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Evaluation of the Bacterial Inhibitory Activity of *Rhodotorula mucilaginosa* on the Sensory Qualities of the Juice Compared with the Synthetic Dye

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ABSTRACT

This study aimed to evaluate the inhibitory activity of the microbial dye extracted from the yeast *Rhodotorula mucilaginosa*, isolated from local oleander flowers. Beta-carotene dye was extracted and identified using high performance liquid chromatography (HPLC) technique. The results showed that the microbial dye exhibited inhibitory activity against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella*. A concentration of (0.1 g/ml) showed no inhibitory effect. At (0.2 g/ml), inhibition was observed only against *Staphylococcus aureus* with a diameter of (10.00 mm). Concentrations of (0.3, 0.4, and 0.5 g/ml) showed inhibition against all tested bacteria, with inhibition zones at (0.3 g/ml) being (5.00, 12.00, and 5.00 mm), at (0.4 g/ml) being (10.00, 15.00, and 8.00 mm), and at (0.5 g/ml) reaching the highest values of (12.00, 15.00, and 10.00 mm), respectively. Sensory evaluation of juice containing the microbial dye compared to synthetic dye showed a significant improvement in color (9.96 vs. 7.96, $p < 0.05$), with no significant differences in taste and flavor. A significant enhancement ($p < 0.05$) was noted in the texture (9.6 vs. 7.86) and overall acceptability (9.74 vs. 7.19) of the microbial dye. The findings demonstrate that the microbial dye not only possesses antimicrobial properties that help extend the shelf life of foods and beverages but is also well accepted by consumers. Additionally, it serves as a source of vitamin A and plays a valuable biological role in human health.

Keywords: Carateniods, Inhibitory effect, *Rhodotorula mucilaginosa*, Sensory evaluation.

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تقييم النشاط المثبط للبكتريا الخميرة *Rhodotorula mucilaginosa* وتأثيرها على الصفات الحسية للعصير مقارنة مع الصبغة الصناعية

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الملخص

هدفت هذه الدراسة إلى تقييم النشاط التثبيطي للصبغة الميكروبية المستخلصة من خميرة *Rhodotorula mucilaginosa*، والمعزولة من أزهار الدفلى المحلية. تم استخلاص صبغة البيتا–كاروتين وتشخيصها باستخدام تقنية HPLC. أظهرت النتائج أن هذه الصبغات تمتلك نشاطًا تثبيطيًا ضد أنواع من البكتيريا، وهي: *Escherichia coli*، *Staphylococcus aureus*، و *Salmonella*. لم يظهر التركيز (0.1 g/ml) أي تأثير مثبط، بينما أظهر التركيز (0.2 g/ml) تأثيرًا ضد *Staphylococcus aureus* فقط بقطر تثبيط (10 mm). أما التراكيز (0.3, 0.4, & 0.5 g/ml) فقد أظهرت تأثيرًا مثبطًا ضد جميع أنواع البكتيريا، حيث ان قطر التثبيط عند تركيز (0.3 g/ml) بلغ (5, 12, & 5 mm)، وارتفع عند (0.4 g/ml) إلى (10, 15, & 8 mm)، وبلغ أقصاه عند (0.5 g/ml) فكان (12.00, 15.00, & 10.00 mm) على التوالي. أظهرت نتائج التقييم الحسي لعصير مضاف له الصبغة الميكروبية تفوقًا معنويًا في درجة اللون مقارنة بالصبغة الصناعية (9.96 مقابل 7.96، p<0.05)، دون فروق معنوية في الطعم والنكهة. كما لوحظ تفوق معنوي في قوام الصبغة الميكروبية (9.6 مقابل 7.86، p<0.05)، وكذلك في التقبل العام (9.74 مقابل 7.19، p<0.05). تشير النتائج إلى أن الصبغة الميكروبية فعالة كمادة مثبطة للكائنات الدقيقة الملوثة للأغذية والمشروبات، مما يزيد من فترة صلاحيتها، إلى جانب كونها مصدرًا لفيتامين A ولها دور بيولوجي مهم للمستهلك.

INTRODUCTION

Microbbial dyes are compounds that are widely used in many industries, including food. Carotenoids are at the forefront of these dyes for their wide spectrum of colors, extending from yellow through orange to red examples of these colours are β-carotene, lycopene, and others, which are used as colorants for juices and children's sweets[1]. Pigments added to foods are divided into natural, microbial, and synthetic dyes. Synthetic dyes are chemical compounds that have high pigmentation ability, but they have side effects on the health of the consumer and are often carcinogenic [2]. As for natural carotenoids, they are divided into two parts, namely plant sources and microbial sources, the plant sources that contain pigments include fruits and vegetables such as carrots, red peppers, melons, and others. These are considered expensive due to the availability of fruits in a certain season without the other, as well as the raw material is expensive. These costs are carried on the dye, making it expensive, which prompted researchers to find alternatives to the sources of these pigments [3]. The second natural source is microbial sources, which are the ideal solution and alternative to natural plant dyes, as they give a high yield of dyes, in addition to not needing expensive primary sources because they are grown on cheap agricultural media, which are called industrial residues such as sugar beet molasses, sugar cane molasses, date molasses, and others, as well as giving a very high pigmentation rate compared to plant dyes. In addition to the inhibitory role that plant and microbial extracts have against certain types of microorganisms, we conclude from this that plant and microbial extracts have inhibitory activity against microbial pollutants[4,5]. The study aimed to use microbially produced the beta-carotene pigment from *Rhodotorula mucilaginosa* to inhibit certain pathogenic bacteria and to evaluate it sensorially in comparison with synthetic pigment when added to juice.

MATERIALS AND METHODS

Yeast isolation sources

(54 samples) were used in the study to isolate agricultural yeasts. These samples included: flowers of defferent plants (30 samples), damaged fruits (9 samples), soil (3 samples), tree leaves (3 sampels), fronds (3 samples), pickles. (3 samples), and milk (3 samples) which was collected from the local markets of the city of Garma.

Extraction of carotenoids

Modified by the method [6], where centrifugation of (50 ml) of fermentation medium was carried out at a speed of (3500 rpm) and washing the precipitate, equal amounts of hexane and acetone were added, mixed well, and passed on an ultrasonic device to break dawn the cells by bubbles and allow the extaction solvent to enter the depths of the cells and to increase the efficiency of extraction, and then the separation was carried out by filtaration.

Inhibitory effect of microbial dye

The inhibition test of microbial carotenoids pigment produced by *Rhodotorula mucilaginosa*yeast on some of pathogenic bacteria, the most were the bacterial samples taken from nature or from contaminated food. This must be clarified as well as major food contaminants, including *E. coli, Salmonella enterica*, and *S. aureus*, obtained in pure from the central laboratories of the faculty of agriculture and activated for (24 hours) by Davis medium at (37 °C), and different concentrations of microbial dye were prepared in advance at concentrations of (0.1, 0.2, 0.2, 0.3, 0.4, and 0.5 g/ml). They were cultured on Muller-Hinton Ager (MHA) cork drillingmethod medium according to the method described by Kirbybauer [7,8], and the inhibitory diameter was read after (24 hours) of incubation.

Preparation of juice containing the dye

The select any type of juice method was conducted according to the method followed [9], which includes dissolving (650 g) of sugar, (5 g) of citric acid, and (2 ml) of orange flavor, all dissolved in (1 L) of water.The microbialcarotenoids pigment was added gradually until the color was added compared to the commercial orange juice (mizo) and no precipitate was left in the container. The juice was cooled in the refrigerator at a temperature of (7 °C), and sensory evaluation.

Sensory evaluation of processed juice

The sensory evaluation of processed juice with added microbial dye compared to commercial juice according to the evaluation form which includes color, taste, flavor, texture, and overall acceptability by experienced evaluators according to the method used by Wafa Salman [10].

RESULTSAND DISCUSSION

Inhibitory effect of bio-produced beta-carotene pigment against test bacteria

The Table (1) indicate the inhibitory effect of beta-carotene on certain types of bacteria, as different concentrations of beta-carotene were used, including (0.1, 0.2, 0.3, 0.4, and 0.5 g/ml), where (0.5 g/ml) gave the highest inhibitory ability on bacterial types include *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus)* and *Salmonella*, the diameter of inhibition was (12.00, 15.00 and 10.00 mm), respectively. The result appeared diameter of inhibition for each of them reached (12.00, 15.00, and 10.00 mm), respectively, while the concentration of (0.1 g/ml) did not give inhibition on all bacterial types used in the study, while the concentration of (0.2 g/ml) showed its inhibitory effect on *S. aureus* only and did not show its inhibitory effect on other species when the inhibitory diameter reached (10.00 mm), as for the concentrations of (0.3, 0.4, and 0.5 g/ml), each one of them gave an inhibitory effect on all types of bacteria used in the study, as the inhibitory diameter for the concentration of (0.3 g/ml) for *E.coli, S.*

aureus, *Salmonella* (5.00, 12.00 and 5.00 mm) respectively. While the concentration of (0.4 g/ml) gave the higher inhibitory effect for the studied bacteria, (8.00, 12.00 and 10.00 mm) respectively, the inhibitory effect increased with increasing concetraction of carotenoids until reached the largest inhibitory diameter at a concentration of (0.5 g/ml) as it reached (10.00, 15.00 and 12.00 mm) respectively. As shown in Figures (1, 2, 3). The same table shows that the higher the concentration, the greater the inhibitory efficacy against bacteria, and the reason for this is that the higher the concentrations of antioxidants, the greater their ability to capture free radicals and oxidative stress through appeaermechanisim and how antioxidant agent effect and inhibitor zone done, which increases and enhances the inhibitory efficacy [11]. These results are consistent with the findings [12] who isolated seven types of carotenoids from fungi and *Lactobacillus plantarum*and showed that these pigments exhibited an inhibitory effect on *S. aureus*, *E. coli* and *P. aeruginosa*. This was confirmed by[13] who indicated that carotenoid dyes produced by different types of microorganisms have an inhibitory effect on different types of pathogenic microorganisms, most importantly *S. aureus*,*E. coli*, and *Salmonella* by a fungal dye produced by different types of microorganisms.

Table 1: Inhibitory effect defferent concentrations of beta-carotene bio pigment on some bacterial species.

Characteristics (mm)			Concentration effect (g/ml)
Salmonella enterica	***S. aureus***	***E. coli***	
0.00 ± 0.00 D	0.00 ± 0.00 D	0.00 ± 0.00 D	0.1
0.00 ± 0.00 D	0.32 ± 10.00 C	0.00 ± 0.00 D	0.2
0.32 ± 5.00 C	0.36 ± 12.00 B	0.32 ± 5.00 C	0.3
0.20 ± 10.00 B	0.40 ± 15.00 A	0.32 ± 8.00 B	0.4
0.32 ± 12.00 A	0.15 ± 15.00 A	0.15 ± 10.00 A	0.5

*Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between the effect of concentrations.

Biologically active compounds are phenolic compounds that can inhibit free radicals in addition to their multiple applications related to human health, including anti-bacterial, anti-viral, anti-inflammatory, and anti-cancer [14].

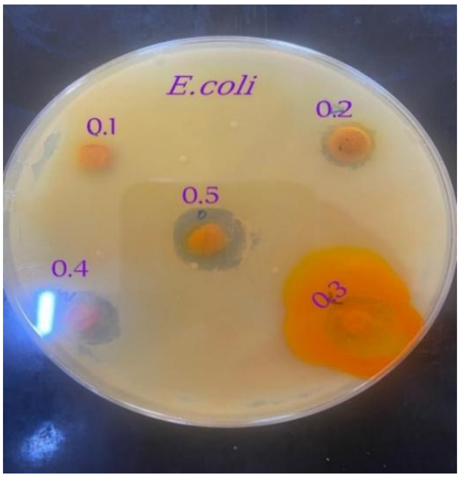

Fig. 1: Inhibitory activity of microbial carotene pigment on *E. coli*.

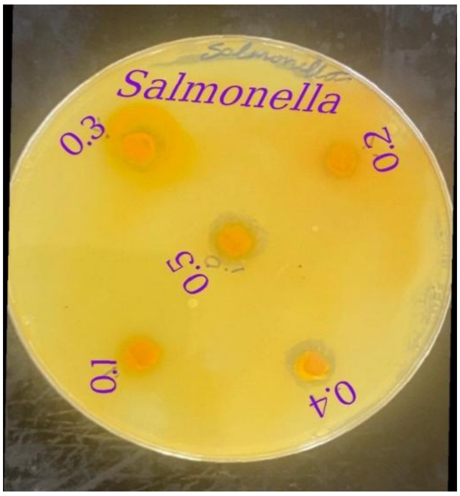

Fig. 2: Inhibitory activity of microbial carotene pigment on *Salmonella*.

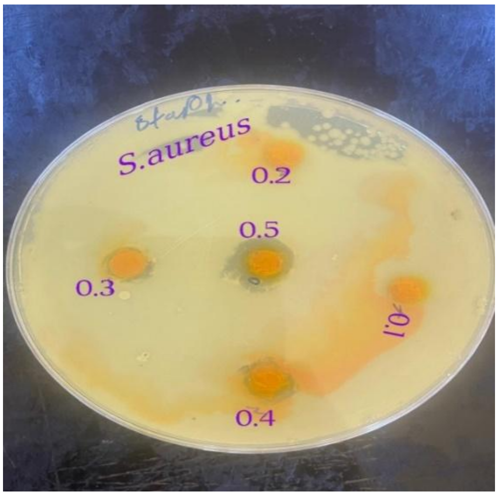

Fig. 3: Inhibitory activity of microbial carotene pigment on *S.aureus.*

Sensory evaluation of juice prepared by adding microbial and synthetic dyes

Table (2) shows the characteristics of the sensory evaluation of the juice made by adding microbial and synthetic dyes, where the results showed that the values of color, taste, flavor, flavor, texture and general acceptance were superior to the microbial dye (9.96, 8.63, 8.30, 9.06 and 9.74), respectively, while the values of the same coefficients when using synthetic dye to prepare the juice were (7.96, 7.63, 8.30, 7.86 and 7.19), respectively. (96, 7.63, 8.30, 7.86, and 7.19), respectively. The results also indicated that there were significant differences in the values of color, texture, and general acceptance, as the microbial dye outperformed the synthetic dye, while the flavor values were equal between the microbial and synthetic dye treatments, reaching (8.30). In general, the results show that the juice with added microbial dye was better in sensory evaluation than the juice with added synthetic dye, which is one of the important factors that distinguish the dyes in addition to their biological effectiveness and their role as a dietary supplement and providing the body with vitamins, the reason may be attributed to the amount of accumulated carotenoids and their chemical composition that leads to a high pigmentation rate comparable to synthetic dyes, which in turn reflects on the texture, taste and general acceptance for the consumer, as shown in Figures (4, 5, 6).

This is consistent with what [15] reported in their study on bio-carbohydrates, carotenoids, and flavonoids, where bio-carotenoids showed their role in improving the sensory, chemical, and physical properties of food products.

Table 2: Sensory evaluation of juice prepared by adding microbial and synthetic dyes.

Characteristics					Effect of coefficients
General Acceptance	**Texture**	**Flavor**	**Taste**	**Color**	
0.19 ± 9.74 A	0.29 ± 9.06 a	0.17 ± 8.30 a	0.20 ± 8.63 A	0.20 ± 9.96 A	**Microbial dye**
0.05 ± 7.19 B	0.29 ± 7.86 b	0.17 ± 8.30 a	0.37 ± 7.63 A	0.20 ± 7.96 B	**Synthetic dye**

*Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between the effect of the coefficients.
*Similar lowercase letters within a column indicate no significant differences ($p > 0.05$) between the effect of the coefficients.

Fig. 4: Processed juice with added microbial dye.

Fig. 5: Processed juice with added synthetic dye.

Fig. 6: Dye production from the yeast *Rhodotorula*mucilaginosa and adding to the juice.

CONCLUSIONS

This study, it is concluded that microbial dye can inhibit certain types of pathogenic and contaminating bacteria in addition to being a food supplement, and it is also characterized by its high staining ability compared to synthetic dye.

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Author contribution: Authors contributed equally in the study.

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