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ARTICLE

Evaluation of β-carotene Isolated From Rhodoturella as Anti-microbial Agents

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Abstract

In this study, we investigated the antimicrobial activity potential of β-carotene as an antimicrobial agent. Where the antimicrobial activity was tested against Candida krusei and Streptococcus pyogenes. Results showing The β carotene pigment revealed a significant antimicrobial activity at a concentration of (10,20,40) µg/ml. And the effect of carotene was increase with rise of concentrations in both of case of bacteria and fungi. Where the high inhibition zone was in (40) µg/ml. It appear highly significant differences between the concentration that used. Also, all concentrations were more effect on *S. pyogenes* than *C. krusie*.

Keywords: *Rhodotorulla*, Antimicrobial, Antifungal, Antibacterial

1. Introduction

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. After the revolution in the "golden era", when almost all groups of important antibiotics (tetracyclines, cephalosporin, aminoglycosides (were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance [1] Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health [2]For this reason, discovery of new antibiotics is an exclusively important objective. Natural products are still one of the major sources of new drug molecules today. Several studies have shown that carotenoids can be used as therapeutic agents various type of cancer and other diseases due to their antioxidant and/or provitamin a properties [3]. This explains the increasing interest in production of microbial carotenoids.

2. Material &methods

Fungal Strain: Rhodotorulla sp was obtained from the laboratory of microbiology in science college \university of Al- Al-Qadisiyah.

2.1. Yeast and bacteria strains

Were obtained from the Microbiology Laboratory at Imam Sadiq Teaching Hospital in Babylon governorate, Iraq, for patients with acute and chronic tonsillitis.

2.2. Extraction of β-carotene pigment

β-carotene pigment was extracted from the isolates of *Rhodotorulla* yeast by using Harborne method [4].

2.3. Antifungal activity

2.3.1. Well diffusion agar

1. Preparation of concentrations of (10, 20, 40) g/µl.
2. Prepare Muller Hinton Agar medium and pour it into sterilized petri dishes.

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3. Take a light smear from the plate containing candida isolate and plan on the surface of the prepared plate in one direction, from top to bottom. so that the dish was rotated with a simple rotational motion at an angle of 60° each time.
4. After the completion of culture, the candida isolates were left for 5–10 min to infuse.
5. Use a corkscrew with a diameter of 6 mm, sterilized with an alcohol flame, to make holes of equal dimensions on the surface of the agar.
6. Put 40 μL of beta-carotene dye, with concentrations of (10, 20, 40) g/μl in each of the first, second, and third holes, respectively.
7. The dishes were closed and left in the culture booth for 15 min, then transferred to the incubator at 37 °C for 24 h.
8. After the end of the incubation period, the diameter of the inhibition zone was measured accurately using a ruler and the results were photographed using a camera [4].
9. Out of concern, the experiment was repeated (2–3) times to obtain accurate results.

2.4. Antibacterial

According to the method of spreading in the agar by drilling, studied the characters of β-carotene pigment against isolation of *streptococcal pyogenes* bacteria which he obtained. The pure bacterial isolates obtained at the beginning of the experiment were renewed on the Nutrient Broth Media. The vaccine was prepared by scraping a number of bacterial colonies separated from each other from a bacterial culture at the age of 18 h, using a platinum inoculation needle, which was dipped in a test tube containing 5 ml of sterile distilled water, with manual mixing well to obtain the bacterial suspension.

2.4.1. Well diffusion agar

1. Preparation of concentrations of (10, 20, 40) g/μl.
2. Prepare Muller Hinton Agar medium and pour it into sterilized petri dishes.
3. A sterile cotton swab was immersed in the test tube containing the bacterial suspension under sterile conditions and swabbed the entire surface of the pre-prepared dish in one direction from top to bottom so that the dish was rotated with a simple rotational motion at an angle of 60° each time.
4. After the completion of culture, the bacterial isolates were left for 5–10 min to infuse.
5. Use a corkscrew with a diameter of 6 mm, sterilized with an alcohol flame, to make holes of equal dimensions on the surface of the agar.
6. Put 40 μL of beta-carotene dye, with concentrations of (10, 20, 40) g/μl in each of the first, second, and third holes, respectively.
7. The dishes were closed and left in the culture booth for 15 min, then transferred to the incubator at 37 °C for 24 h.
8. After the end of the incubation period, the diameter of the inhibition zone was measured accurately using a ruler and the results were photographed using a camera [4].
9. Out of concern, the experiment was repeated (2–3) times to obtain accurate results.

2.5. Statistical analysis

Results of the present study were illustrated as mean ± standard devition (SD). The values were statistically analyzed by using ANOVA Different latters denote to the significant differences at $p < 0.05$. The highly significant differences at $p < 0.001$.

3. Results

3.1. Antifungal activity

The results of the inhibition zone are shown in Table (1) (Figs. 1 and 2).

3.2. Antibacterial

Table (2) and Figs. (3, 4) show antibacterial activity of β-carotene against one of Gram-positive bacteria, *S. pyogenes*. It appears highly significant between the

Table 1. In-vitro antifungal activity of β-carotene.

Candida	Concentration μg/ml			
	10	20	40	P value
Mean ± SD of Inhibition zone	**6.0 ± 0.9** ^{A}	9.0 ± 1.2 ^{B}	13.0 ± 3.2 ^{C}	**0.001**[a] **HS**

SD: standard deviation; **HS**: Highly significant at $P > 0.001$.
Different latter's denote to the significant differences at $p < 0.05$.
[a] One-way ANOVA.

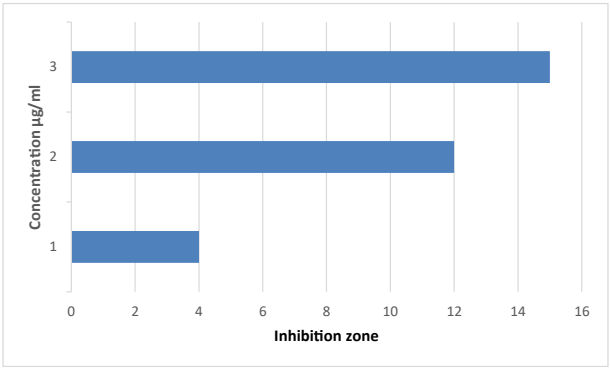

Fig. 1. Diameter of inhibition zone of Candida krusie *treated with β-carotene.*

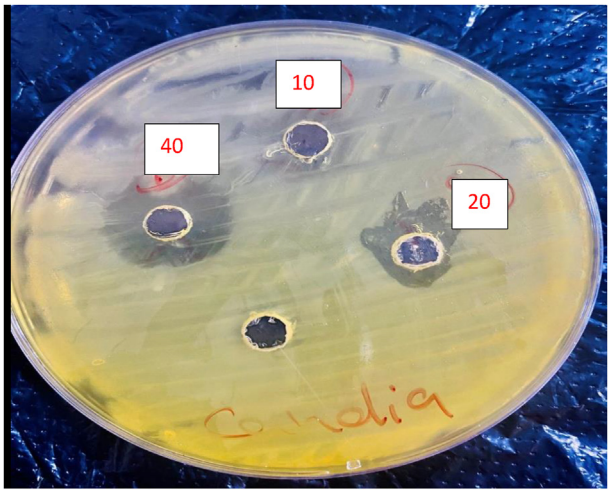

Fig. 2. Inhibition zone of Candida krusie *treated with β-carotene.*

concentration that used. And this activity increased with increased of concentrations.

4. Discussion

This study indicates antifungal activity of β-carotene in vitro, Also, this activity increased with increased of concentrations that used. Also, This study shows antibacterial activity of β-carotene against one of Gram-positive bacteria, *S. pyogenes*. It appears highly significant between the concentration that used. And this activity increased with increased of concentrations. From above results concluded that antimicrobial activity showed no uniform response between strains to the pigment. The difference in sensitivity could be attributed to differences in cell wall composition [5]. indicated that, the difference in cell wall structures makes the antibacterial efficacy to be selective [6]. stated that the a staxanthin dye was found to be significantly more effective against all species of pathogens tested such as *S. typhi* producers, *P. aeruginosa, B. subtilis* and *S. aureus* [7]. reported that the extracellular pigment *Penicillium purpurogenum* was found to be significantly more effective against all microbial species tested which included Candida albicans, *Escherichia coli*, *P. aeruginosa*, *Staphylococcus aureus*, and *Brucella subtilis*. Also [8], observed that the antimicrobial activity of xanthine dye at a concentration higher than 400 µg ml^{-1} results in the lysis of pathogenic bacterial cells.

Yonuis [9] findings that at a dosage of 100 mg/mL, the -carotene pigment showed substantial antibacterial action against K. pneumonia, *E. coli*, and *S. aureus*. K. pneumonia had the highest level of bacterial growth suppression (40 mm), followed by *E. coli* (36 mm) and *S. aureus* (31 mm), while *P. aeruginosa* exhibited no effect. So, carotene pigment may be used as a promising natural alternative to antibiotics and other synthetic substance with concentrations as high as 1200 g/ml against some pathogen strains. Beta-carotene demonstrated strong antibacterial action. Gram-positive bacteria were shown to be substantially more affected by -carotene than Gram-negative bacteria. For the purpose of inhibiting bacterial activity [10]. The current study agrees with [10–12] and the effect of -carotene on Gram-positive bacteria was shown to be significantly higher than that on yeast may be because the yeast was eukaryotic.

Table 2. Inhibition zone of S. pyogenes.

S. pyogenes	Concentration µg/ml			
	10	20	40	P value
Mean ± SD of Inhibition zone	**4.0 ± 0.5** ^{A}	12.0 ± 1.0 ^{B}	15.0 ± 1.5 ^{C}	**0.001**[a] **HS**

SD: standard deviation; **HS**: Highly significant at $P > 0.001$.
Different letters denote to the significant differences at $p < 0.05$.
[a] One way ANOVA.

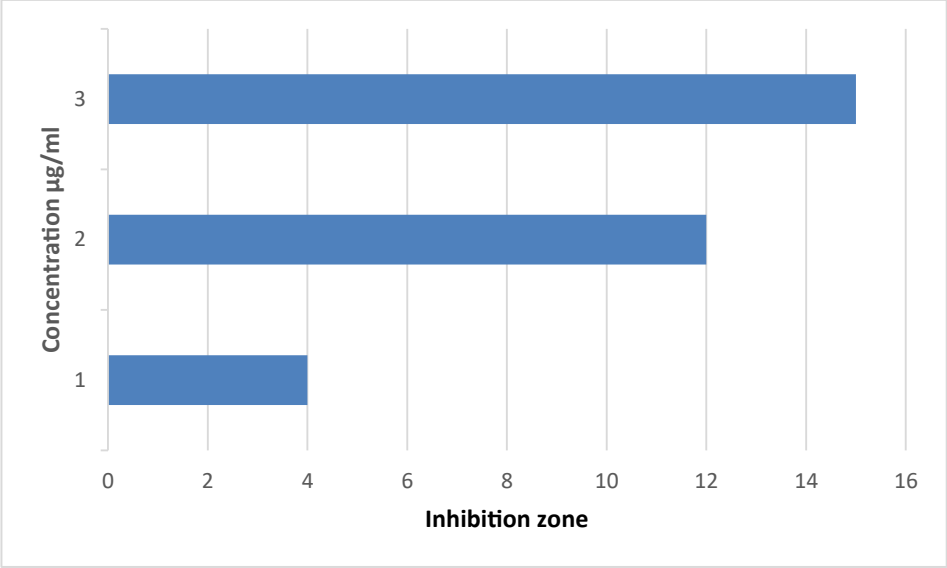

Fig. 3. Diameter of inhibition zone of S. pyogenes *treated with β-carotene.*

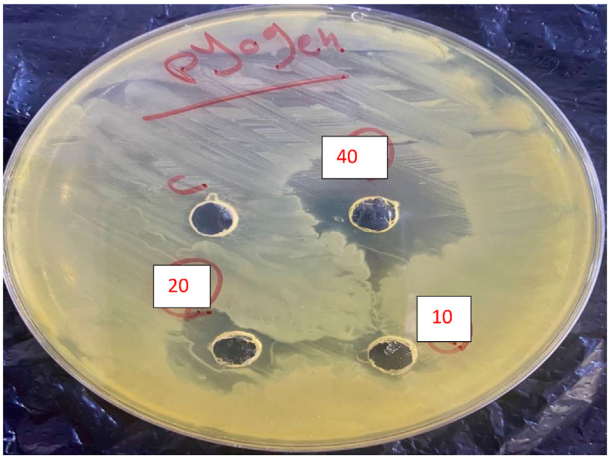

Fig. 4. Inhibition zone of S. pyogenes *treated with β-carotene.*

4.1. Conclusion

In conclusion, the b-carotene pigment demonstrated the highest antimicrobial activity at a concentration of 40 mg/mL against S. pyogenes and C. krusie. Additionally, due to the effectiveness of b-carotene's antibacterial effects as a natural pigment, it may be used as an antibacterial agent to clean and sanitize various surfaces as well as other things.

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