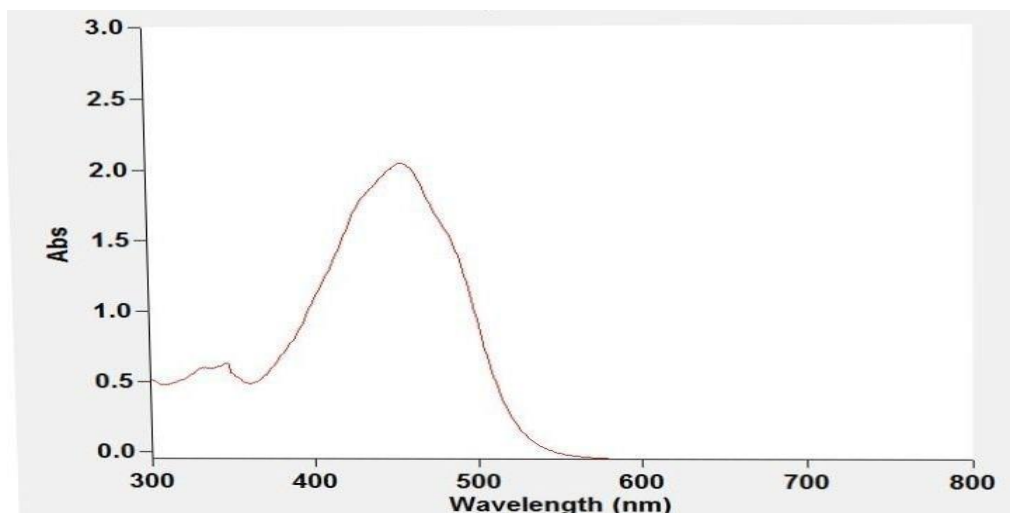


Figure 7:UV-vis spectrum for Staphyloxanthin purified from Staphylococcus aureus (S4) showing characteristics $\lambda_{max} \approx 460$ nm

3.9. Antibacterial Activity of purified staphyloxanthin pigment

The antibacterial activity of purified staphyloxanthin pigment was determined using the well diffusion method against several pathogenic bacteria including (*Pseudomonas aeruginosa*, *staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Escherichia coli*, *Enterococcus faecalis*) at different concentrations (100, 50, 25, and 12.5 ug/ml).

The results showed variable activity depending on the bacterial species a The maximum inhibition zones were observed against *Enterobacter. cloacae*(ZOI=22±1mm), followed by *Klebsiella pneumoniae* (ZOI=20.5±0.5mm), and then *Enterococcus faecalis*(ZOI=16.5±2.1mm), While weak activity was observed against *Escherichia coli* (ZOI=10±1mm),, and no inhibition activity was recorded against *Pseudomonas aeruginosa*, *staphylococcus aureus* (ZOI=0mm), Detailed results are presented in Tabel (1) as illustrated in Figure (8,9), which shows the diameters of the inhibition zones for each bacterial species. Statistically significant differences showed in inhibition zone diameter among bacteria isolates included *K.pneumoniae*, *E.clocaea* ,*E. faecalis*, *E.coli* ($p \leq 0.05$),while bacteria isolates,*S.aureus* ,*P.aeruginosa* did not exhibit zone diameter (ZOI=0mm) not included statistical analysis.

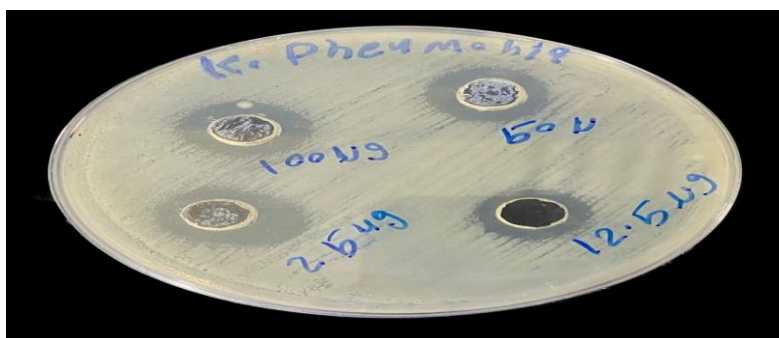


Figure 8: zone inhibition of Staphyloxanthin (STX) against *K. Pneumonia* at different concentrations (100, 50, 25, 12.5 ug).

Tabel 1: zone inhibition diameter (ZOI) of staphyloxanthin purified against pathogen bacteria isolates, representing express zone diameter as Mean $\pm$ SD.

Bacterial isolates	Tested concentrations of Staphyloxanthin				P value
	zone mm/100 ug Mean $\pm$ SD	zone mm/50 ug Mean $\pm$ SD	zone mm/25 ug Mean $\pm$ SD	zone mm/12.5 ug Mean $\pm$ SD	
<i>K.pneumoniae</i>	A 20.5 $\pm$ 0.5	B 17.5 $\pm$ 1.5	B 18 $\pm$ 1	C 14.5 $\pm$ 0.5	0.02
<i>E.clocae</i>	A 22 $\pm$ 1	B 22.5 $\pm$ 0.5	C 20 $\pm$ 1	D 18.5 $\pm$ 1.5	0.01
<i>E.coli</i>	10 $\pm$ 1	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	N.C
<i>E.faecalis</i>	A 16.5 $\pm$ 2.1	A 15.5 $\pm$ 0.7	B 13 $\pm$ 1.4	0 $\pm$ 0	0.05
P value	0.001	0.001	0.003	0.001	

LSD test identified substantial differences between means, with letters (A, B, C, D) representing significance levels A for extremely significant and decreasing to the last. Matching letters signify” There were no statistically noteworthy differences between the means. $p \leq 0.05$ was suggestive of significance

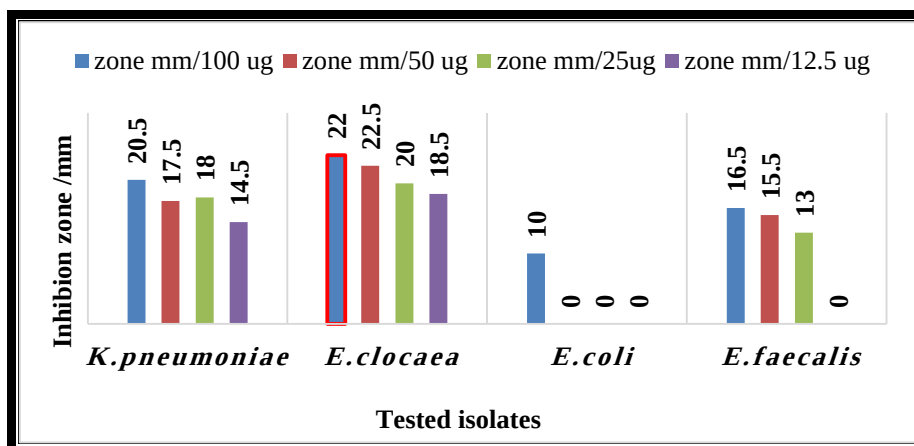


Figure 9:zone inhibition diameter of Staphyloxanthin among tested bacterial isolates

This consistent Local study conducted in Iraq-Baghdad by (Abd Algabar & Authman, 2009) showed that purified staphyloxanthin is effective against *Klebsiella pneumoniae* and *Enterobacter*, while the results differed regarding *Pseudomonas aeruginosa*, as our study did not demonstrate any Activity against this bacterium.

While internationally study by (Saranraj, 2025) showed high inhibition zones against pathogenic bacteria including *Pseudomonas aeruginosa* followed by *Salmonella typhi* *Escherichia coli*, and *Streptococcus pyogenes*.

The no antibacterial activity against *Staphylococcus aureus*, *pseudomonas aeruginosa* may be attributed to having intrinsic resistance mechanisms included efflux pump They result in the unique intrinsic resistance of these bacteria to antibiotics, detergents, dyes, and organic solvents. They also expel a notably wide variety of antimicrobial substances from the cells. (Zgurskaya & Nikaido, 2000).

Variation in antibacterial activity staphyloxanthin in different studies may be attributed to different clinical isolates, concentration of pigment, and mechanisms of resistance toward pigment.

The Antibacterial activity of staphyloxanthin suggest by (Saranraj, 2025) ability to cellular permeability is altered, oxidative stress is produced, and ion-in-press membrane connections are disrupted.

Furthermore, staphyloxanthin not only virulence factor but also bioactive compound can be therapeutic application.

10-Antibacterial Activity of Ciprofloxacin

Susceptibility of ciprofloxacin against bacterial isolates was assessed using Agar well diffusion at different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125), result revealed lowest concentration of ciprofloxacin was giving inhibition zone measurable were considered lowest effective concentration and selected for subsequent combination experimental showed in Tabel(2).

Tabel 2: lowest effective concentration of Ciprofloxacin produced zone inhibition measurable.

Isolates	Lowest effective concentration (ug/ml)
P.aeruginosa	No inhibition zone
E.coli	5
K.pneumoniae	0.625
S.aureus	2.5
E.faecalis	1.25
E.clocaea	0.312.5

11-Screning Synergistic Effect of Staphyloxanthin and Ciprofloxacin

In the present study the synergistic effect of staphyloxanthin purified and ciprofloxacin was evaluated against bacteria isolates that demonstrate sensitivity to lowest concentration of STX , The combination was tested using the lowest effective concentrations of both ciprofloxacin and staphyloxanthin.

The result showed combination of Staphyloxanthin with ciprofloxacin enhanced antibacterial as evidenced by an increased the diameter inhibition zone was observed at bacteria *E.clocaea* (5mm), *Klebsiella pneumoniae*(3mm), *E.faecalis*(4mm) with statistically significant differences compared to ciprofloxacin alone ($p \leq 0.05$), while in *E.coli* combination effect not showed any increased in zone inhibition diameter compared to ciprofloxacin alone. Detailed results are presented in Table (4), as illustrated in Figure (10,11). These results suggest a synergistic

effect that may contribute to reducing the use of Antibiotics dose and delaying development of resistance, minimizing side effects of Antibiotics.

Previous local study conducted in Iraq by (Raja & Alattar, 2024b) demonstrated the combination of staphyloxanthin pigment with ciprofloxacin improved activity against *K.pneumoniae* by decreasing MIC value of ciprofloxacin suggest synergistic effect ,

The increased activity of ciprofloxacin in combination with staphyloxanthin may be attributed to ability staphyloxanthin pigment carotenoid to alter permeability membrane bacterial cell and cause oxidative stress according to previous study by (Saranraj, 2025) such alteration may be facilitated penetration of ciprofloxacin antibiotics into cell therapy enhances antibacterial activity.

Tabel 4: Antibacterial activity comparison of STX=staphyloxanthin alone, CIP=ciprofloxacin alone, CIP+STX at lowest concentration , statistically significant differences ($p \leq 0.05$)

Bacterial isolates	CIP (ug)	CIP(ug)zone mm	STX(ug)zone mm Lowest concentration	Cip+STX zone mm	P value
<i>K.pneumoniae</i>	0.625	22.5±0.5	15.5±0.5	A 25.5±1.5	0.002
<i>E.faecalis</i>	1.25	B 18±1	C 12±0	A 22.5±2.5	0.001
<i>E.clocae</i>	0.3125	B 25.5±0.5	C 18.5±0.5	A 31±1	0.001
<i>E.coli</i>	5	B 15.5±0.7	C 10±1.4	A 16±0.5	0.9
P value		0.001	0.05	0.001	

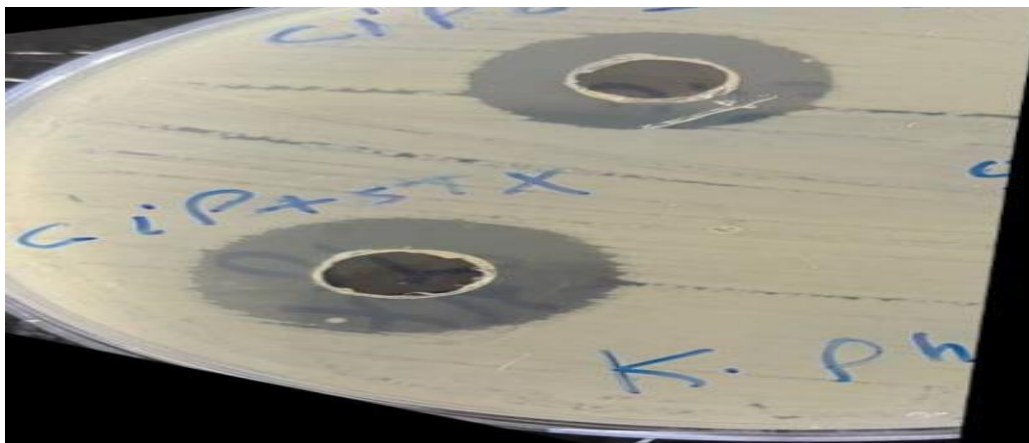


Figure 10: inhibition zone comparison of , CIP=ciprofloxacin alone, STX +CIP combination in *K.pneumoniae*.

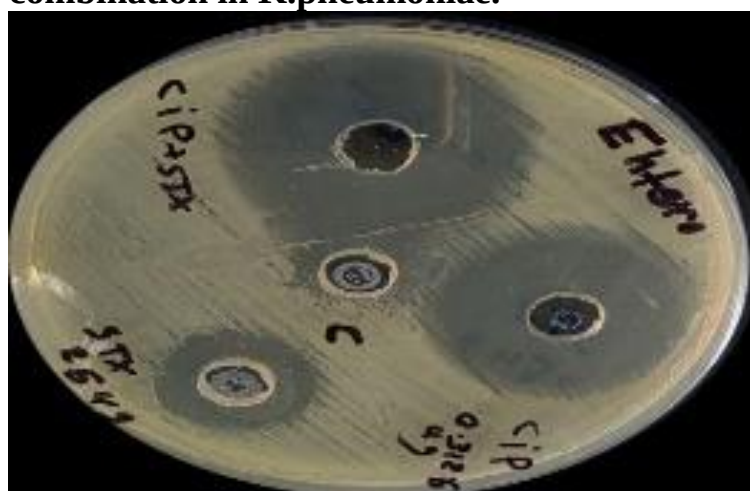


Figure 11: inhibition zone comparison of STX=staphyloxanthin , CIP=ciprofloxacin alone, STX +CIP combination in *Enterobacter cloacae*

Conclusion

most *staphylococcus aureus* isolates produced of Staphyloxanthin pigment on Skime milk agar, purified staphyloxanthin exhibited Antibacterial activity against some pathogenic bacteria ,the combination of Staphyloxanthin with ciprofloxacin enhanced the activity of ciprofloxacin, as indicated by the increased inhibition-zone diameter compared to ciprofloxacin alone.



Recommendation

Study combination of purified Staphyloxanthin with other class Antibiotics and evaluated effect on MDR pathogenic bacteria, investigated Antibiofilm Activity of Staphyloxanthin with ciprofloxacin and Study effect on biofilm Formation and inhibition

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References

1. Abd Algabar, F. A., & Authman, S. H. (2009). Extraction of Staphyloxanthin from *Staphylococcus aureus* and studying some biological effect of Staphyloxanthin pigment. *J Global Pharma Technol*, 11(04).
2. Aziz, Z. S., & Hassan, M. A. (2019). Phenotypic and molecular study of *mecA* gene in MRSA isolated from clinical cases in misan province/Iraq. *Indian J. Public Health*, 10(553), 10-5958.
3. Barretto, D. A., & Vootla, S. K. (2018). In vitro anticancer activity of staphyloxanthin pigment extracted from *Staphylococcus gallinarum* KX912244, a gut microbe of *Bombyx mori*. *Indian journal of microbiology*, 58(2), 146-158.
4. Chang, J., Yue, M., Zhao, G., Tang, B., & Shi, C. (2025). Advancements in function recognition of staphyloxanthin and targeted inhibitor development. *Current Opinion in Food Science*, 63, 101300.
5. Chaturvedi, S. B., Mainali, S., & Chaudhary, R. (2024). Antibacterial activity of pigment extracted from bacteria isolated from soil samples. *BMC Research Notes*, 17(1), 169.
6. CLSI Performance Standards for antimicrobial susceptibility testing, 29th edition. CLSI supplement M100. Wayne, PA: Clinical and Laboratory standard Institute; 2025. ISBN Number: 978-1-68440-033-1
7. Coker MS, Forbes LV, Plowman-Holmes M, Murdoch DR, Winterbourn CC, Kettle AJ. 2018. Interactions of staphyloxanthin and *Enterobacter* with myeloperoxidase and reactive chlorine species. *Arch Biochem Biophys* 646:80–89

8. Dhairh, S. H., & Al-Azawi, I. H. (2022). Comparative study between standard pyocyanin and extracted pyocyanin and synergistic with antibiotics against multidrug bacteria isolated from different clinical samples. *International Journal of Health Sciences*, 6(S6), 4320-4334.
9. Dong, P. T., Mohammad, H., Hui, J., Leanse, L. G., Li, J., Liang, L., ... & Cheng, J. X. (2019). Photolysis of Staphyloxanthin in methicillin-resistant *Staphylococcus aureus* potentiates killing by reactive oxygen species. *Advanced science*, 6(11), 1900030.
10. Elmesserli, R. A., Saleh, S. E., Elsherif, H. M., Yahia, I. S., & Aboshanab, K. M. (2022). Staphyloxanthin as a potential novel target for deciphering promising anti-*Staphylococcus aureus* agents. *Antibiotics*, 11(3), 298.
11. Gade, N. D., & Qazi, M. S. (2013). Fluoroquinolone Therapy in *Staphylococcus aureus* Infections: Where Do We Stand? *Journal of Laboratory Physicians*, 5(02), 109–112. <https://doi.org/10.4103/0974-2727.119862>
12. Grinsted, J., & Lacey, R. W. (1973). Ecological and genetic implications of pigmentation in *Staphylococcus aureus*. *Microbiology*, 75(2), 259-267.
13. Hauschild, T., Sacha, P., Wiczorek, P., Zalewska, M., Kaczyńska, K., & Tryniszewska, E. (2008). Aminoglycosides resistance in clinical isolates of *Staphylococcus aureus* from a University Hospital in Bialystok, Poland. *Folia Histochemical et Cryobiological*, 46(2), 225–228. <https://doi.org/10.2478/v10042-008-0034-3>
14. Hushyar, S., Doghaheh, H. P., & Arzanlou, M. (2025). Evaluation of aminoglycoside- and methicillin-resistant *Staphylococcus aureus*: phenotypic and genotypic insights from clinical specimens in Ardabil, Iran. *BMC Infectious Diseases*, 25, 285. <https://doi.org/10.1186/s12879-025-10659-2>
15. Judi, H. K., & Naser, H. H. (2025). Application of staphyloxanthin-silver nanoparticles combination as a sustainable therapeutic platform with anticancer potential. *Agricultural Biotechnology Journal*, 17(4), 415-438.
16. Kim, C., Milheirico, C., Gardete, S., Holmes, M. A., Holden, M. T., de Lencastre, H., & Tomasz, A. (2012). Properties of a novel PBP2A



protein homolog from *Staphylococcus aureus* strain LGA251 and its contribution to the β -lactam-resistant phenotype. *Journal of Biological Chemistry*, 287(44), 36854-36863.

17. Kim, M., Heo, S. R., Choi, S. H., Kwon, H., Park, J. S., Seong, M. W., ... & Kim, E. C. (2008). Comparison of the Micro Scan, VITEK 2, and Crystal GP with 16S rRNA sequencing and Micro Seq 500 v2. 0 analysis for coagulase-negative *Staphylococci*. *BMC microbiology*, 8(1), 233.

18. Liu GY, Essex A, Buchanan JT, et al. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J Exp Med*. 2005;202:209–215.

doi:10.1084/jem.2005084616009720

19. Ma, S., Xu, Y., Ma, J., Luo, D., Huang, Z., Wang, L., ... & Wang, Z. (2025). Mechanisms of *Staphylococcus aureus* antibiotics resistance revealed by adaptive laboratory evolution. *Current Microbiology*, 82(1), 46.

20. Marshall, J. H., & Wilmoth, G. J. (1981). Pigments of *Staphylococcus aureus*, a series of triterpenoid carotenoids. *Journal of bacteriology*, 147(3), 900-913.

21. Mishra, N. N., Liu, G. Y., Yeaman, M. R., Nast, C. C., Proctor, R. A., McKinnell, J., & Bayer, A. S. (2011). Carotenoid-related alteration of cell membrane fluidity impacts *Staphylococcus aureus* susceptibility to host defense peptides. *Antimicrobial agents and chemotherapy*, 55(2), 526-531.

22. Mohammed, H. A., Kandala, N. J. and Al-Ogaidi, I. (2017). Use multiplex PCR techniques for distribution the accessory gene regulatory polymorphisms among Baghdad clinical *Staphylococcus aureus* isolates and its correlation to cassette type. *World J Exp Biosci*. 5(1): 23-29.

23. Mulani, M. S., Kamble, E. E., Kumkar, S. N., Tawre, M. S. & Pardesi, K. R. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. *Front. Microbial*. 10, (2019)

24. Múnera-Jaramillo, J., López, G. D., Suesca, E., Carazzone, C., Leidy, C., & Manrique-Moreno, M. (2024). The role of staphyloxanthin in the regulation of membrane biophysical properties in *Staphylococcus*



aureus. *Biochemical et Biophysical Acta (BBA)-Bio membranes*, 1866(3), 184288.

25. Nosair, A. M., Abo-Kamar, A. M., Al-Madboly, L. A., Farghali, M. H., & Abdelaziz, A. A. (2026). Staphylococcus aureus' golden-yellow pigment staphyloxanthin: production enhancement, analytical characterization, and biological attributes. *Microbial Cell Factories*.

26. Plez, A.; Wieland, K.P.; Putzbach, K.P.; Hentschel, P.; Albert, K.; Cötzt, F. (2005). Structure and biosynthesis of staphyloxanthin from Staphylococcus aureus. *The J. Biological Chem.* 280, 32493-32498.

27. Poddar, K., Padhan, B., Sarkar, D., & Sarkar, A. (2021). Purification and optimization of pink pigment produced by newly isolated bacterial strain Enterobacter sp. PWN1. *SN Applied Sciences*, 3(1), 105.

28. Saranraj, P. (2025). Extraction of Natural Food Colorant Staphyloxanthin from Staphylococcus aureus and Analysis of its Antibacterial Activity. *Indian Journal of Natural Sciences*.

29. Selvaraj A, Valliammai A, Muthuramalingam P, Priya A, Suba M, Ramesh M, Karutha Pandian S. 2020. Carvacrol targets SarA and CrtM of methicillin-resistant Staphylococcus aureus to mitigate biofilm formation and staphyloxanthin synthesis: an in vitro and in vivo approach. *ACS Omega* 5:31100–31114.

30. Taylor, T.A.; Unakal, C.G. Staphylococcus aureus Infection. In Stat Pearls; Stat Pearls Publishing: Treasure Island, FL, USA, 2025. Available online: <http://www.ncbi.nlm.nih.gov/books/NBK441868/> (accessed on 20 February 2025)

31. Zgurskaya, H. I., & Nikaido, H. (2000). Cross-linked complex between oligomeric periplasmic lipoprotein AcrA and the inner-membrane-associated multidrug efflux pump AcrB from Escherichia coli. *Journal of bacteriology*, 182(15), 4264-4267.

تقييم التأثير المضاد للبكتيريا المشترك لكل من السيبروفلوكساسين وصبغة الستافيلوزانثين من المكورات العنقودية ضد بعض البكتيريا الممرضة

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مستخلص البحث:

الستافيلوزانثين هو صبغة كاروتينويدية ذهبية صفراء برتقالية تنتجها معظم بكتيريا المكورات العنقودية الذهبية، وتلعب دوراً هاماً في عوامل الضراوة التي تحمي البكتيريا من أنواع الأكسجين التفاعلية التي ينتجها الجهاز المناعي. تهدف هذه الدراسة إلى تقييم النشاط المضاد للبكتيريا لستافيلوكسانثين، وتأثيره عند دمجه مع المضاد الحيوي سيبروفلوكساسين، ضد بعض البكتيريا الممرضة. تم تحديد 25 عزلة من المكورات العنقودية الذهبية من مصادر سريرية مختلفة في مستشفى ببغداد. كشفت اختبارات حساسية المضادات الحيوية عن أعلى مقاومة للبنسلين ج (100%)، والسيفوكسيتين والأوكساسيلين (96%)، بينما كانت أقل مقاومة للأميكاسين والجنتاميسين، والكلينداميسين (20%)، و16%، و12% على التوالي. تم الكشف عن إنتاج صبغة ستافيلوكسانثين على وسط أجار الحليب منزوع الدسم، حيث كانت 84% من العزلات منتجة، وتم تقدير الكمية باستخدام مطياف ضوئي. أظهرت قياسات الامتصاص الضوئي عند 450 نانومتر أن العزلة S4 كانت الأعلى إنتاجاً (متوسط الامتصاص الضوئي $\pm$ الانحراف المعياري = 1.248). تم استخلاص صبغة ستافيلوكسانثين باستخدام الميثانول وتنقيها بواسطة كروماتوغرافيا العمود. تم تقييم النشاط المضاد للبكتيريا لمادة ستافيلوكسانثين النقية باستخدام طريقة الانتشار في الآبار على وسط الآغار ضد أنواع البكتيريا التالية: (الزائفة الزنجارية، المكورات العنقودية الذهبية، الكلبييلة الرنوية، الإشريكية الكلوكية، الإشريكية القولونية، المكورة المعوية البرازية) بتركيزات مختلفة (100-12.5 ميكروغرام/مل). لوحظت أكبر مناطق تثبيط ضد الإشريكية الكلوكية (1 ± 22 مم)، تليها الكلبييلة الرنوية (0.5 ± 20.5 مم)، ثم المكورة المعوية البرازية (2.1 ± 16.5 مم)، بينما لوحظ نشاط ضعيف ضد الإشريكية القولونية (1 ± 10 مم). أظهر التحليل الإحصائي فروقا دالة إحصائية ($p \leq 0.05$)، ولم يُسجل أي نشاط ضد (الزائفة الزنجارية، المكورات العنقودية الذهبية). أظهر التأثير المشترك بين الستافيلوكسانثين والسيبروفلوكساسين فعالية مضادة للجراثيم عالية، حيث زاد قطر منطقة التثبيط مقارنة بالسيبروفلوكساسين وحده في البكتيريا التالية: الإشريكية الكلوكية، الكلبييلة الرنوية، المكورة المعوية البرازية ($p \leq 0.05$)، بينما لم يظهر أي زيادة في التأثير المشترك مقارنة بالسيبروفلوكساسين وحده في بكتيريا الإشريكية القولونية. الكلمات المفتاحية: ستافيلوزانثين، نشاط مضاد للبكتيريا، سيبروفلوكساسين. ملاحظة: هل البحث مستل من رسالة ماجستير او اطروحة دكتوراه؟ نعم