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Effect of staphyloxanthin from staphylococcus aureus on breast cancer cell line

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Abstract---One hundred and six of different specimens were collected from patients in Baghdad hospitals with different ages and genders during the period between December to March, 2022. All bacterial Isolates have been submitted to the cultural, microscopically, biochemical examination and Vitek2 system. Fifty-two bacterial isolates 49% belonged to *Staphylococcus aureus*. The results showed the percentage of infection of *S. aureus* from Nasal cavities of (medical staff and patient) Burns and wound 38.9% Which was the most accessible site for *S. aureus* infection. As well as the results showed the percentages of infection of *S. aureus* isolated from urine and blood were (22%) and (27%) respectively and from eyes (20%), while isolates obtained from other specimens were lower. The ability of *Staphylococcus aureus* isolates to produce staphyloxanthin pigment was tested and (TID14) isolate gave the highest pigment production (1.87). In (MCF-7) cell line maximum ratio of inhibition was 84% at conc. of 1000µg/ml, 77% at 500µg/ml, 74% at 250µg/ml and 71% at 125µg/ml, while in (REF) normal cell line at 72hrs exposure time inhibition ratio was 29% at conc. of 1000µg/ml, 26% at 500µg/ml, 23% at 250µg/ml and 20% at 125µg/ml.

Keywords---staphyloxanthin, MCF7, REF, TID14.

Introduction

Staphylococcus aureus is a widely known human pathogen responsible for various clinical manifestations (Lowy, 2018). It is Gram-positive bacterium, cocci-shaped arranged in clusters “grape-like.” On culture media, they can tolerate up to 10% NaCl, and the grown colonies are often golden or yellow (aureus means golden or yellow). They tend to be facultative anaerobes and can grow at temperatures between 18°C and 40°C. The routine identification testing include catalase, coagulase and mannitol fermentation (Rasigade and Vandenesch, 2014). Some strains of *S. aureus* produce a carotenoid pigment called staphyloxanthin. The carotenoids belong to the class of biologically active compounds that have a high anti-cancer and antioxidant potential (Liu *et al.*, 2005). The staphyloxanthin is a membrane-bound carotenoids and is chemically composed of triterpenoid carotenoids, that have a C30 chain that differ it from C40 carotenoid found in other organisms (Clauditz, 2006). MCF-7 is a commonly used breast cancer cell line, that has been propagated for many years by multiple groups (Baguley and Leung, 2011). It proves to be a suitable model cell line for breast cancer investigations worldwide, including those regarding anticancer drugs (Shirazi, 2011). This study aimed to investigate the cytotoxicity effect of bacterial staphyloxanthin on breast cancer cell line MCF7 and normal cell line (REF).

Materials and Methods

A one hundred and six of different specimens were collected from patients in Baghdad hospitals with different ages and genders during the period between December to March, 2022 . Specimens included nasal, urine, blood, burns and wounds, and from eyes.

Detection the Ability of Isolates to Produce Staphyloxanthin

For detection of staphyloxanthin production, 10 ml of brain heart infusion broth was inoculated with *S. aureus* isolates (100ml from each isolate) and incubated at 37°C for 18h in order to get 1.5×10^8 CFU/ml compared with McFarland standard. Skim milk agar was inoculated with 100 ml of each isolate and incubated for 2 days at 37°C and later incubated at 20°C for another 2 days. Appearance of growth with pigment (orange, yellow) indicates a positive result (Marshall and Wilmoth, 1981). Extracting by Ethanol and Ethyl Acetate The pigment of *S. aureus* (TID14) was extracted from S.22 isolate according to (Pelz *et al.*, 2005). The procedure for using ethanol and ethyl acetate was done by the following steps; grown bacterial cells were recovered from the milk agar plates, and double distilled water was added to rinse agar surfaces. The cells were centrifuged at 6000 rpm for 15 min. The supernatant was discarded and the pellet was suspended in 10 ml of ethanol. The extract was incubated for 20 min at 40 °C. For pigment detection, the supernatant should be concentrated in vacuum. Then ethyl acetate 1.7M aqueous NaCl (1:1 v/v) was added for extraction, and vortexed. The extract was removed by vacuum (crude extract) and the quantity of pigment from crude extract was measured (Marshall and Wilmoth, 1981).

Staphyloxanthin Pigment Assay

According to method described by Tao *et al.* (2010) , the staphyloxanthin pigment was measured according to the equation (Steven *et al.*,2015):

$$\text{Total carotenoid unite/cell} = V (A0.0051) / 0.175W \quad (1)$$

A: absorbance value of the diluted staphyloxanthin extraction at 450nm.

V: final volume of the extract staphyloxanthin.

W (g) : the weight of the dried powder of staphyloxanthin.

0.175: extraction coefficient of carotenoid.

Purification of staphyloxanthin by Column Chromatography

After extraction of staphyloxanthin TID pigment (highly productive), it was purified by column chromatography with silica gel by using ethanol for dissolving silica gel (20g, Merck), and the crude extract was run through column chromatography (1.5*60) cm. Ethyl acetate was used to elute fractions and they were evaporated to dryness. All purification steps were carried in the dark due to light sensitivity of the pigments. The collected fractions were assayed and absorbance was measured at 450nm. The purified fractions were stored at at - 20°C. The staphyloxanthin peak fraction was pooled to assay pigment absorption (Tao *et al.*,2010).

Treatment of cancer cell line by Staphyloxanthin

According to Freshney (2010), Plates that contain cancer cell lines was checked under inverted microscope to ensure that the cells was grown well then these steps was done as the following:

- Serial one-half dilutions was prepared in sterile test tubes to get the following concentrations: (125 , 250 , 500 , 1000) µg/ml. all these dilutions was prepared currently at the time of the work.
- Concentrations of Staphyloxanthin pigment was prepared by dissolving 0.01 gm of the pigment in 0.4 ml of DMSO , then the volume was completed to 10 ml of RPMI contain no serum. This conc. Was regarded the first 1000 µg/ml and serial dilutions was done by transferring 5ml from the first dilution to the next one to get the mentioned above dilutions.
- Culture medium was poured out from the well of falcons after removing of transparent adhesive paper , then 0.2 ml/well from each conc. was added about 3 well per each conc. and one column of wells regarded as negative control by adding 0.2 ml of RPMI free of serum. All these additives was done rapidly as could and recover the plates with adhesive paper with gently stirred .
- All plates were incubated at 37°C, Exposure time was 24 hrs only except for plate of Rat Embryo Fibroblast (REF) Exposure time was 72 hrs.

Staining of tissue culture plates

Crystal violet pigment was used to detect the cytotoxic effect of prodigiosin on tumor cells like indicated by Mather and Robert (1998). as the following steps:

- After exposure time of pigment on tumor cells was ended after 24hrs., all plate wells content was poured out and 0.1 ml of crystal violet solutions was added to all wells of the plates. then all plates was incubated at 37°C for 30 minutes.
- Crystal violet was then poured out and excess of this pigment was disposed from wells by washing the plates by tap water gently then washed by distilled water and turned over and allowed to dryness at room temp.
- Optical density for absorbed cells of crystal violet was recorded at 492 nm by using ELISA micro plate spectrophotometer.
- Inhibition rate (IR) percentage was calculated according to Gao *et al.*,2003) as the following equation:-

$$\text{Inhibition rate \%} = (\text{O.D control} - \text{O.D test}) / (\text{O. D control}) * 100 \quad (2)$$

The data of optical density was subjected to statistical analysis in order to calculate the concentration of compounds required to cause reduction in cell viability for each cell line.

Results and Discussion

Cultural Characteristics

The cultural characteristics for *S. aureus* isolates appeared when isolated bacterial samples grown on its selective media, the colony morphology of colony on blood agar and mannitol salt agar, and these isolates were characterized by translucent with varied pigmentation, smooth, raised, and glistening. The bacterial cells appeared as cocci arranged in clusters form, Gram positive, non-motile, which agrees with Missiakas *et al.*, (2013). *S. aureus* isolates have been identified there ability for staphyloxanthin production by culturing of *S. aureus* isolates on skim milk agar. only ten isolates from 52 isolates of *S. aureus* that producing staphyloxanthin and this isolate (TID14) isolated from burn infection and recorded high productivity (1.87). The highly concentration of staphyloxanthin was obtained from extraction by ethyl acetate and ethanol, with absorbance of staphyloxanthin that recorded (0.628) at 450nm, and the amount of pigment produced by (TID14) isolate indicated (170.997) U/cell. These results correlated to the result of Abd Algabar and sawsan(2019) in which the amount of pigment produced was (154.948) U /cell.

Treatment of cancer cell line by Staphyloxanthin

Prodigiosin pigment in fourth concentration at exposure time 24 hrs was used in treating MCF7 cell line. As shown in table (1-1) inhibition ratio was significant ($p < 0.05$) and maximum ratio of inhibition was 84% at conc. of 1000 µg/ml, 77% at 500 µg/ml, 74% at 250 µg/ml and 71% at 125 µg/ml. also the table showed that there was no effect or slightly effect of the solvent DMSO on tumor

cells. (Inhibition was 21% for conc. 1000 µg/ml, 15% for conc. 500 µg/ml, 12% for conc. 250 µg/ml and 5% for conc. 125 µg/ml).

Table 1-1
Inhibition ratio of MCF7 cell line when treated at 24 hrs with purified staphyloxanthin

Conc. µg/ml	IC of staphyloxanthin %	IC of DMSO %	X ² test/P value
1000	84	21	0.000
500	77	15	0.000
250	74	12	0.000
125	71	5	0.000

Abdalgabar *et al.* (2019) showed that staphyloxanthin pigment caused inhibition of MCF-7 cell line and the cell viability was 35% when 400 µg/ml of pigment was used. Sruthy *et al.* (2014) showed in their study that prodigiosin pigment was most active on MCF-7 cell lines and induced apoptosis in a dose dependent manner. Other studies showed the considerable *in vitro* anticancer activity against standard 4 different cancer cell lines (Barretto and Vootla, 2018); This confirms that staphyloxanthin can be a strong anticancer agent that can kill the cancer cells even at very low concentrations without affecting the normal cells. The anticancer nature of the pigment maybe due to the antioxidant nature of the molecule as the research by (Nisha and Deshwal, 2011) highlights that the antioxidant nature of several carotenoid molecules is the key factor to fight cancer by avoiding the damage of DNA and other biological molecules like proteins and lipids. A review by Sharoni *et al.*, (2012) describes that the apocarotenoids may acts as anticancerous agents and thus can be useful in the prevention of cancer and other degenerative diseases.

Effect of staphyloxanthin on normal cell line (REF)

Staphyloxanthin pigment in fourth concentration was used in treating REF normal cell line at 72 hrs exposure time. As shown in table (1-2) inhibition ratio was Non significant (P:0.69-0.24 N.S. P>0.05) and 29% inhibition ratio was seen at conc. of 1000 µg/ml, 26% at 500 µg/ml, 23% at 250 µg/ml and 20% at 125 µg/ml. also, the table shows that there was no effect or slightly effect of the solvent DMSO on normal cells. (Inhibition was 13% for conc. 1000 µg/ml, 9% for conc. 500 µg/ml, 8% for conc. 250 µg/ml and 5% for conc. 125 µg/ml).

Table 1-2
Inhibition ratio of REF cell line when treated at 72 hrs with purified staphyloxanthin

Conc. µg/ml	IC of staphyloxanthin %	IC of DMSO %	X ² test/P value
1000	29	13	0.69
500	26	9	0.34

250	23	8	0.19
125	20	5	0.24

Alsamaraie *et al.*(2012) illustrated in their study that there was no effect on REF normal cells when treated by staphyloxanthin at exposure time 72 hrs. Guryanov *et al.*(2013) showed that staphyloxanthin pigment does not exhibit genotoxicity on normal cell , So the pigment is a good candidate in pharmaceutical development.

Conclusions

The purified extract of Staphyloxanthin pigment showed cytotoxicity effect against MCF-7 cell line while had no effect on normal REF cell line.

References

- Abdalgabar,F.A. ; Authman,S.H. and Khalaf,K.J.(2019) Antibacterial and anti tumor effect of staphyloxanthin produced by *Staphylococcus aureus* (ph.D thesis). Mustansiriyah university ,college of biology science.
- Alsamaraie, A.F. ;Nadir,M.A. and Hussein,S.M. (2012). Extraction and purification of prodigiosin from *Serratia marcescens* and study it's effect on cancer cell lines.(M.SC thesis); Institute of Genetic Engineering and Biotechnology - University of Baghdad.
- Baguley, B.C. and Leung, E.(2011). Heterogeneity of Phenotype in Breast Cancer Cell Lines. In: Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways (Gunduz M, Gunduz E (eds.). Rijeka, InTech, pp. 245-256.
- 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.
- Clauditz, A. A. ; Resch, K. P.; Wieland, A.; Peschel and Götz, F.(2006). Staphyloxanthin plays a role in the fitness of *Staphylococcus aureus* and its ability to cope with oxidative stress. *Infect. Immun.* 74:4950-4953.
- Freshney , R.I. (2010). Culture of Animal Cells: A Manual of Basic Technique and .John Wiley and Sons ,Ins, USA.
- Gao, S. ; Yu, B. P. ; Dong, W. G. ; Luo, I. S. and Li, Y. (2003). Antiproliferative Effect of Octreotide on Gastric Cancer Cells Mediated by Inhibition of Akt/PKB and Telomerase. *World J. Gastroenterol.*, 9(10): 2362-2365.
- Guryanov, I. D. ; Karamova, N. S. ; Yusupova, D. V. ; Gnezdilov, O. I. and Koshkarova, L. A. (2013). Bacterial pigment prodigiosin and its genotoxic effect. *Russ. J. Bioorganic Chem.* 39, 106–111.
- Liu, G. Y., A. Essex, J. T. Buchanan, V. Datta, H. M. Hoffman, J. F. Bastian, J. Fierer, and Nizet. V. (2005). *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J. Exp. Med.* 202:209-215.
- Lowy, F.D.(2018). *Staphylococcus aureus* infections. *N Engl J Med.* 20;339(8):520-32.
- Marshall, J.H. and Wilmoth, G.J. (1981a). Pigments of *Staphylococcus aureus*, a series of triterpenoid carotenoids. *J. Bacteriol.*, 147:900-913.

- Mather, J.P. and Roberts, P.E. (1998). Introduction to cell and tissue culture theory and technique. Plenum Press, New York.
- Missiakas, D. M., and Schneewind, O. (2013). Growth and laboratory maintenance of *Staphylococcus aureus*. *Current protocols in microbiology* , Chapter 9 , Unit–9C.1.
- Nisha K, Deshwal RK. Antioxidants and their protective action against DNA damage. *Int J Pharm Pharmaceut Sci*. 2011;3:28–32.
- Pelz, A. ; Wieland, K.P. ; Putzbach, K. ; Hentschel, P. ; Albert, K. and Götz, F. (2005). Structure and biosynthesis of staphyloxanthin from *Staphylococcus aureus*. *J. Biol. Chem.*, 280(37): 32493-32498.
- Rahmadeni, A. S. ., Hayat, N. ., Alba, A. D. ., Badri, I. A. ., & Fadhila, F. . (2020). The relationship of family social support with depression levels of elderly in 2019 . *International Journal of Health & Medical Sciences*, 3(1), 111-116. <https://doi.org/10.31295/ijhms.v3n1.188>
- Rasigade, J.P. and Vandenesch, F.(2014). *Staphylococcus aureus*: a pathogen with still unresolved issues. *Infect Genet Evol.* ;21:510-4.
- Sharoni Y, Linnewiel-Hermoni K, Khanin M, Salman H, Veprik A, Danilenko M, Levy J.(2012). Carotenoids and apocarotenoids in cellular signaling related to cancer. *Mol Nutr Food Res*.56:259–269.
- Shirazi, F.H.(2011). Remarks in Successful Cellular Investigations for Fighting Breast Cancer Using Novel Synthetic Compounds. In: Breast Cancer – Focusing Tumor Microenvironment, Stem Cells and Metastasis (Gunduz M, Gunduz E (eds.). Rijeka, InTech, pp. 85-102.
- Sruthy, P.B. ; Anjana, J.C. ; Rathinamala,J. and Jayashree,S.(2014). the role of red pigment prodigiosin from bacteria of earthworm gut as an anticancer agent. *J Microbiol Biotech Food Sci* 15 : 4 (3) 246-251.
- Steven, Y. C. ; Tonga, J.S. ; Davisa, E.E. ; Thomas, L.H. and Vance, G.F.(2015). *Staphylococcus aureus* Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. *Clin. Microbiol. Rev.*, 28 (3): 603-661.
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, 5(3), x-xv. <https://doi.org/10.53730/ijhs.v5n3.2938>
- Tao, N. ; Gao, Y. ; Liu,Y. and Ge, F. (2010).Carotenoids from the Peel of Shatian Pummelo (*Citrus grandis* Osbeck) and Antimicrobial Activity. *American Eurasian J. Agric. & Environ. Sci.*, 7(1): 110-115.