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RESEARCH ARTICLE

Extraction of Staphyloxanthin from *Staphylococcus aureus and* Studying Some Biological Effect of Staphyloxanthin Pigment

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Abstract

Objective: This study aimed to extraction and purification of staphyloxanthine and to determine antibacterial effect of purified extract against pathogenic bacteria. Design and methods: To reach this goal from 306 samples of different clinical sources, *Staphylococcus* (22.8%) had been isolated and examined as *S. aureus* depending on cultural morphology, biochemical tests and Vitek2 system. Staphyloxanthin pigment was extracted by using ethyl acetate with ethanol ,as wells the using qualitative and quantitative screening method to detect antibacterial effect of purified extract then the results were statistically analyzed by SPSS to perform the data analysis. Results: The results show significant difference in absorption of staphyloxanthin and the extracts; higher absorption of staphyloxanthin was recorded in tube 7 (0.998). Our study proved that purified extract of staphyloxanthin has an anti-bacterial effecting agent, It made inhibition zone against several kinds of gram negative bacteria at concentration (2, 20) mg/ml. This extract produces high inhibition zone (15mm) against *Pseudomonas aeruginosa*. In conclusion: our finding to confirm the ethyl acetate and ethanol method is very significant for extracting staphyloxanthin pigment. Our results indicate that column chromatography is one of the most important and widely used techniques for separation of pigment (staphylxanthin); also the purified extract of staphyloxanthin was established as anti-bacterial activity against several gram negative bacteria with highly inhibition zone against *Pseudomonas aeruginosa.*

Keywords: *Staphyloxanthin, Antibacterial, Purified extract, Well diffusion.*

Introduction

Staphylococcus aureus is a major bubble normal health provoking critical infections extending from junior skin infections to life-danger infections, such as bacteremia, endocarditis, necrotizing pneumonia and toxic shock syndrome (TSS) in the community and hospital setting [1].

This bacterium definite several possible pathogenic factors such as surface protein that support colonization and invasions, of host tissues also surface influences used to block engulfment by phagocytic (capsule and protein A) in addition to biochemical properties such as (carotenoids and catalase production) definite to improve their survival in phagocytosis (immunological disguises (Protein A and coagulase) [2].

Staphyloxanthine was produced in secondary phase; it is playing an additional role sweeps free radicals by conjugated double bonds. Because staphylolxanthi discovered in the cell membrane, it is enhancing the virulence and fitness of the cells by playing an additional role in the defense against damage by ROS [3].

Staphyloxanthin is a carotenoid pigment that was produced by some strains of *S. aureus* .Carotenoids are one among definite classes of biologically active compounds that have been noted to occupy greater antioxidant and anti-cancer action [4]. *S. aureus* is simulated to produce staphyloxanthin that is located in cell membrane. And their chemical structures of pigment which are all triterpenoid

carotenoids possessing a C30 chain instead of the C40 carotenoid structure detected in majority other organisms [5]**.** Extraction of metabolic compounds depending on nature of compounds that produced from microorganism or pigment characteristic and location in the cell, basically carotenoids are lip soluble, the use of organic solvents and alcohol lead to melt fat connected in the cell wall such as ethyl acetate**,** chloroform and methanol [6].

Column chromatography is a physical separation method based on the distribution of the bio components in a mixture, Wiktor [7] explained the column chromatography step increased the purity of the isolated pigments, while the spectral quality of the main constituent remained. Secondary metabolites like statins, naphthoquinones and carotenoids had produced by microorganism and these metabolites have pharmaceutical purpose and occupy antimicrobial, antioxidant and anticancer activities. Microbial carotenoids have been acceptable benefit in nutrition considering of their role as antioxidants, also in animals and humans, it have important role in improving immune responses [8]. The presented study was designed to detection and extraction and purification of staphyloxanthin, also to determine the effect as antibacterial agent against different type of pathogenic bacteria.

Material and Methods

Collection of Sample and Diagnosing

A total of 70 isolate of staphylococcus (22.8%) isolated from 306 samples of different clinical sources that isolated and examined as *S. aureus* depending on cultural morphology and biochemical tests. These features incorporate colonial morphology, size of colony, color and efficacy on the media such as blood hemolysis, pigments emerge on milk agar and capability to ferment mannitol.

Then bacterial isolates were examined and identified by cultural, microscopic, biochemical test and Vitek2 system characteristics [9]. Also, the 16th isolates from different genes of pathogenic bacteria *(Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumonia, and Enterobacter species*) isolated from different clinical sources depending on Vitek2 system characteristics.

Detection the Ability of Isolates to Produce Staphyloxanthin

In order to identify the ability of *S. aureus* isolates for staphyloxanthin production, 100 ml of S. aureus isolate (70 isolates) separately inoculated with 10ml of BHIB and incubated at 37°C for 18h in order to get 1.5×108 CFU/ml by compare with McFarland standard. A volume of 100 µl of the inoculum from each isolate was streaked on skim milk agar medium, incubated at 37°C for two days and then incubated at 20°C for two days. Appearance of growth with pigment (orange, yellow) indicates a positive result [10].

Extracting by Ethanol and Ethyl Acetate

The pigment of *S. aureus* (STX) was extracted from S.25 isolate according to [11]. By using ethanol and ethyl acetate as follows: recovering of bacterial cells from the milk agar plate after incubation these bacterial cells at 37°C for 72h., in addition double distilled water used for rinsing agar surfaces .Then centrifuging these cells at 6000 rpm for 15 mm.

This supernatant was discarding and this pellet were suspending in 10 ml of ethanol and then incubating this extract for 20 min at 40 °C. After that to detect of pigment, the supernatant must be concentrated in vacuum and then to apply extraction with ethyl acetate 1.7M aqueous Na Cl (1:1 v/v) after vortex the colored ethyl acetate, extract contains the pigment was removed in vacuum (crude extract), and the amount of pigment from crude extract (4ml) was calculated by using the following equation [10].

Staphyloxanthin Pigment Assay

According to method described by Tao [11] the staphyloxanthin pigment was measured as follows:

$$\text{Total carotenoid unite / cell} = V(A - 0.0051)/0.175W \quad (1)$$

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 by ethanol and ethyl acetate from *S. aureus* isolate (S25) (highly productivity of pigment), The pigment purified by using column chromatography with silica gel as follows : ethanol was used for dissolving the silica gel (20 gm, Merck) and then crude extract of pigment subjected in column chromatography (1.5× 60) cm.

The pigmented fractions were eluted with ethyl acetate, and evaporating the individual fractions to dryness. In the dark, the all steps of purification were carried out for the light sensitivity of the pigments. The fraction 5ml were collected and assayed for absorbance at 450 nm The purified pigment was stored at -20°C. The staphyloxanthin peak fraction was pooled to assay pigment absorption [12].

Staphyloxanthin as Anti-bacterial Agent

Antibacterial activity of purified staphyioxanthin was determined based on [13]. Qualitative and quantitative screening method as following: Four bacterial isolates from each genus *(Pseudomonas aeruginosa, Serratia marcescen, Klebsiella pneumonia, and Enterobacter species*) were selected and the inoculum was prepared compared with McFarland turbidity standard. Purified stapyloxanthin was prepared via D.W at concentrations (2, 20) mg/ml.

Well-diffusion Methods

Qualitative screening of antibacterial activity was carried out by inoculating of indicator bacteria on MHA plate, the wells were adjusted in the plate with 4mm diameter sterile cork borer and these wells were filled with 100 µl of purified extracts with concentration (2, 20) mg/ml and then incubated the plates at 37°C for 24hrs and the inhibition rate dejected by zone of clearing around well that contain purified extract of pigment [14].

Turbidity Method

The turbidity assay was performing to assess the sensitivity of the test pathogen in liquid culture according to [13]. In this assay 100 µl of inoculum of indicator bacteria 1.5×108 CFU/ml was added to the test tubes containing 5ml of nutrient broth, then added 100 µl of purified extract of staphyloxanthin at concentration (20)mg/ml, and the tubes incubated at 37°C for 24hrs. After that turbidity of the bacterial cultures was measured spectrophotometrically at 600 nm. The readings were measured by using spectrophotometer and compare between the control (tube containing nutrient broth and indicator bacteria) and inhibition of bacteria growth in tubes (containing the indicator bacteria and purified extract).

Results and Discussion

In this section, the results will be discussed in detail as the following subsections:

Cultural Characteristics

Cultural characteristics for *S. aureus* isolates appeared when isolated bacteria grown on its selective media, the colony morphology of isolates on blood agar and mannitol salt agar, and these isolates were characterized by raised, smooth, glistering, and translucent with varied pigmentation. The bacterial cells appeared as cocci arranged in clusters, positive for gram stain, non-motile, which agrees with [15].

Detection and Extraction and Purification of Staphyloxanthin

S. aureus isolates have been identified there ability for staphyloxanthin production by culturing of *S. aureus isolates* (70) on skim milk agar, In the assay Figure1. only ten isolates from 70 isolates of *S. aureus* that producing staphyloxanthin and this isolate (S. 25) isolated from burn infection as well as, this (S.25 recorded high productivity (1.56) ,also excellent identification (96%) by Vitek2 System has been recorded by (S.25) isolate.

Medium component supported the production of staphyloxanthin and might critical for production of pigment, so that the fatty acid, glucose, sucrose and xylose must be support carbon sources as well as leads to cause variation in the productivity of pigment as well as the complexity of medium component is required to profit from it [11].

The highly concentration of staphyloxanthin was obtained from extraction by ethyl acetate and ethanol, with absorbance of staphyloxanthin that recorded (0.683) at 450nm, and the amount of pigment produced by (S.25) isolate indicated (154.948) unit /cell (4 cuvate).

Column Chromatography techniques were available for the separation and analyses of complex organic mixtures. The gel may be regarded as a typical polar sorbent was used in separation and purification of steroids, lipids, amino acids and dye [16].As a result the presence of one peak, it represented in fraction (5-11) and the fraction (1-13) is containing the purified extract of staphyloxanthin (3ml), and this purified extract is reading at 450 nm and the curve was plotted between the absorbance and fraction number. Column chromatography step increases the purity of the extracted pigments, while the spectral quality of the main constituent remains comparable [17].

The results were showed in (figure 2. and table 1.) significant difference in absorption of staphyloxanthin in tubes containing the purified extract as well as the extracts (in tube 7) recorded (0.998) highly absorption of staphyloxanthin, therefore this tube contain extract of pigment (highly purified).

Antibacterial Activities of Purified Extract of Staphyloxanthin by using Well Diffusion Method on Agar

The presented work was the first study to find the anti-bacterial activity of staphyloxanthin was examined against several pathogenic bacteria *(Pseudomonas aeruginosa, Klebsiella pneumonia, Serratia marcescens*, and *Enterobacter species*).Results were reported to contain significant activity of purified extract of staphyloxanthin that isolated from *S. aureus* (S.25, which appeared inhibition zone against several gram negative bacteria at concentration (2,20)mg/ml , and the highly inhibition zone (15mm) of pigment was recorded against *Pseudomonas aeruginosa* [figure 2].

While the staphyloxanhin is carotenoid pigment and compared with previous study [18] the results were demonstrated the anti-bacterial activity of carotenoid isolated from *Rhodotorula glutinis* strains against different types of pathogenic bacteria. Also, in other study the carotenoid pigment extracted from *Micrococcus yunnanensis* showed high antibacterial activity against *Pseudomonas aeruginosa* Devihalli *et al.*[19] and Boontosaeng [20] were showed the capability of carotenoid pigments to repress pathogenic bacteria in agar well diffusion method.

Accordingly, the results shows in quantitative method the (purified) extract gives strong bactericidal activity at concentration (20 mg/ml) toward Pseudomonas isolates ,this result was similar to that report by [21] they showed Streptomyces has ability to produce pigment that containing yellow color sugar and this pigment has ability to inhibit microbial activity of pathogens could enhances drug resistant (ß-lactamase) such as E. coli, Pseudomonas aeruginosa and Klebsiella. Also in other study by Manimala and Murugesan [22] they found the carotenoid pigment more effective against E. coli, S. aureus and P. aeruginosa.

Turbidity Method

The results show in table (3) and figure (3) the antibacterial activities of purified extract with significant differences in imbibition rate against pathogenic bacteria especially against *Pseudomonas aeruginosa* that was recorded (0.451) compared with control (tube contain the pathogenic bacteria without extract), also the results indicate the inhibition rate is recorded (0.096) from effect staphyloxanthin extract against *Klebsiella pneumonia* and inhibition rate against *Serratia marcescens* is recorded (0.115) as well as against Enterobacter species is recorded (0.077).

Sanjay [23] excited xanthine pigment that induced antimicrobial activity at higher concentration (400 µg/ ml) from pigment , this leading to lysis of pathogenic bacterial cells, therefore the health benefits of astaxanthinas pigment by prevention cardiovascular disease, supporting immune system, and it is owning high antioxidant activity due to cataract prevention [24].

Statistical Analysis

All results were expressed as mean± SD. SPSS version 21.0 was used to perform the data analysis, while an ANOVA test with LSD used to significant compare between means according o to Morgan *et al* [25].

References

1. Steven Y C, Tonga JS, Davisa EE, Thomas LH, Vance GF (2015) *Staphylococcus aureus* Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clin. Microbiol. Rev., 28 (3): 603-661.

2. Scott DK, Natalia M, Frank RD (2015) Pathogenesis of Staphylococcus aureus Abscesses. Am. J. Pathol., 185(6): 1518-1527.
3. Liu GY, Nizet V (2009) Color me bad: microbial pigments as1 virulence factors. Trends Microbiol., 17: 406-413.
4. Liu GY, A Essex, JT Buchanan, V Datta, HM Hoffman, JF Bastian, J Fierer, Nizet V (2005) Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. J. Exp. Med., 202:209-215.
5. Clauditz AA, Resch KP, Wieland A, Peschel 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.
6. Ahmad WA, Ahmad YW, Zakaria ZA, Yusof N Z (2012) Application of Bacterial Pigments as Colorant.ch4., 57-68.
7. Wiktor T, Stanisław L, Joanna P, Piotr L, Dariusz L (2016) Column chromatography as a useful step in purification of diatom pigments. Acta Biochim Pol., 63(3):443-7.
8. Manimala MRA, Murugesan R (2014) In vitro antioxidant and antimicrobial activity of carotenoid pigment extracted from Sporobolomyces sp. isolated from natural source JANS., 6 (2): 649 - 653.
9. Sushma K, Mahalakshmi M, Rajan SM (2012) Morphological and biochemical characteristics and antibiotic resistance pattern of Staphylococcus aureus isolated from grapesIN," International Journal of Nutrition and pharmacology, 2(1): 70-73.
10. Marshall JH, Wilmoth GJ (1981a) Pigments of Staphylococcus aureus, a series of triterpenoid carotenoids. J. Bacteriol., 147:900-913.
11. Pelz A, Wieland KP, Putzbach K, Hentschel P, Albert K, Götz F (2005) Structure and biosynthesis of staphyloxanthin from Staphylococcus *aureus*. J. Biol. Chem., 280(37): 32493-32498.
12. Tao N, Gao Y, Liu Y, 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.
13. Shyamapada M, Manisha D, Nishith K, Krishnendu S (2012) Antibacterial activity of honey against clinical isolates of Escherichia coli, Pseudomonas aeruginosa and Salmonella enterica serovar Typhi . Asian Pacific Journal of Tropical Medicine, 40:2594-2597.
14. Mounyr B, Moulay SS, Saad KI (2015) Methods for in vitro evaluating antimicrobial activity: Journal of Pharmaceutical Analysis, 6(2):1-79.
15. Sushma K, Mahalakshmi M, Rajan SM (2012) Morphological and biochemical characteristics and antibiotic resistance pattern of Staphylococcus aureus isolated from grapes IN," International Journal of Nutrition and pharmacology, 2(1): 70-73.
16. Raoof WM, Latif AR In vitro study of the swarming phenomenon and antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from different human infections. Eur. J. Sci. Res, 47: 405-421.
17. Wiktor T, Stanisław L, Joanna P, Piotr L, Dariusz L (2010) Column chromatography as a useful step in purification of diatom pigments. Acta Biochim. Pol., 63(3):443-7.
18. Turkan MK, Zerrin E, Esra T, Umit K (2013) Antioxidant and Antibacterial effects of Carotenoids Extracted from Rhodotorula glutinis Strains. Asian Journal of Chemistry, 25(1): 42-46.
19. Devihalli C, Mohana S, Thippeswamy R.&Umesh AAntioxidant, antibacterial, and ultraviolet-protective properties of carotenoids isolated from Micrococcus sp. Radiation Protection and Environment . 2013;V : 36 (4) 168-174.
20. Boontosaeng T, Nimrat S, Vuthiphandchai V (2016) Pigments Production of Bacteria Isolated From Dried Seafood and Capability to Inhibit Microbial Pathogens. J E.S., 2319-2402.
21. Selvameenal L, Radhakrishnan M, Balagurunathan1 R (2009) Antibiotic Pigment from Desert Soil Actinomycetes; Biological Activity, Purification and Chemical Screening Indian J. Pharm. Sci.. 71(5): 499-504.
22. Manimala M, Murugesan R (2014) In vitro antioxidant and antimicrobial activity of carotenoid pigment extracted from Sporobolomyces sp. isolated from natural source. J. A. N.S., 6(2): 649-653.

23. Sanjay KR (2009) Characterization of Aspergillus carbonarius mutant in relation to xanthin production toxicity studies and fermentation conditions for pigment production. Ph.d Degree, CFTRI, Mysore.

24. Kirti K, Amita S, Priti S, Mukesh K A, Jyoti S (2014) Colorful world of microbes: carotenoids and their applications. Advances in Biology, 1-13.

25. Morgan K, George A, Leech NL, Gloeckner GW, Barrett IBM (2011) SPSS for introductory statistics: use and interpretation, 4th ed., Rout ledge Academic.

26. Chhibber PK, Majumdar SK (1999) Foreign ownership and profitability: Property rights, control, and the performance of firms in Indian industry. Journal of Law & Economics, 42 (1):209-238.

