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Department of Biology

Antimicrobial and Anticancer Characters of β-Carotene Pigment Extracted from *Rhodoturella sp.*

A Thesis

Submitted to the Council of the college of Science / University of Al-Qadisiyah in a partial Fulfillment of the Requirements for the Degree of Master of Science in Biology / Microbiology

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

{يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾}

صَدقَ اللَّهُ العلي العَظيم

سورة المجادلة: 11

Dedication

To My Dear Parents

To My Dear Sisters

To My Dear Brothers

To My Dear Friends

And to all people whom I love, I dedicate this work

Ghouson

Acknowledgment

Thanks, and appreciation to God Almighty for all the blessings he bestowed upon me and has succeeded in overcoming the difficulties that we faced. Thanks, appreciation and gratitude to the dear supervisor, Prof. Dr. Neeran Obied Jasim, for her support and assistance to me to complete this work.

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Big thanks and appreciation to and everyone who supported me with good words.

Ghouson

Summary

Microorganisms that produce pigment are among the natural sources that have the ability to help solve today's problems. Additionally, natural colors have additional benefits like antioxidant and anticancer capabilities. In the present study analyze the biological and clinical characteristics of microbial pigments, such as their anticancer capabilities. so this study, β-carotene was extracted from *Rhodoturella* yeast using acetone as a dye solvent and investigated the therapeutic potential of β-carotene as antimicrobial and anticancer agent.

The study included the isolation and identification of yeasts and bacteria from the oral cavity period that lasted from October 1, 2022 to February 28, 2023. 150 isolates of *Rhodotorula* yeast with a percentage of 83.3%, two isolates of Candida yeast with a percentage of 1.11 , and 3 isolates of *Streptococcus pyogenes* with a percentage of 60.0% were obtained. The isolates were diagnosed based on phenotypic, culture and biochemical tests.

The results of extracting the β-carotene pigment showed that *Rhodotorula* yeast was a good natural producer of the pigment, and show the efficiency of the method used for extraction to concluded concentrated pigment. Also HPLC demonstrated that the main carotenoid in extract ,is β-carotene .

Results of antimicrobial activity appear, antifungal activity of β-carotene against of *C. Krusei* at different concentrations (10,20,40) µg/ml, the diameter of inhibition zones were (6,9,13) mm respectively. Also it appears as antibacterial against *S. pyogenes* the diameter of inhibition zones was (4,12,15) mm respectively. And this activity increase with high concentrations.

Summary

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Different amounts of the pigment carotene (0.3215, 0.625, 1.25, 2.5, 5, and 10) g/ml were applied to each cell line of bladder and colon cancer. and demonstrated that beta-carotene clearly causes pathological and cytological changes.

Results show that the purified β-carotene have variant cytotoxic activity against colon cancer cells with different concentrations, where showed higher cytotoxic activity (88.5%) in concentration 10 µg/ml against colon cancer cells . While the lowest inhibition percentage (-3.43%) was at the concentration used (0.3125 µg/ml) in cell line of colon cancer (LS174T). The results also showed the highest viability at concentration 0.3125 µg/ml which is 103.44% after the absorbance reading with the MTT technique (a colorimetric test to evaluate a cell's metabolic activity) and lowest viability rate, at 10 µg/ml and it reached 11.5% . The mean concentration of IC50 of purified β-carotene was 4.83,

Also, results show that the purified β-carotene have variant cytotoxic activity against bladder cancer cells with different concentration of β-carotene, where showed higher cytotoxic activity (85.3%) in concentration 10 µg/ml against bladder cancer cells. While the lowest inhibition percentage (3.06%) was at the highest concentration used (0.3125 µg/ml) in cell lines of bladder cancer (EJ138). The current results showed the highest viability at concentration 0.3125 µg/ml which is 96.93% after the absorbance reading with the MTT technique and To demonstrate the cytotoxicity of β-carotene. The mean concentration of IC50 of purified β-carotene was 5.21.

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List of Abbreviations

Abbreviation	Meanings
CVC	Central venous catheter
DMSO	Dimethyl sulfoxide
GAS	Group A streptococci
RT	Recurrent tonsillitis
CT	Chronic tonsillitis
SDA	Sabouraud's Dextrose agar
RPMI-1640	Rosswel park Memorial institute
PDA	Potato Dextrose Agar
MHA	Muller Hinton Agar
LS174T	Colon cancer cell line
EJ-138	Urinary bladder cancer cell line
Nacl	Sodium chlorid salt
RVE	Rotary Vacuum Evaportory
FBS	Fetal born serum
ROS	Reactive Oxygen Species
IC50	Half-lethal concentration

1- Introduction

Carotenoids are natural pigments responsible for the pleasing colors of many foods and have important biological activities (Berman *et al*., 2015). Some carotenoids are precursors of vitamin A which have beneficial effects on human health including enhancement of the immune system and reduction of the risk for degenerative diseases such as cancer, cardiovascular diseases and cataract (Garrido-Fernandez, 2010). Thus, carotenoids constitute one of the most valuable classes of compounds for industrial applications, e.g. in pharmaceutical, chemical, food and feed industries, color is an important attribute that determines consumers acceptance of foods, recently, a large market of pigments is actually satisfied through synthetic way. However, in food and cosmetic industries, the application of chemically synthetic carotenoids is restricted because of their toxicity (Maldonade, 2007).

Rhodotorula sp. is carotenoid synthesizing yeast, part of the *Basidiomycota* phylum, easily identifiable by distinctive yellow, orange/red colonies. The main carotenoids produced in *Rhodotorula* species are torularhodin, torulene and γ-carotene and minute quantity of β-carotene (Smith *et al*., 2021). Among the main biological properties described carotenoids, they stand out for their antioxidant capacity and ability to quench singlet oxygen species (Aziz *et al*., 2020)

The efficiency of carotenoids use is known for the protection and therapy of various chronic diseases. They exhibit an anti-inflammatory property and may activate the immune response of an organism (Haddad and Levy 2012). It was shown that the use of lycopene-enriched foods might decrease the risk of developing atherosclerosis and other cardiovascular diseases, carotenoids might have beneficial effects on weight management and obesity (Eggersdorfer and Wyss 2018) It is expected that future studies could reveal a positive role of carotenoids in

the treatments of other diseases, i.e., skin disorders, rheumatoid arthritis, periodontal diseases, and others (Bonet *et al.*, 2020). Carotenoids have characteristics of antioxidants. They quench 1O2 and in-crease the levels of glutathione and glutathione peroxidase (Niranjana *et al.*, 2015).

β-carotene reduces the risk of developing neoplastic diseases, and also inhibits the promotion and progression of neoplasm. Very promising findings were also obtained on the putative efficiency of using carotenoids against some types of cancer (Cardoso *et al.*, 2016). The anticancer activity of some others, was studied regarding prostate, breast, colon, lung, oral, gastric, and skin cancers, in addition to hematoma, leukemia, uveal melanoma.

Due to the fact that their cells are significantly smaller and grow at greater rates in a cheap culture media, microorganisms offer several advantages over plants and animals for the creation of pigments, Additionally, microbes are highly productive and can continuously create a product. Moreover, a variety of microorganisms, including bacteria, fungi, and protozoa, create natural pigments including carotenoids, the greatest array of pigments can be produced by fungi on a range of substrates, Fungi create these pigments as secondary metabolites.

So, this study performed to clarify the biological characters of β-carotene that purification from *Rhodotorula sp*, including, antimicrobial activity against fungi and bacteria and anticancer activity. To achieve the aim of this study, we carried out the following study steps:

1-Isolation and identification of yeast *Rhodotorula sp*.

2- Isolation and identification pathogenic fungi and bacteria from oral cavity.

3. Extraction of Pigments from *Rhodotorula Spp.*

4. The antimicrobial effect of the extracted pigment against potent pathogenic fungi and bacteria.

5. anticancer effect of extracted pigment by used cell line

2.1. Rhodotorula

A genus of colored yeasts belonging to the class Basidiomycota. That produces tiny capsules, rudimentary hyphae, and spherical to ellipsoidal budding yeast (Wirth and Goldani, 2012). The typical flora of the human gastrointestinal, respiratory, and vaginal systems as well as the moist part of the skin include it, and it is widely disseminated in the environment (Fleet and Weiss, 2006). When grown on Sabouraud's dextrose agar (SDA), its characteristic orange/red colonies make it easy to identify. Rhodotorula yeast, also referred to as carotenogenic yeasts, are extremely valuable industrially because they can synthesize carotenoids. Has been scientific classified (Lambert *et al*., 1957).to:

Kingdom: Fungi

Division: Basidiomycota

Class: Microbotryomycetes

Order: Sporidiobolales

Family: Sporidiobolaceae

Genus: Rhodotorula

Rhodotorula species are ubiquitous saprophytic yeasts that can be recovered from many environmental sources (Ekendahl *et al*., 2003). *R. mucilaginosa* is commonly isolated in foods and beverages. Several studies have reported the presence of *R. mucilaginosa* in peanuts, apple cider, cherries, fresh fruits, fruit juice, cheese, sausages, edible molluscs, and crustaceans (Tournas *et al*., 2006). *Rhodotorula* was reported as the causative agent in some papers, including dermatitis in sea lions, chickens, and cats, and lung infections and otitis in sheep and cattle. This fungus can also be found in pools where sea animals are kept in captivity.

Previously considered nonpathogenic, Rhodotorula species have emerged as opportunistic pathogens with the ability to colonies and infect susceptible patients. *Rhodotorula* in humans primarily cause bloodstream infections that are associated with central venous catheter (CVC) use (Tuon and Costa. 2008). Risk factors include solid and hematologic malignancies in patients who receive corticosteroids and cytotoxic drugs, the presence of CVCs, and the use of broad-spectrum antibiotics. Unlike Fungemia, localized infections caused by *Rhodotorula*, including skin, ocular, meningeal, prosthetic joint, and peritoneal infections, are not necessarily linked to the use of CVCs or an immunosuppression (Wirth, 2012)

Actually, there are 8 species of the genus *Rhodotorula* known and several of them are found in humans for example *R. glutinis, R. minuta*, and *R. mucilaginosa*. Since these fungi are tolerant to dryness, they are able to survive in the environment. Hence, this genus can be found almost everywhere in the surroundings, namely in the air – *Rhodotorula* is one of the most common fungi transmitted by air – in soil and water, namely ocean seas as well as lakes, in many kinds of fruits and berries (for example strawberries), milk, toothbrushes and shower curtains. Since these yeasts are endowed with a definite affinity for plastic materials (Wirth, 2012), they can be often detected in medical devices, for example on plastic catheters (Kitazawa *et al*., 2018) and dental equipment where they will grow in form of biofilms. Furthermore, they colonize often hands of medical staff.

They are taken up regularly by mammals as well as humans via food, which may lead to intermittent or even permanent gut colonization even in healthy subjects (Schinder *et al*., 2017) not least because they are relatively resistant to bile in comparison to *Candida albicans* being susceptible to bile acids to some extent.

The ability and potential of *Rhodotorula sp.* yeast to manufacture carotenoids on a bigger scale. (Lau *et al*., 2018). Also (Garcia *et al.*, 2021) reported that, one of the colors produced by the fungus *Rhodotorula sp*, which was identified from a food sample, was beta-carotene.(Zhao *et al.*, 2019) mention The isolated yeast *Rhodotorula sp.* can potentially be used as a promising microorganism for the commercial production of carotenoids

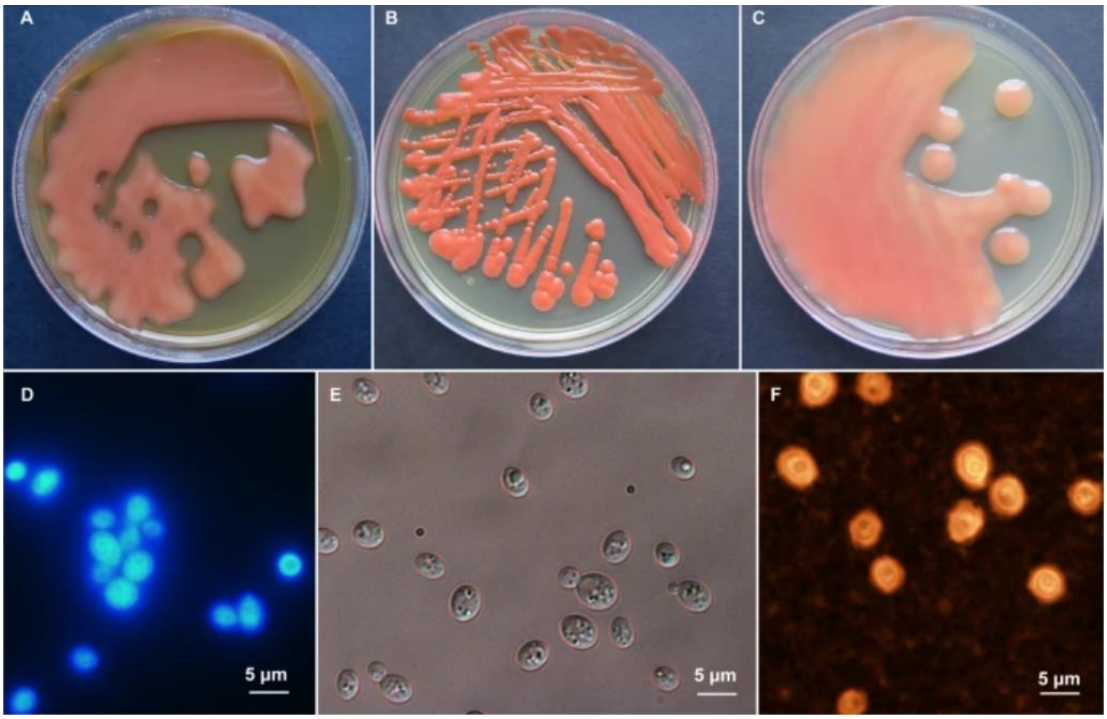

Figure (2-1): ***Rhodotorula*** sp.(A,B,C :colony .Fluorescent microscopy .E: viewed by DIC .F: light microscopy)
(Touchette,*et.al*,2022)

2.2. Carotenoids

Carotenoids are a group of natural pigments produced by a wide variety of plant, algae, and microorganisms such as bacteria, molds, and yeast (Perez -Fons *et al*., 2011).

Carotenoids have applications in various areas of industry, medicine, agriculture, and ecology. That give many foods their appealing colors and have significant biological functions. In addition to strengthening the immune system and lowering the risk of degenerative diseases like cancer, cardiovascular disease, and cataracts, some carotenoids, which are precursors to vitamin A, have positive effects on human health. Garrido – Fernandez.2010)

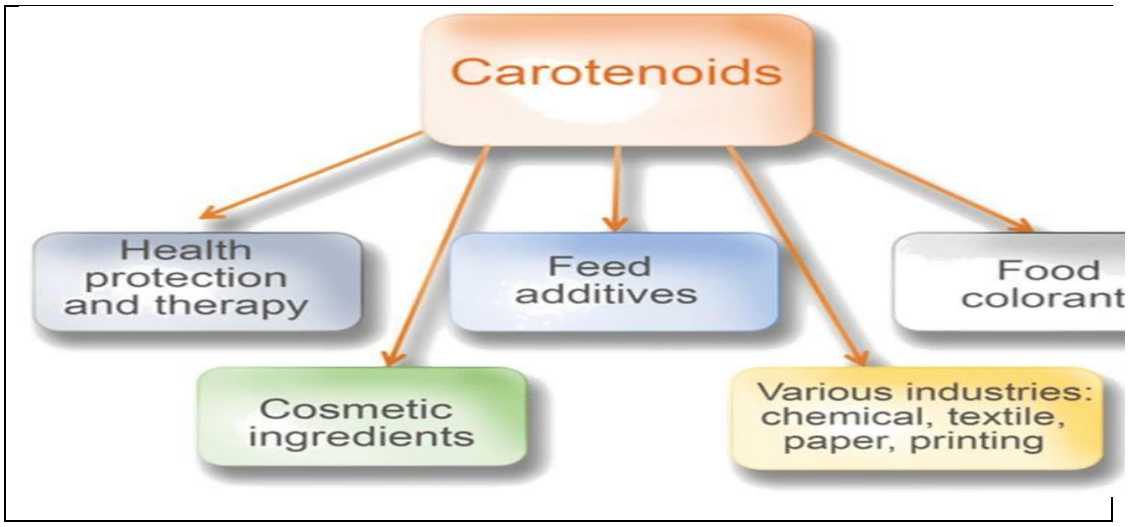

Figure (2-2): Current and potential use of carotenoids.

They are highly unsaturated organic compound with the chemical class of polyamide compound with the same general molecular formula (C40H56) .it belong to the various turbine kinds. Due to the isoprene units that make up its chemical structure. The yellow, red, and pink colors of the carotenoids and their susceptibility to oxidation are due to the presence of many admixtures that enter into their composition (Rodriguez-Amaya, 2001).

Around 80 compounds were considered to be naturally occurring carotenoids in 1960; this figure rose to 500 in 1980; and as late, there are currently more than 700 different varieties. It is one of the most important types of carotenoids produced by yeasts: -

2.3.1 β-carotene

It is used as a nutritional supplement and food coloring in concentrations ranging from 2-50 ppm. It is a precursor to vitamin A. Juices, beverages, and some goods like cheeses, butter, and ghee all contain it (Britton *et al*., 1995)

2.3.2 Astaxanthin

A red pigment that gives fish, birds, and marine invertebrates their beautiful colors. It is a coloring agent, just like the carotenoid pigment, which gives salmon, trout, and shrimp their pink and red hues (Jackson *et al.*, 2008).

2.3.3. Torulene

The thirteen double bonds it possesses make it more effective as an antioxidant than β-carotene, which has less double bonds in its makeup. It is distinguished by its appealing colors, and its usage as an antioxidant is made possible by its possession of this amount of double bonds (Afroz *et al*., 2023).

2.3.4. Canthaxanthin

It is a globally important keto-carotene pigment, it is mainly used in the food and cosmetic industries (Hannibal *et al.,* 2000).

Carotenoids are divided according to the presence of oxygen in their composition (Breithaupt. 2007).

1.Carotenes

They are colored lipids of oxygen-free compounds, which are polyunsaturated hydrocarbons, soluble in polar solvents, mainly containing 40 carbon atoms per molecule and variable numbers of hydrogen atoms. They are tetracyclic, which means that they are composed of eight isoprene units.

2.Xanthophylls

Oxygen-containing compounds include in the form of active groups. They are similar to carotenoids in their carbonic structure. They are considered ketones of carotenoids, and each xanthophyll compound often has a corresponding compound of carotenoids.

2.3.5 Carotenoid pigment

The carotenoid pigments of red, yellow, pink, orange and coral red represent a group of natural antioxidant pigments that can be produced through biotechnological processes. Which led to an increase in their use in the food, cosmetics and pharmaceutical industries, and these compounds are responsible for the intense coloring of fruits and plants because of its abundance in them, such as citrus fruits, carrots, apricots.

Technologically important pigments are produced from various organisms (Malisorn and Suntornsuk, 2008) such as bacteria of the species *Corynebacterium michiganense*, *Micrococcus sroseus*, *Brevibacterium* spp., and *Gordonia jacobaea* (Saini and Keum, 2019) and many algae like *Dunaliella, Dictyococcus, and Haemato* coccus and some species of filamentous fungi and a few fungi that belong to the class of Ascomycetes as well as yeasts such as *Cryptococcus, Phaffia, Rhodospridium, Rhodotorula, and Sporobolomyces* (Marova *et al*., 2012).

It has recently been noted that the production of carotene from plants has become limited. this is due to the high production costs

compared to the percentage of returns, which led to the orientation of most research and studies to obtain carotene from microorganisms, which have become alternative sources for plants, given the low cost of production and avoiding environmental pollution with residues resulting from agricultural and industrial waste(Mata-Gomez *et al*.,2014) as well as The carotene produced by these microorganisms avoids the problems resulting from seasonal and geographical changes that confront its production from plants(Frengova and Beshkova,2009).

Yeasts are more suitable for producing carotene than other microorganisms, especially algae, due to their being single-celled organisms as well as their rapid growth rate (Perez- Fonz *et al*., 2011)

2.3.6. Types of carotenoids and their chemical composition

There are three types of carotene: alpha-carotene, beta-carotene and gamma-carotene. Figure (2-3) shows the chemical structure of the three types of carotene.

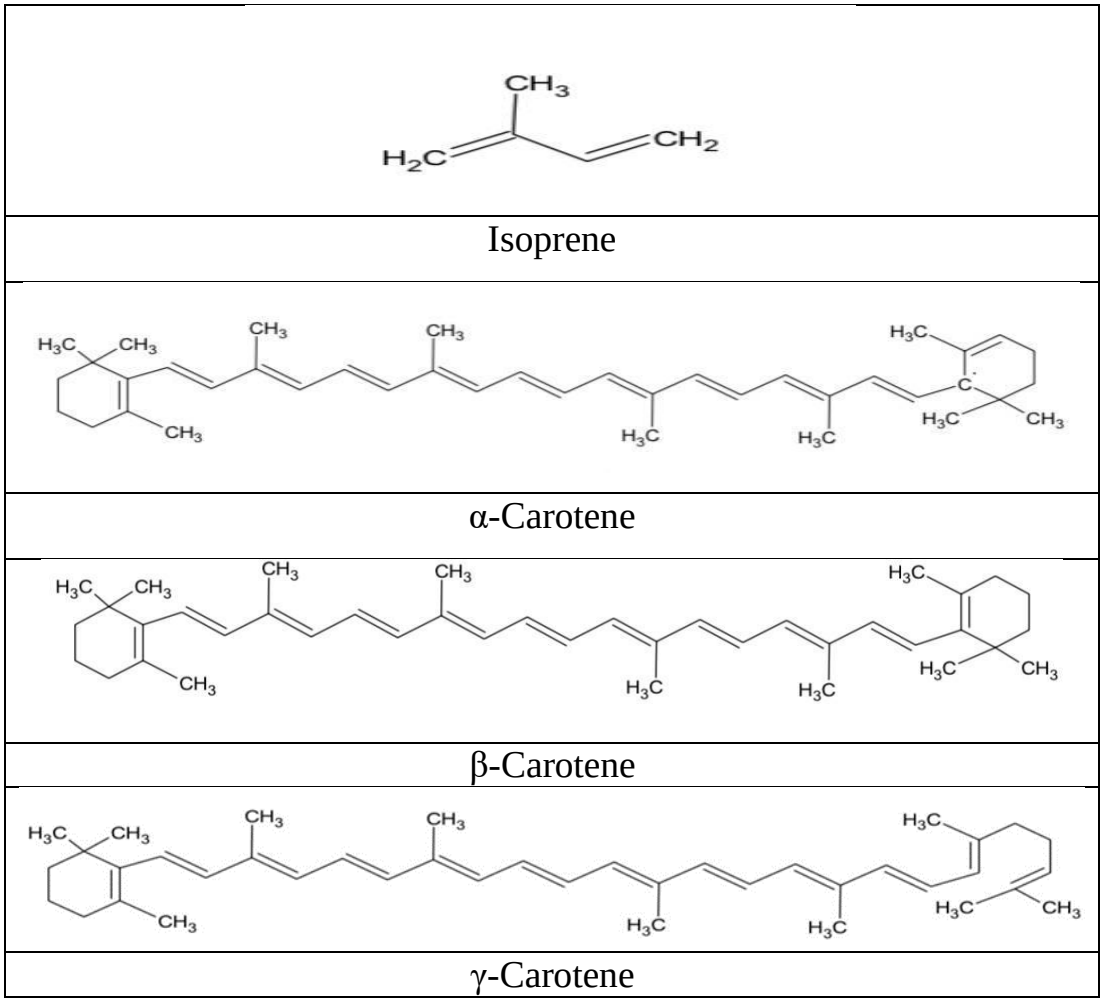

Figure (2-3) chemical structure of the three types of carotene.
(Grotewold, 2006)

2.4. β-carotene

β-carotene is one of the important bioactive components with potent antioxidant activity. Apart from its nutritive value and health benefits, β-carotene can serve as a substitute to synthetic dyes as the poor stability and solubility issues can be addressed by encapsulation. A number of potential health benefits have been associated with the consumption of this bioactive at appropriate levels; however, despite large number of publications the exact characteristics that make this carotenoid effective are still at large unknown.

More research is needed to fully understand the antioxidant mechanisms involved in β-carotene physiology in addition to knowing the exact possibility of this carotenoid acting as an anti-carcinogen at appropriate dosage (Donhowe *et al*., 2014)

2.4.1. Importance and applications of β-carotene

One of the most significant and prevalent natural carotenoids produced by plants and microorganisms, beta-carotene makes for roughly 70% of all carotene (Eldashan and Singab, 2013). Due to the overwhelming amount of information demonstrating its advantages and significance for human health, interest in it has significantly increased recently.

It serves as a source of vitamin A in the body, which is then transformed into vitamin A in the human intestine and essential for maintaining vision. It also functions as an antioxidant to defend the body against free radicals, It strengthens the body's immunological system and aids in promoting cell growth and differentiation (Terlecki *et al*., 2014) Therefore, it is given to those who suffer from a deficiency in the body in daily doses, It has been demonstrated through research (Giuseppe *et al*., 2007) that it aids in shielding lymphocytes from the threat of free radicals,

particularly hydrogen peroxide(H_2O_2) and nitrogen peroxide(NO_2) which can damage cells and their membranes.

It is a particularly powerful antioxidant against several vegetable oils, including corn oil, soybean oil, and sunflower oil, Various carotenoids, found in different foods, such as β-carotene, lycopene, lutein, and zeaxanthin are believed to have a role in maintaining bodily functions and preventing diseases. β-carotene, along with many other carotenoids, is a source of provitamin A. Vitamin A, a fat soluble vitamin, is an important nutrient not only for the vision and, preventing nyctolapia (night blindness) and xeropthalmia (lack of tears/abnormal dryness), but it also helps to strengthen the ability of immune system to resist infections, proper growth, development, gastrointestinal function, and functioning of reproductive systems (Haskell., 2012) Humans lack the ability to synthesize vitamin A.

Therefore, must get proper amounts of it from the dietary sources, rich in β-carotene, like dark green leafy vegetables (e.g. spinach), fruits, and vegetables (e.g. carrot, orange, and mango) (Gul *et al.*, 2016). The rising awareness of the potential health benefits of β-carotene has lead to the development of functional foods enriched with β-carotene (Sy *et al*., 2013).

In addition to using it as a colorant for food and beverages, giving it an attractive color, flavor and taste, and being a safe and non-harmful substance for human health instead of industrial dyes that are not authorized to be used in food (Mohsen, 2010; Venil *et al*. 2013), It is also used in coloring cosmetics. Besides, being attractive natural colorant, β-carotene provides additional advantages due to its provitamin and antioxidant properties.

As β-carotene has highest vitamin A activity among other provitamin A carotenoid (α-carotenes and cryptoxanthins) (Donhowe *et al*., 2014) and most efficient conversion to vitamin A. It stands as one of the most widely studied of all carotenoids present in nature.

Apart from the primary role that β-carotene plays in being as the nutrient source of vitamin A, β-carotene has a myriad of health benefits associated to it when consumed at appropriate levels. It is a potent antioxidant and can function as a lipid scavenger and a singlet oxygen quencher due to the unique structure of conjugated double bonds and in one rings (Grune *et al*., 2010).

Epidemiological studies and clinical trials have established a number of potential health benefits of β-carotene, e.g. decreased risk of some cancers, cardiovascular disease, age related macular degeneration, and cataracts, and increased immune response (Boon & Clements, 2010).

The physiological benefits that have been proposed to account for the health benefits include preventing oxidative damage, quenching singlet oxygen, altering transcriptional activity, and serving as a precursor of vitamin A (Lee and Park, 2011).

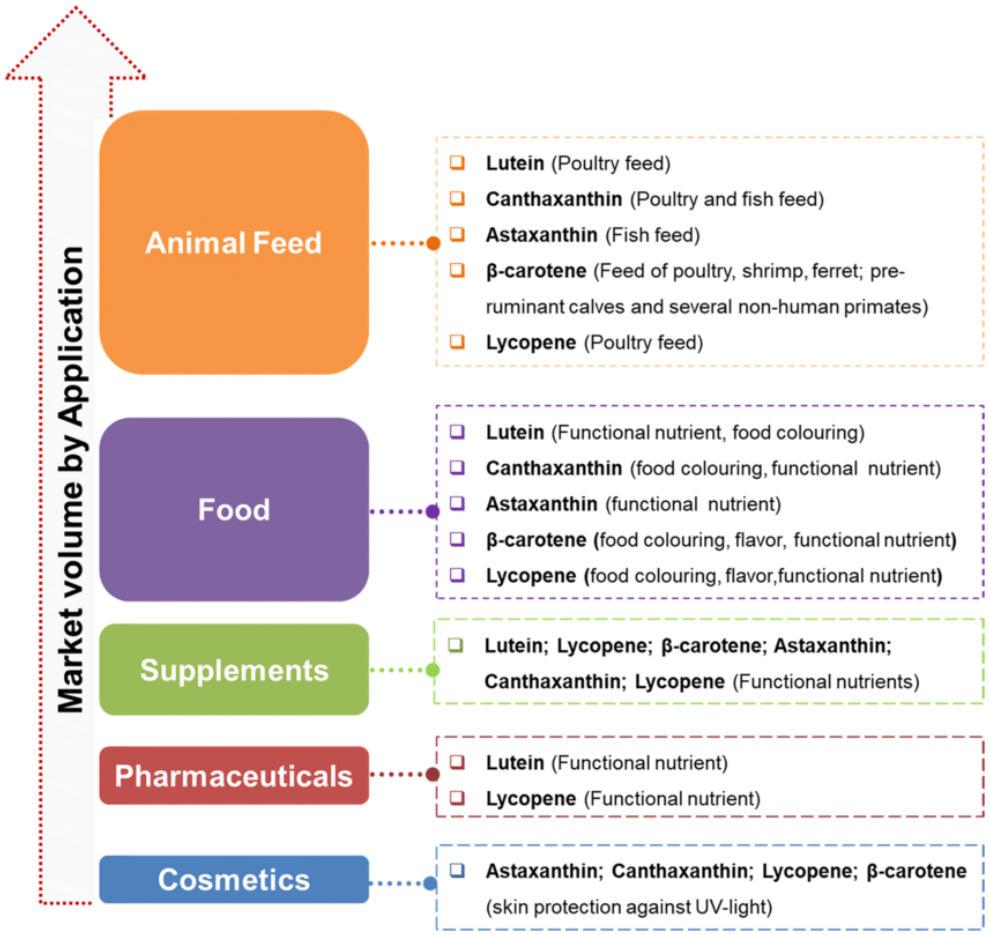

Figure (2-4) Carotenoids and their industrial applications (Mussagy,*et.al*,2019)

2.4.2 Carotene extract

Carotenoids accumulate in certain parts of the cell, but it has not yet been inferred that they are present in the plasma membrane only or in the cell wall as well. In addition, the distribution of carotenoids and their derivatives in parts of the cell has not been studied (Mata-Gomez *et al*, 2014).

Many techniques were used to extract carotenoids from yeasts, but they faced many difficulties and limitations due to the characteristics of the cell wall, which constitutes an obstacle to the extraction processes, and this calls for improvement and development in the techniques used. Many types of techniques were used, including mechanical, chemical and enzymatic, in tearing the cell walls. Carotenoids were obtained from yeasts Phaffia rhodozgma. With high concentrations of 190.35 µg / g when applying the technique of freezing the live mass and enzymatic hydrolysis, despite this, many patents related to the production of carotenoids from microorganisms were registered in various parts of the world.

One of the most important techniques used to break down cell walls in yeasts and obtain one of the most important techniques used to break down cell walls in yeasts and obtain carotene is soaking and freezing in diatomaceous earth, treatment with carotene is soaking and freezing in diatomaceous earth, treatment with Dimethyl sulfoxide (DMSO), Enzymatic hydrolysis using Ultrasound Waves (Michelon *et al*, 2012), use Glass beads (Aksu and Eren, 2007), dry freeze (Park *et al*, 2007), soaking and freezing with liquid nitrogen (Valduga *et al*, 2009), use a mixture of solvents (DMSO), acetone and petroleum ether (Taskin *et al*, 2011) or use acetone and hydrochloric acid.

2.5 Antimicrobial agents

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 (Mayers *et al*.,2017)

Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health (Guschin, *et al*, 2015)

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 property (Ungureanu *et al*., 2011). This explains the increasing interest in production of microbial carotenoids.

2.5.1 Antifungals

All chemical substances, pharmaceuticals, and organic items used to treat fungi are collectively referred to as "antifungals. (Wilkinson et al., 2021). The majority of illnesses are brought on by opportunistic fungus, such Candida yeasts, therefore cases of infections brought on by fungi that are resistant to treatment have risen over the past several decades, raising concerns among doctors (Lestrade *et al*., 2019; Denning *et al*., 2014).

The treatment of fungal diseases has progressed over the past few years by selecting the appropriate antifungal type at the appropriate time,

resulting in rapid improvement and recovery (Bassetti *et al*., 2016). In low concentrations, antifungals either kill fungi or prevent them from growing, but in other cases, they are either ineffective or do not prevent fungal cells from growing. And these antifungal have multiple side effects, as the antifungal Amphotericin B causes low blood pressure, fever and chills, and treatment using antifungals is difficult because of the toxicity to humans so scientists tended to produce natural antifungals to reduce side effects.

As well as increasing development in therapeutic methods for fungal diseases by selecting the required and correct antifungal type at the appropriate time, as this leads to good results, which are reflected in improvement and rapid recovery (Bassetti *et al*., 2016), Among the most important groups of antifungals for the treatment of various fungal infections are the following:

1-The polyene group, which includes Nystatin and Amphotericin B in its various preparations.

2-The azole group and its derivatives such as Fluconazole, Iitraconazole, Clotrimazole and Ketoconazole

The polyene group is one of the important antifungal groups that interacts with Ergosterol within the fungal cell membranes and their contents leak out, and then the fungal cell dies (Szomek *et al*., 2021), one of the most important types is nystatin, which is one of the most important antifungal drugs with high effectiveness and broad spectrum, the mechanism of action of this antagonist is represented by two mechanisms. The first is the interaction of this antagonist with ergosterol which is a major component of fungal cell membranes, where it proves the construction of the membrane and thus the death of the cell, The second mechanism is represented in the participation of this antifungal in a series

of oxidation-reduction reactions and interferes with the lipoproteins that are found within the fungal cell membrane This leads to poor permeability of the membrane (Mesa-Arango *et al*.,99 2012). Nystatin is highly effective against pathogenic fungi, especially fungi that infect children under the age of the33 weeks (Ganesan *et al*., 2009).

In front of the azole group, it is one of the largest groups of antifungals that affect the production of Ergosterol, which is one of the components of fungal cell membranes (Hof, 2006), there are important types of antifungal Fluconazole, which is one of the most widely used types of antifungals, as this drug is used to treat oral and pharyngeal candidiasis, urinary tract infection and systemic candida infection of all kinds, The mechanism of fluconazole is represented by their interaction with compound 14α- demethylase which is the enzyme cytochrome P-450 And what is responsible for stimulating the process of converting the compound Lanosterol to the Ergosterol present in the composition of the fungal cell membrane, fluconazol works by inhibiting the production of Ergosterol and then increasing cell permeability, which leads to cell death as well as preventing the cell's cellular respiration process (Berkow and Lockhart, 2017), it should also be noted that the loss of sterols occurs in conjunction with the accumulation of compound 14- Methylsterols found in fungi, and this is the reason for the sensitivity of fungi to this drug, Also, fluconazole is effective against *Candida* species and *Cryptococcus* species, and it has activity against yeast *C.glabrata*, but it has no activity against yeast *C.krusei* (Spampinato and Leonardi, 2013;Hornik *et al*., 2021).

2.5.1.1 Candidiasis

The term "candidiasis" is used to describe fungal diseases brought on by species of the genus *Candida* spp. These infections are numerous and widespread since the mucous membranes of the mouth, vagina, and the lining of the alimentary canal are home to these species naturally (Mahmoudabadi *et al*., 2013).

Candida albicans is the most common worldwide, although cases of other types of *candida* have been reported (Castelo *et al*., 2004). Candida yeasts succeeded in causing infections due to their possession of virulence factors that make them resistant to the surrounding conditions.

They have the ability to withstand temperatures of 37℃ degrees, dimorphism, and produce their lysing enzymes that help them penetrate the tissues of the host and have the ability to adhere to the cell wall and penetrate the tissues of the host (Mayer *et al*., 2013). Due to the various fungal infections and their wide spread, antifungals were used to treat these infections, but a significant development was observed in the resistance of these fungi towards antifungals, and some of them failed to treat most cases (Silva *et al*., 2011).

Antifungal resistance has increased in recent years due to the indiscriminate and increasing use of antifungals (Yang *et al*., 2003). Resistance to these antibiotics has increased with the increase in their prevalence rate, widespread use of them, and the weakened immunity of patients with serious diseases (Bassetti *et al*., 2015). The increase in the resistance of Candida yeasts to antifungals is not limited to one type, but includes all types (Alexander *et al*., 2013).

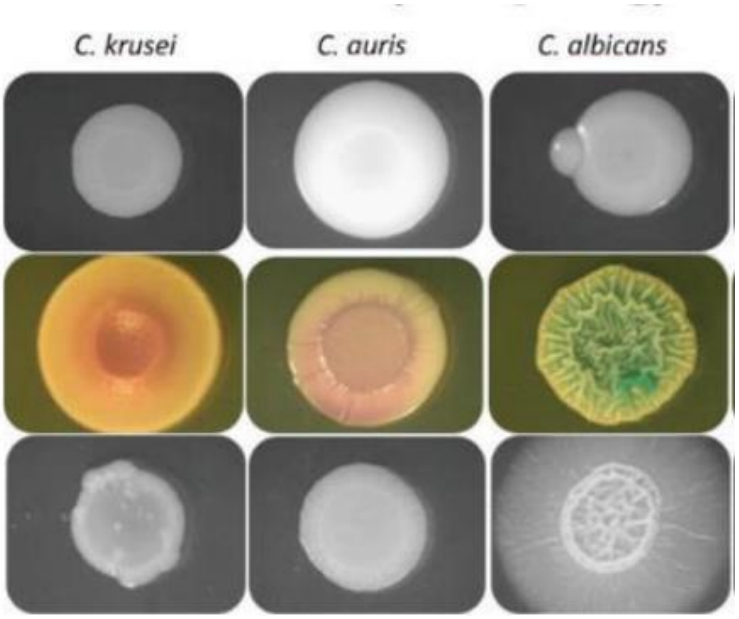

Figure (2-5) colony of *candida sp*.

2.5.1.2 Sensitivity of Candida to Antifungals

Candida is characterized by its resistance to antifungals, and this is considered a bad scientific reality, as fungi, including yeasts, over time, acquire virulence factors, mechanisms, and resistance to used antibiotics and drugs, as the frequent and frequent use of drugs, antibiotics, and medications works to reduce the work of the immune system and increases the chance of infection with fungal yeasts.

Therefore, it was necessary to find new antibiotics and drugs that work to reduce infection or reduce deaths to the lowest level, and disease infection can be overcome by administering a vaccine that consists of fungus antigens in order to stimulate the host's immune system to show actual positive responses against the disease (Zheng *et al*., 2005).

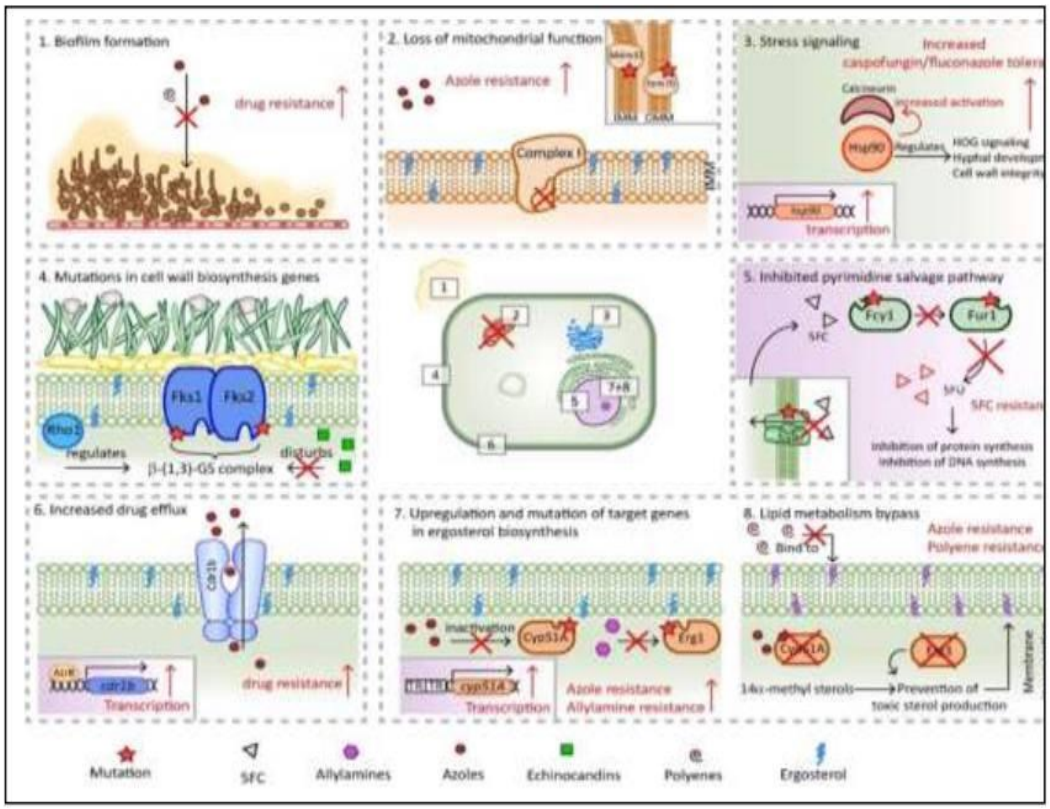

Figure (2-6): Mechanisms of antifungal resistance to fungi (Rajendran *et al*., 2016).

2.5.2 Antibacterial

Waxman first used the term "antibiotics" in 1942 to refer to any chemical created by microbes that inhibits the development of other germs in a highly diluted medium (Waksman.,1942). In bacterial pathogens is a worldwide challenge associated with high morbidity and mortality (Akova, 2016). Multidrug resistant patterns in Gram-positive and -negative bacteria have resulted in difficult-to-treat or even untreatable infections with conventional antimicrobials. Because the early identification of causative microorganisms and their antimicrobial susceptibility patterns in patients with bacteremia and other serious infections is lacking in many healthcare settings, broad spectrum antibiotics are liberally and mostly unnecessarily used.

Dramatic increases in emerging resistance occurs and, when coupled with poor infection control practices, resistant bacteria can easily be disseminated to the other patients and the environment. Availability of updated epidemiological data on antimicrobial resistance in frequently encountered bacterial pathogens will be useful not only for deciding on treatment strategies but also for devising an effective antimicrobial stewardship program in hospitals (Brotz and Brunner, 2008)

2.5.2.1. *Streptococcus pyogenes*

Streptococcus pyogenes is a gram positive beta-hemolytic bacteria, also known as group A streptococci (GAS) a facultative anaerobe, non-motile, no spore-forming, which occurs in chains or pairs, having a diameter of 0.5-1.0 mm (Amano *et al*., 2006).

The group A *streptococci* are fastidious organisms that have complex growth requirements are generally grown on agar media supplemented with blood. This technique allows the detection of β-

hemolysis, which is important for subsequent identification steps (Shulman *et al*., 2012). Globally, GAS has been estimated to cause more than 500,000 deaths every year, making it one of the world's leading pathogens (Cho and Caparon, 2008).

2.5.2.1.1 *Streptococcus pyogenes* susceptibility to antibiotic

Antibiotics are organic substances produced by microorganisms and capable at low concentration of inhibiting or destroying the growth of another microorganism most often isolated from bacteria and fungi (Patini *et al*., 2020). The most important mechanisms action of antibiotic are includes inhibition of cell wall remodeling, interference with protein synthesis, interference with nucleic acid synthesis and inhibition metabolism (Katzung, 2012).

Since the discovery of naturally occurring antibiotics from microbial sources, resistance has rapidly emerged, often soon after their introduction into clinical use began. Antibiotic activity and resistance is influenced by some differences the structure of Gram-positive and Gram- negative bacteria (Haque *et al*., 2018). The use of antibiotics against causative agents that cause chronic tonsillitis (CT) and recurrent tonsillitis (RT) consider general trend. Antibiotic therapy usually fails to prevent the recurrence of chronic tonsillitis (CT) and recurrent tonsillitis (RT) because inappropriate usage against the pathogen in deep tonsillar tissue leads to the continuation of infection and re-inoculation causing recurrence leaving the patient with no choice but surgery (Hammouda *et al.*, 2009). Due to increased incidence of resistance for antibiotics that used for the treatment of tonsillitis therefore evaluating antibiotic susceptibility to pathogen that causes tonsillitis will be useful in a more careful and planned use of antibiotics. Antibiotic sensitivity testing is a simple technique that demonstrates the susceptibility or resistance of various microorganisms to

the antibiotics tested. This is useful in guiding the physician's choice of antibiotics by revealing the changing trends in susceptibility (Alasil *et al*., 2011).

2.6 Anticancer

Cancer is a disease in which the somatic cells grow uncontrollably and spread to other body parts. The growth, differentiation, and death of body cells are generally well- programmed. In contrast, some cells, especially those ones bearing somatic and/or epigenetic mutations, may escape destiny and grow out of control. This kind of abnormal growth can form the so-called neoplasm. A localized neoplasm restricted to the tissue of origin is a benign tumor, while neoplasms able to invade other tissues and form secondary tumors is a malignant tumor, namely cancer (Boutry et al., 2022)

Cancer refers to a disease involving abnormal cells that proliferate uncontrollably and can invade normal body tissue. According to the World Health Organization (WHO), cancer is the second leading cause of death worldwide (Sitzmann *et al*., 2020). It was estimated that at least 9 million patients are killed by cancer annually. Conventional therapies for cancer include surgery, chemotherapy, and radiotherapy. However, the downside of these traditional cancer treatment methods is that patients often suffer from various side effects during treatment. In particular, conventional treatment exhibits low specificity, leading to drug resistance in cancer cells.

There is extensive evidence that high intake of fruits and vegetables is associated with decreased risk of many types of cancers. Thus, it is widely accepted that diet changes are a powerful means to prevent cancer. Although there is a growing interest in the role of the

tomato carotenoid lycopene in cancer prevention and treatment, carotenoids seem to play a fundamental role, their presence in the human diet being considered positively because of their action as pro-vitamin, antioxidant or possible tumors-inhibiting agents (Seel *et al*., 2020).

Due to the growing scientific evidence on the potential benefits of carotenoid pigments in human and animal health, there has been an increasing commercial interest during the past years in natural sources. The consumption of a diet rich in carotenoids has been epidemiologically correlated with a lower risk for several diseases (Amr *et al*, 2012).

Thus, carotenoids constitute one of the most valuable classes of compounds for industrial applications, e.g. in pharmaceutical, chemical, food and feed industries. Carotenoids pigment biosynthesis is a characteristic ability of the genus Rhodotorula (Seel *et al*., 2020). Easily identifiable by distinctive yellow, orange/red colonies. The main carotenoids produced in Rhodotorula species are torularhodin, torulene and β-carotene (Aziz *et al*., 2020).

2.6.1: Colon Cancer

The colon and rectum comprise the final portion of the human digestive tract, commencing at the ileocecal valve that marks the end of the small intestine, terminating at the anus, and measuring roughly one yard in length. Cancers of the colon and rec- tum are the second leading cause of cancer incidence and cancer death among adult Americans, with 135,000 new cases and 57,000 deaths in 2001, and with a 6% lifetime risk of developing the disease (Greenlee *et al*., 2001).

Encouraging declines in the death rate from colorectal cancer in the last decade speak to the potential effectiveness of recent advances in prevention, screening, and therapy. Cancers of the colon arise from the

colonic epithelial cells that line the lumen of the organ, which renew themselves every five days from a stem cell population located at the base of colonic epithelial cell crypts. Colon cancers are the end result of a multistep process of colon neoplasia that extends over several years. First, neo- plastic tubular colon adenomas arise as pedunculated polypoid structures growing into the colon lumen. With time, they acquire increasingly disordered villous histology and dysplastic cellular cytology, and are recognized as frank cancers only when invasive cells breach the underlying epithelial basement membrane.

Reproducible increases in incidence of the disease in populations that have migrated from low to high incidence regions of the world show the importance of environmental factors (Markowitz et al., 2002). Cohort studies have rejected variations in intake of fiber, vegetables, and antioxidant vitamins as causative factors, but support that risk increases with red meat consumption, low folate intake, and sedentary lifestyle (Hu, 2001).

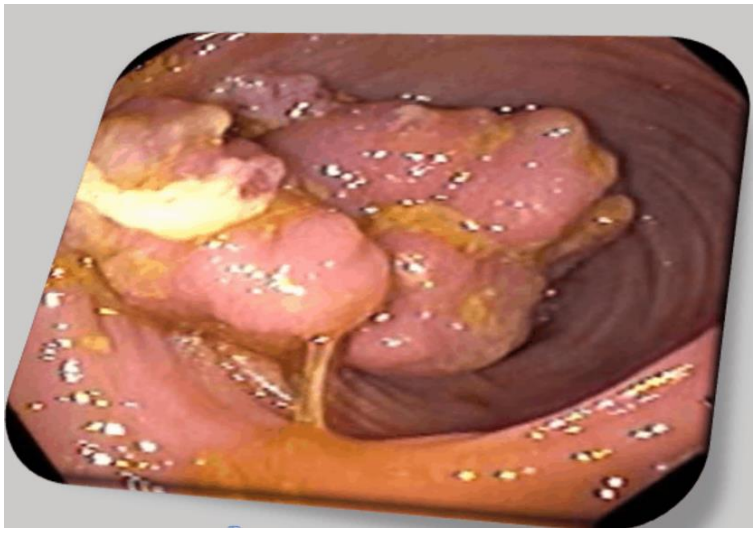

Figure (2-7): colon cancer (Mármol,*et.al*,2017)

2.6.2: Urinary Bladder Cancer

Bladder cancer is a heterogeneous disease, with 70% of patients presenting with superficial tumours, which tend to recur but are generally not life threatening, and 30% presenting as muscle-invasive disease associated with a high risk of death from distant metastases (Kaufman *et al*., 2009).

The main presenting symptom of all bladder cancers is painless hematuria, and the diagnosis is established by urinary cytology and transurethral tumor resection. Intravesical treatment is used for carcinoma in situ and other high grade non-muscle-invasive tumors (Urist et al., 2002).

The standard of care for muscle-invasive disease is radical cystoprostatectomy, and several types of urinary diversions are offered to patients, with quality of life as an important consideration. Bladder preservation with transurethral tumor resection, radiation, and chemotherapy can in some cases be equally curative. Several chemotherapeutic agents have proven to be useful as neo adjuvant or adjuvant treatment and in patients with metastatic disease. We discuss bladder preserving approaches, combination chemotherapy including new agents, targeted therapies, and advances in molecular biology (Habuchi *et al*., 2005).

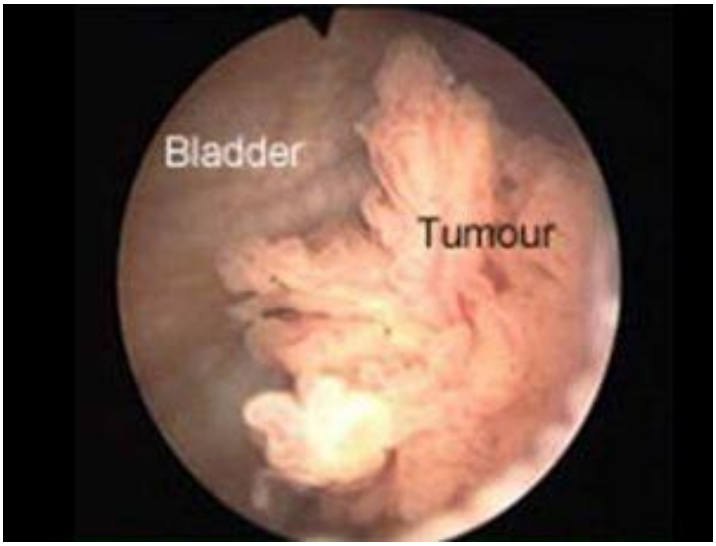

Figure (2-8):**Urinary Bladder Cancer**

(https://www.med.unc.edu/urology/patientcare/cancer/bladder-cancer/)

3.1: Materials

3.1.1: Equipment and Apparatus

Table (3-1) tools and devices utilized in the study and their origin

Device	Company	Origin country
Incubator	Fisher	England
Water bath	Gallen kamp	England
Refrigerator	Ishtar	China
Hotplate		
Sensitive electronic balance	KERN	Germany
Light microscope	Olympus	Japan
Vortex mixture	KERN	Germany
Autoclave	Express	England
Burner	Amal	Turkey
Hood		Germany
Disposable petri dish	Alhani	Lebanon
Micropipette	Appendorff	USA
Cotton swab	Alhani	Lebanon
Loop	Himedia	India
Filter paper		China
Millipore filter		China
Oven		England
Magnetic stirrer		Germany
Flask		China
Slide and Cover		China
Cylinders		China
Beakers		China
Separating funnel		China
HPLCE System		Kanuer Germany

3.1.2: Chemicals

Table (3-2) the materials used in the study and their origin

Material	Company	Origin country
Acetone	Kumpulan	Malaysia
Glass beads		Iraq
Methanol	Emsure	Germany
Ether	Emplura	Germany
Normal Saline	pioneer	Iraq
DMSO	BDH	England
Chloramephincol	Wockhardt	Uk
Tetracycline	Samarra	Iraq
Lactophenol	BHD	England
Nacl		Iraq
Lactophenol cotton blue	BHD	England
Trypsin Enzyme	US Biological	USA
HiCandida identification kit	BHD	England
H_2O_2 (3٪)		USA

3.1.3: Media

Table (3-3) The media used in the study and their origin

Media	Company	Origin country
Sabouraud's Dextrose agar (SDA)	Hi- media	India
Potato Dextrose Agar (PDA)	Oxoid	England
Yeast extract Agar	Oxoid	England
Blood Agar	Hi-media	India
Muller Hinton Agar	Hi- media	India
Rosswel park Memorial institute(RPMI-1640)		Laboratory prepared
Freezing media		Laboratory prepared
Chrome Agar	Hi-media	India

3.1.4: cell line

Table (3-4) The cell line used in the study and their origin

Cell line name	Origin
LS174T	**Urinary bladder cancer cell line**
EJ-138	**Colon cancer cell line**

3.2: Methods

3.2.1: Sterilization

A. Wet heat sterilization

Solutions, dyes and media sterilize some of them using auto clave at 121℃ and a pressure of 15 1b/in^2 for 15 minutes (Agalloco *et al*., 1998)

B. Dry heat sterilization

In this way, the glassware used to prepare these materials was sterilizes in an electric oven at a temperature of (160) ℃ for 1 hour (Alkadhim, 2018).

C. Sterilization by filtration

The rest of the materials, such as sugars, were sterilized by filtering with micro filters with a diameter of 0.22 μ (Rajniak *et al*., 2008).

3.2.2: Laboratory prepared culture media

3.2.2.1: Sabouraud Dextrose Agar (SDA)

It was used for routine cultivation of fungi, especially clinical samples, Prepared according to the manufacturer's instructions:

Powder of medium	65 gm
Distilled water	1000 ml

3.2.2.2: Potato Dextrose Agar (PDA)

It was used for routine cultivation of fungi, Prepared according to the manufacturer's instructions:

Powder of medium	38 gm
Distilled water	1000 ml

3.2.2.3: Yeast Extract Agar (YEA)

It is a medium for the growth of microorganism, Prepared according to the manufacturer's instructions:

Powder of medium	23 gm
Distilled water	1000 ml

3.2.2.4: Muller Hinton Agar (MHA)

It was used for sensitivity test by disk diffusion method, Prepared according to the manufacturer's instructions:

Powder of medium	38 gm
Distilled water	1000 ml

3.2.2.5: Blood Agar

It is enrichment medium for the isolation and identification of bacteria prepared according to the manufacturer's instructions:

Powder of medium	40 gm
Distilled water	1000 ml

After that, it was sterilized with the autoclave, and after completing the sterilization process, it was cooled to (45-50) degrees, and human blood was added to it at ratio of (3-5ml).

3.2.2.6: Rosswell Park Memorial Institute (RPMI-1640)

Used to develop and save the cellular lines used in the current study, use ready according to the manufacturer

3.2.2.7: Freezing media

Use to freeze cell lines, it is prohibited to add

DMSO	10%
Serum	30%
Meadia	60%

3.2.2.8: Candida Chrom Agar

It was used to differentiate between the Candida species, prepared according to the manufacturer's instructions:

Powder of medium	47 gm
Distilled water	1000 ml

3.2.3: Specimen collection

3.2.3.1: Collection of Yeasts Specimen

150 clinical specimens were collected from patients infected with diabetes, it was done in specialized dental center in Babylon governorate, Iraq during the

research period starting from 1 October 2022 to 28 February 2023. Sample were taken from the oral, a sterile cotton swabs were rotated inside the patient's mouth cavity and then kept in plastic containers until use.

3.2.3.2: Collection of Bactria Specimen

Five specimens were collected from patient with tonsillitis in Imam Al-Sadiq Teaching Hospital in Babylone governorate.

3.2.4: Specimens cultivation

The swabs streaked directly onto SDA, the dishes were incubated at a temperature of 35℃ for 24 hours to check and record the growth of yeast. Then for subculture we used another culture media like Yeast Extract Agar, potato Dextrose Agar for diagnostic reasons.

3.2.5. Maintenance of isolates

Isolates were Maintenance by inoculate SDA slants, which were then allowed to develop at 37 °C and kept cool. Every three months, a new activation of each culture was made. Cultured isolates in tubes with SDA medium and 15% sterile glycerol added for long-term preservation should be kept at -4 degrees.

3.2.5: Identification of microorganisms

3.2.5.1 Identification of yeast

Depending on the morphological characters of colonies and microscopic examination as stated in (Hasan *et al*., 2023)

3.2.5.1.1 Macro-features

After the appearance of growth on SDA, Yeast extract Agar, the shape, color, texture and height of the colonies were observed on the culture medium.

3.2.5.1.2 Micro-features

The examination was done by making a glass slide of a model of the colony with a drop of lacto phenol and covered with the cover of the slide, then the model was examined with a light microscope to observe the shape of cells for yeasts (Kozel & Wickes, 2014).

3.2.5.1.3 Biochemical test by using HI Candida Identification

A ready-made diagnostic kit was used in the biochemical diagnosis to verify the isolated species according to the manufacturer's instructions. The system consists of a tape containing 12 wells containing basic materials. This kit was used to determine the patterns of the sugar's assimilation and urease production (Hedayati *et al*., 2015), where the fungal suspension, after regrowth on PDA, is added to each well as follow:

1- The kit was opened in a sterile way. Then the packaging foil is removed.

2- The surface inoculation method was used to inoculate each well with 50 µl of the aforesaid inoculum.

3- Incubation, at 22.5 C ± 2.5 C. for 24-48 hours.

4- Interpretation of results, the results was interpreted as per the standards given in the identification index.

3.2.5.2: Identification of Bacterial

3.2.5.2.1 Morphological Tests:

3.2.5.2.1.1 Colonial morphology and microscopic features:

A single colony was taken from each primary positive culture. Its identification depended on the morphology properties (colony size, shape, color, translucency, edge, and elevation of texture). The colonies were then investigated by gram stain to observe bacterial cells. Specific biochemical tests were done to reach the final identification.

3.2.5.2.2 Biochemical Tests:

The following biochemical tests were performed for the identification of Streptococcus pyogenes isolates from other isolation

3.2.5.2.2.1 Catalase Test:

Blood agar base was streaked with the selected bacterial colonies and incubated at 37ºC for 24 hrs. Then transfer the growth by the wooden stick and put it on the surface of a clean slide and add a drop of catalase reagent. Formation of gas bubbles indicates positive results (Forbes et al., 2007).

3.2.5.2.2.2 Oxidase Test:

A strip of filter paper (what man No.1) was soaked with a little freshly made 1% solution of tetra Methyl-P-phenylene - diamine dihydrochloride, and the colony to be tested was picked up with a sterile wooden stick and smeared over the filter paper. A positive result was indicated by an intense deep purple color which appeared within 5-10sec. (Forbes *et al*., 2007).

3.2.5.2.2.3 Bacitracin Sensitivity test:

The blood agar was streaked with bacterial culture and the bacitracin disk was put in the center of the plate. The diameter of inhibition zone was equal to or more than 12 mm, indicating a positive result. This test was used to differentiate between Strep. Pyogenes and other β hemolytic streptococci (McFadden, 2000).

3.2.6: Extraction of β-carotene pigment

β-carotene pigment was extracted from the isolates of *Rhodotorulla* yeast. According to Harborne (1984) method, if 3 gm of yeast grows for 48 hours on the solid food media (SDA) with a sensitive electronic balance placed in a beaker containing 20 ml of acetone and 150 mg of glass beads completely covered with aluminum foil to prevent exposure to light and light oxidation. Put the beaker on the magnetic stirrer for 48 hours to break the walls of a cell yeast. The walls of break cells were excluded by filtering through the filter paper Whatmann No. 1Putting the filter in separating funnel of 250 ml and add a mixture of methanol solvent and ether at ratio 2: 15ml, respectively, with a small amount of sodium chloride salt (Nacl), the funnel was shaken well and left for two minutes until two layers (polar and non polar). Represent the upper layer ether solvent that contains carotene pigment and the lower layer represents the rest of compound. Neglected the lower layer and took the solvent used containing β-carotene pigment. The solvent was evaporator using the Rotary Vacuum Evaporator (RVE). At a temperature of 40-60 and preserved β-carotene pigment in dark bottles and in dark conditions at -18 degree to avoid oxidizing and damaging the pigment.

3.2.6. Identification and quantification of carotenoids (β-carotene)

Carotenoids (**β** -carotene) were quantified and identified using HPLC analysis.

The extraction ,detection and quantitation of b carotene was performed according to (Barba *et al*., 2006).

The separation on C18 column (Knuaer , Germany) (250 _ 4.6 mm i.d., 5 µm particle size, 80 Å pore size)

mobile phases were methanol/ACN (90/10 v/v) +TEA 9 lM,, flow rate 1ml/min

detection on 475nm

The detection of each compound was performed by matching retention time and absorbance spectrum of the standards ,

the concentration was calculated by serial concentrations of external standard materials to build calibration curve between concentration and its equivalent peak area .

3.2.7: Antimicrobial activity of β-carotene pigment

3.2.7.1: Antibacterial activity

Agar-well diffusion method used to evaluate the antibacterial activity of beta carotenoid against *Streptococcus pyogenes.* Bacterial suspension was prepared by adding 2-3 pure colonies of already diagnosed bacterial isolate to 5ml of sterile Brain Heart Infusion broth and incubated at 37°C for 18h. after incubation period, 1ml of this broth culture was diluted by sterile normal saline in sterile plane tube to

produce a standard bacterial suspension with moderate turbidity equal to McFarland standard tube.

3.2.7.2: Well Diffusion Agar

1. Preparation of concentrations of (10, 20, 40) µg/ml.

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 degrees each time.

4. After the completion of culture, the bacterial isolates were left for 5-10 minutes 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 microliters of beta-carotene dye, with concentrations of (10, 20, 40) µg/ml in each of the first, second, and third holes, respectively.

7. The dishes were closed and left in the culture booth for 15 minutes, then transferred to the incubator at 37°C for 24 hours .

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 (Obeidat et al., 2012).

9. Out of concern, the experiment was repeated (2-3) times to obtain accurate results.

3.2.7.2: Antifungal activity

According to the method of spreading in the agar by drilling, studied the characters of β-carotene pigment against isolate of Candida yeast which he obtained as mentioned in the paragraph (3.2.3.1). The pure yeast isolates obtained at the beginning of the experiment were renewed on the yeast extract media.

3.2.7.3: 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. 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 degrees each time.

4. After the completion of culture, the candida isolates were left for 5-10 minutes 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 microliters 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 minutes, then transferred to the incubator at 37°C for 24 hours.

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 (Obeidat et al., 2012).

9. Out of concern, the experiment was repeated (2-3) times to obtain accurate results.

3.2.8: Anticancer characters of β-carotene pigment

3.2.8.1: Cancer Cell Lines Used

To complete the experiment as it is designed for it, used line cells included line cancer cells for colon cancer EJ-135 and the urinary bladder cancer line LS174T.The lines were prepared by Rawafed Scientific Research Company. Save the two types of lines in liquid nitrogen, it was developed and perpetuated and examined in Rawafed Science Research Company.

3.2.8.2: Growth Of Cancer cell lines

To grow cancer cell lines, the method described by Freshney (2012) followed the following steps.

1. Cancer cell lines were incubated in a water bathe at a temperature of 37°C.

2. Cancer cell lines were placed in special falcon plastic flask of size 25cm^2. It contains the culture medium RPMI-1640 and 10% of FBS, the culture vessels containing the cell suspension and the culture medium were incubated in 5% CO_2 incubator at 37°C for 24 hours.

3. After the end of the incubation period and ensuring that there is growth in the cell culture and that it is free of contamination, subcultures were conducted.

4. The cells were then examined using an inverted microscope to ensure that vitality and growth to the required number (500-800 cells/ml)

5. The cells were transferred to the growth booth and the used culture medium was discarded.

6. Followed by washing the cells with phosphate buffer solution, the washing process was repeated twice for 10 minutes each.

7. This was followed by adding a sufficient amount of trypsin enzyme to the cells and incubated for 10-30 minutes at a temperature of 37℃, they were monitored until they changed from a single cell layer to single cells, then the enzyme is stopped by adding a new culture medium containing fetal bovine serum.

8. Then it is ready to use.

3.2.8.3: Maintenance Of Cancer Cell Line

The cancer cell lines were maintained by observing the cells, and when they formed a complete monolayer, transfer to a secondary culture was carried out, which was done by first removing the old growth medium, followed by washing the cells with phosphate buffer solution and adding 3 ml of trypsin enzyme, while stirring the vial gently and carefully. Followed by getting rid of the trypsin enzyme and incubating the cells in a co incubator at 37℃ until the cells separated, then adding a new growth medium for the cells and redistributing them in special falcon flask and keeping them at 37℃.

3.2.8.4: Testing the effectiveness of β-carotene pigment against cancer

To evaluate the effectiveness of beta-carotene dye purified from *Rhodoturella* yeast on colon cancer cell lines (LS174T) and urinary bladder cancer cells (EJ134) and the possibility of using them as promising anticancer drugs.

1- Cancer cells of colon and urinary bladder were prepared as described in the paragraph (3.2.7.2), and were cultured in 96 well microtiteration, So that each well contains a final volume of 200µl of complete culture medium. The plate was covered with a piece of sterile Para film with gently stirring and left in an incubator equipped with 5% CO_2 at 37°C for 24 hours.
2- After 24 hours of culturing the cell lines in 96-well microtiteration, they were examined by inverted microscope to ensure their growth and formation of an integrated cell layer of 85-90%.
3- Cell lines were exposed to serial concentrations of β-carotene pigment (0.3125, 0.625, 1.25, 2.5, 5, 10), by placing 2µl of each concentration in each well of the micron titer plate and it was by four replicates for each concentration and the same number of replicates for the positive and negative control group, each of which put 2µl.
4- The plate were covered and the lid was sealed with Para film, then returned to the incubator at a concentration of 5% CO_2 at a temperature of 37°C for 24 hours.
5- Read the results after 24 hours by the Huma Reader Hs .

3.3. Statistical analysis

Microsoft Office Excel 2010 and the statistical program for social sciences (SPSS) version 26 were used to gather, summarize, analyze, and present the data. The mean, standard deviation, and range of numerical data were displayed. unbiased samples When comparing the means of any two groups, the t-test was utilized, assuming that the variable was normally distributed.

4. Results and Discussion.

4.1. Isolation and Identification:

In this study, 180 oral cavity specimens were taken from patients suffering from diabetes, the results of isolation and identification results showed,150 (83.3%) isolates for *Rhodotorulla sp*. Only 2 (1.11%) for *Candida* sp, among 5 specimens collected from tonsils, only 3 (60.0٪) were *strep. pyogenes*

Table (4-1): Isolation and Identification in the current study

Microorganism	No. of specimens	Number of isolates
Rhodoturella sp	180	150 (83.3%)
Candida Krusei	180	2 (1.11%)
S. pyogenes	5	3 (60.0%)

This results accordant with Troska *et.all* (2017) which isolated *Rhodotorula* and *Candida* from oral cavity in percentage 83% for *Rhodotorula*. In the diabetic patients, the increasingly frequent appearance of the species of fungi indicates the progressing risk posed by opportunistic types, which includes *Rhodotorula* species.

4-1-1- *Rhodoturella sp*

Colonies on SDA were coral pink, smooth, moist to mucoid yeast, orange- pink colony on PDA, orange-red bulged colony on yeast extra agar-like (Fig. 4-1) Microscopic morphology showed, spherical to elongated budding yeast-like cells or blastoconidia (Fig.4-2). According to this description and biochemical tests, (Fig.4-3), this isolate identified as *Rhodoturella*. sp. (DeHoog *et.al*., 2005)

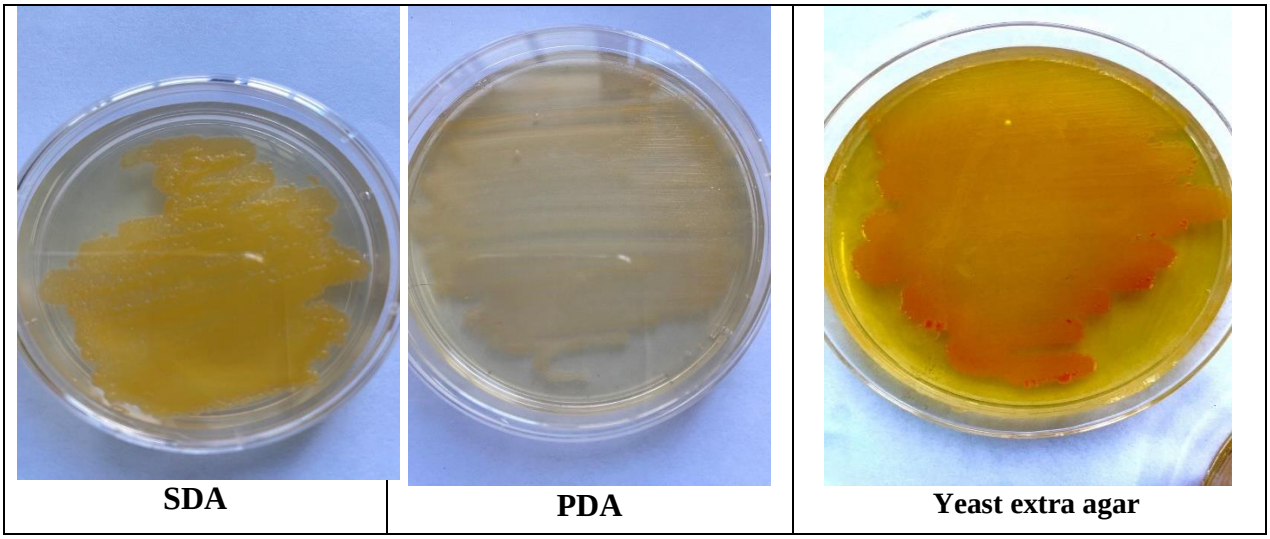

Figure (4-1): Colonial morphology of *Rhodoturella sp* (on SDA, PDA and Yeast extra agar) in the current study

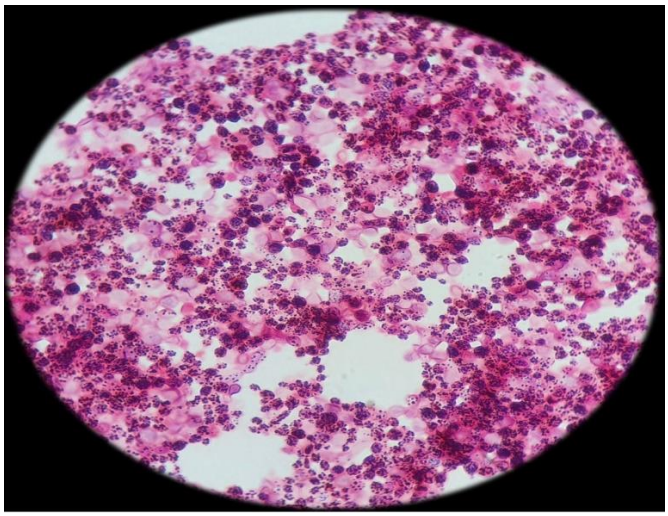

Figure (4-2): *Rhodoturella* under microscope in the current studay

4-1-2 Biochemical Test

The results of this test shown the ability of *Rhodoturella species* to detect Urease enzyme and utilize carbohydrate fermentation, this is what is shown in the table (4-2) and in (Figure 4-3)

Table (4-2) Results of using HiCandida identification kit

Test / Species	Urease	Melibios	Lactose	Maltose	Sucrose	Galactos	Cellobio	Inositol	Xylose	Dulcitol	Raffinos	Trehalos
Rhodoturell sp	+	-	-	-	-	-	-	-	-	-	-	-

+positive reaction, -: negative reaction, * strain variation (for carbohydrates: Red: Negative Result,) (for Urease: Pink: Positive result)

The results of this test were the same as those of Hedayati et al. (2015) in which the data showed that *Rhodoturella species* were accurately identified using the HiCandida identification kit, which is also conforms to Singh et al. (2021) who stated that Identification was accomplished with the support of diagnostic by using this kit**.**

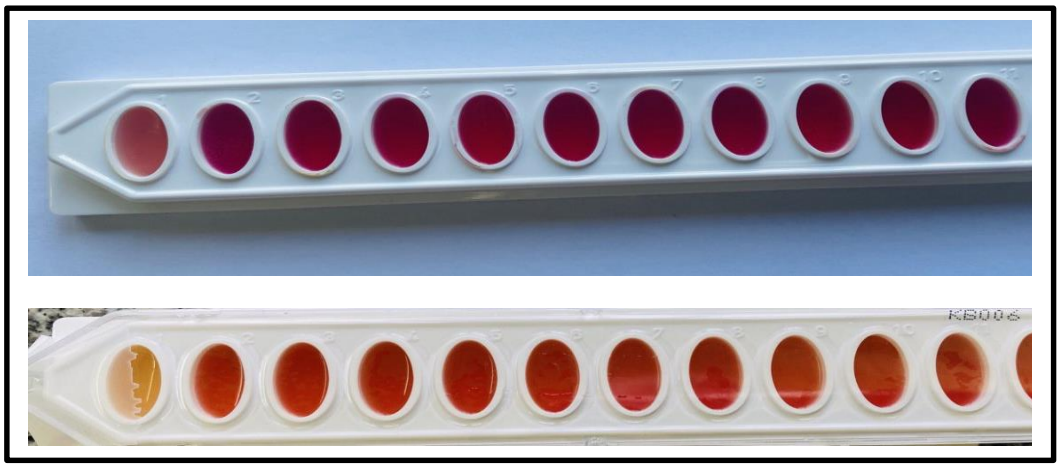

Figure (4-3): HiCandida identification kit

4-1-3-*Candida* sp

Colonies on SDA are white to cream colored, smooth, glabrous and yeast-like in appearance (Fig.4-4), Microscopic morphology showed spherical to sub spherical budding yeast-like cells or blastoconidia (Fig.4-5). According to these characteristics and biochemical tests (Fig.4-6) this isolate identified as *C. krusei* (DeHoog *et.al*., 2005)

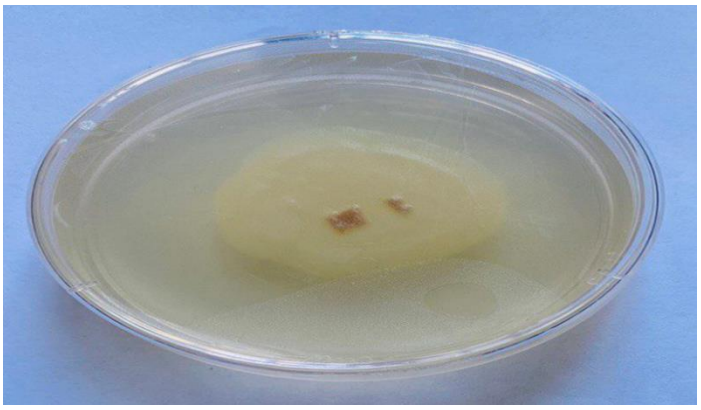

Figure (4-4): *Candida krusei.* on SDA

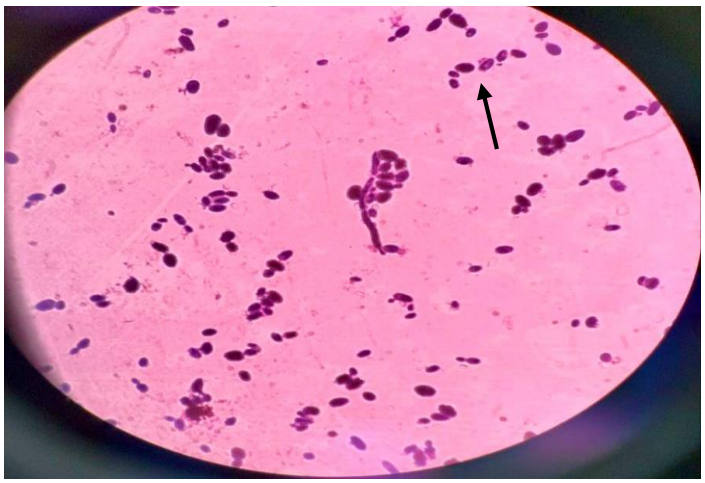

Figure (4-5) ***Candida*** *krusei*. Yeast cells under microscope from colony at 37℃ for 24h

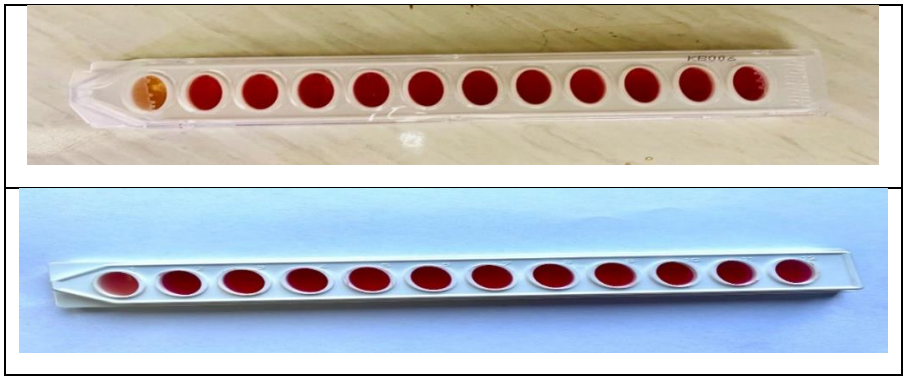

Figure (4-6) HiCandida identification kit

All the colonies of *Candida* had no definite borders, tended to spread, and had a pink color of varying intensity on chrome agar and HiCandida biochemical kit.Therefore, according to (San-Millan,*et.al*.1996) and according to phenotypic characteristics of (Nadeem,*et.al*,2010) these isolates were identified as *Candida krusei*, Fig. (4-7)

Figure (4-7) *Candida krusei*. on Chrome agar at 37℃ for 24h.

4-1-4- ***Streptococcus pyogenes***

The diagnosis of *Streptococcus pyogenes* in laboratory is based on phenotypic characteristics including morphology of colony on blood agar. Its colonies were small, 0.5 in diameter greyish white, round, with slightly convex, the hemolytic activity was observed and the kind of hemolysin (alpha, beta, and gamma) .as Figure (4-8). Microscopic examination showed that the cells were spherical in shape, Gram- positive and arranged in chains, as Figure (4-9). They are negative for catalase and require amount of CO_2 ranging from 5-10٪

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Figure (4-8): *Streptococcus pyogenes* on blood agar

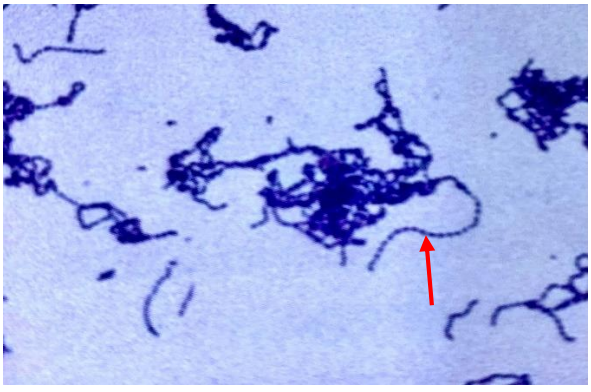

Figure (4-9): *Streptococcus pyogenes* under microscope

4.1.4.1- Sensitivity to bacitracin

The positive bacterial culture of *S. pyogenes* tested for bacitracin sensitivity and the results was shown in figure (4-10). *Streptococcus pyogenes* (GAS) is inhibited by a small amount of bacitracin (0.04 U) in the disk. After an overnight incubation at 35°C in 5% CO2, a zone of inhibition surrounds the disc, indicating the strain's susceptibility (Sayyahfar *et al.*, 2015).

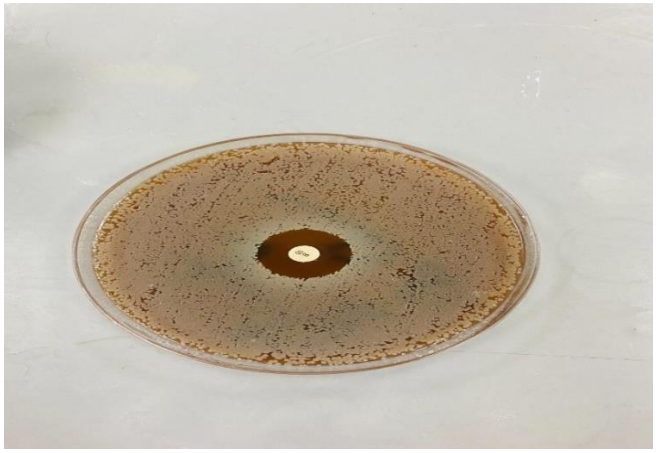

Figure (4-10): Bacitracin sensitivity test of *S. pyogenes*

4-2- Extraction of β-carotene pigment

Fig (4-11) shows the efficiency of the method used for extraction of β-Carotene , where the method concluded in the end to obtain a stain with a high concentration, and this result is consistent with (Naghavi *etal.*, 2015), concluded that using each of ether and methanol in extracting dyes, including β-carotene. Also with (Malla Obaeda, 2017) which used this method in extraction β-carotene.

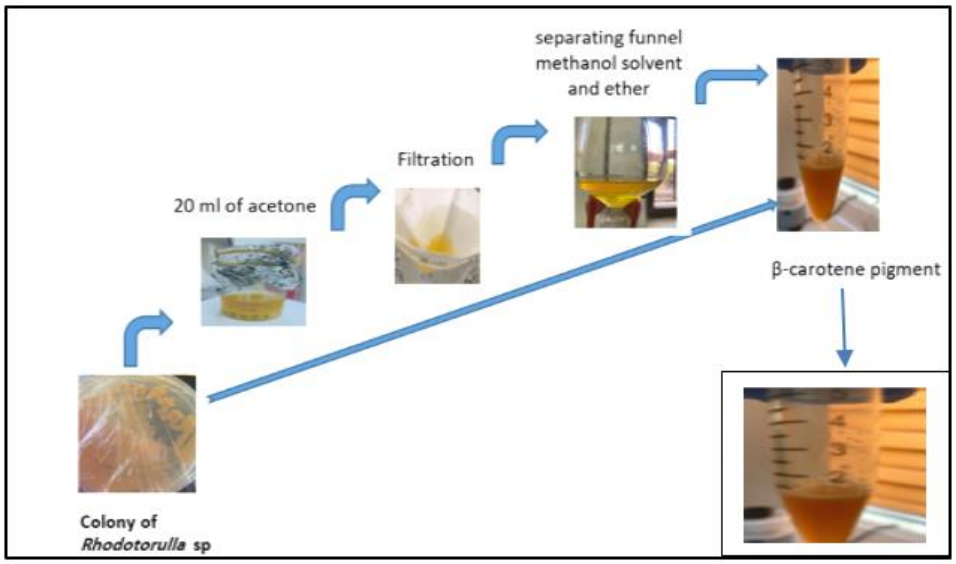

Figure (4-11): scheme of extraction β-carotene

4-3- Identification and quantification of β-carotene

β-Carotene pigments have been identified by HPLC equipment. Figure (4-12): displays how the carotene pigments that were isolated from *Rhodoturella*. and separated and identified by HPLC. According to spectral analysis and HPLC separation, β-carotene is the only significant carotene component in *Rhodoturella*. Also, Table (4-4), show that concentration of β-carotene extraction from *Rhodoturella* was 2.708936 µg/ml.

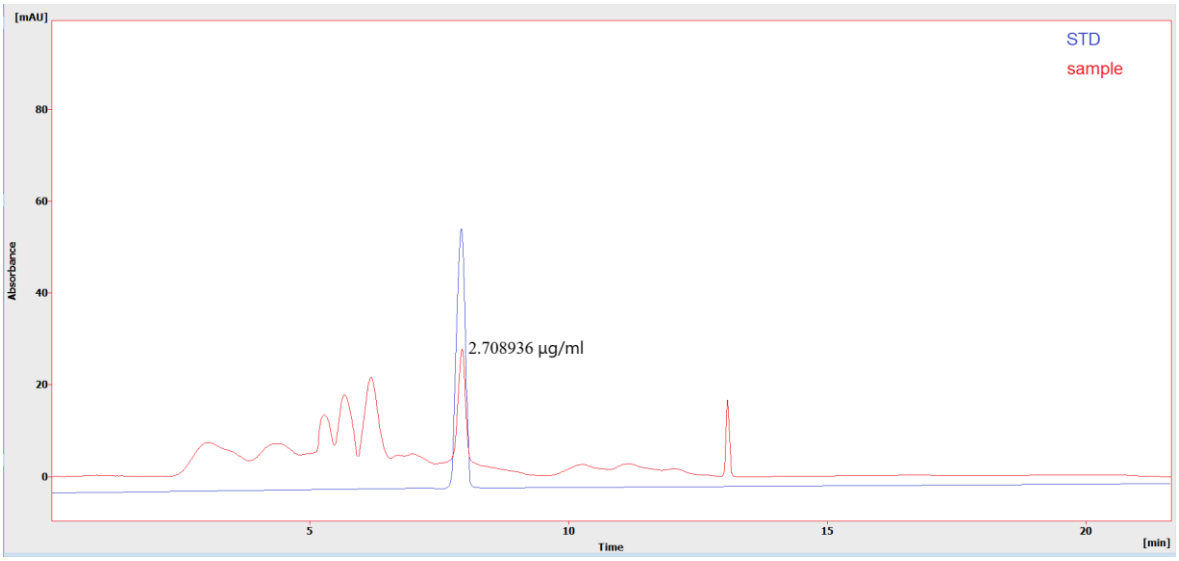

Figure (4-12): Identification and quantification of β-carotene

Table (4-3): concentration of β-carotene

sample	area	µg/ml sample
1	102.224	2.708936

This results agree with (El-Rahman,et.al,2019) which found the major carotene when used HPLC was β-carotene with concentration 2.505 µg/ml. And these results indicate the efficiency of the method used in extraction

4-4-Antimicrobial characters of β-carotene

4-4-1-Antifungal

As shown in table (4-5), figure(4-12) and figure(4-13), there is a high antifungal activity of beta carotene against *Candida krusie* with high significant differences according with the concentrations of beta carotene, where , this activity increases with increased concentration.

Table (4-4): In-vitro antifungal activity of β-carotene against *Candida Krusie*

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** † **HS**
Different latter's denote to the significant differences at p < 0.05				

SD: standard deviation; †: one-way ANOVA; **HS**: Highly significant at $P < 0.001$

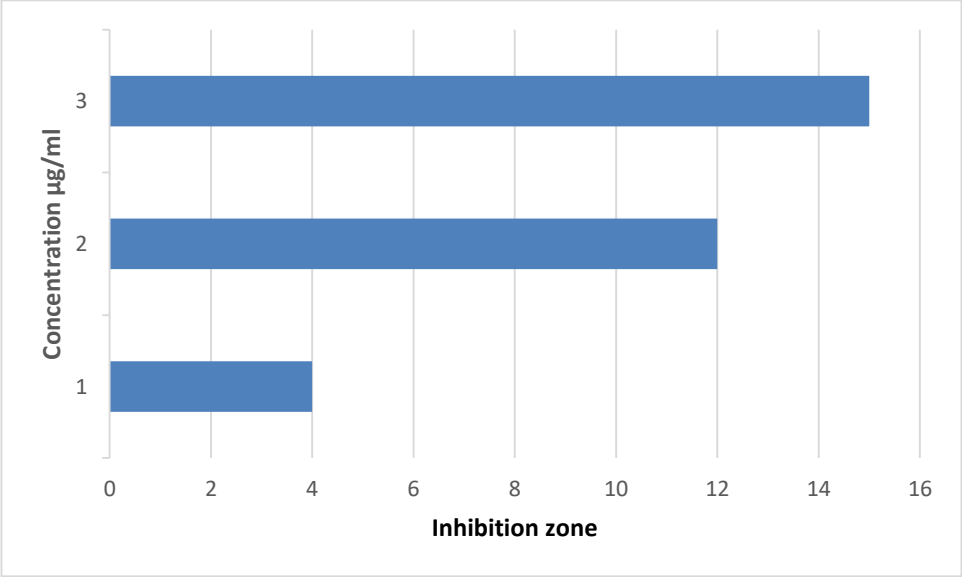

Figure (4-13) Diameter of inhibition zone of *Candida krusie* treated with β-carotene

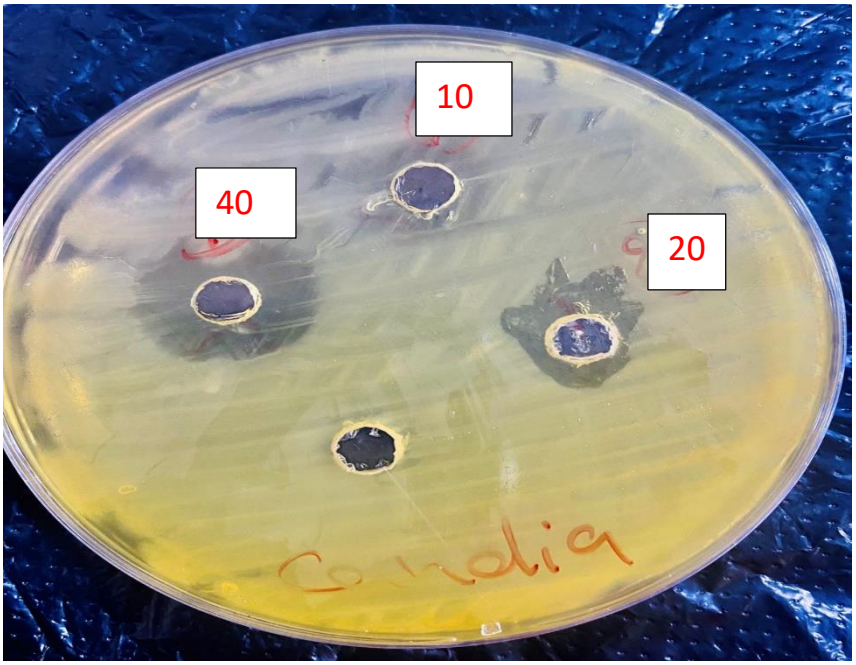

Fig (4-14) inhibition zone of *Candida krusie* treated with β-carotene

4-4-2-Antibacterial

As shown in table (4-6), Figure (4-14) and Figure (4-15), there is a high antibacterial activity of beta carotene against *S. pyogenes* with high significant differences according with the concentrations of beta carotene, where, this activity increases with increased concentration.

Table (4-5):In vitro antibacterial activity of β-Carotene against *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** † **HS**
Different letters denote to the significant differences at p < 0.05				

SD: standard deviation; †: one-way ANOVA; **HS**: Highly significant at $P < 0.001$

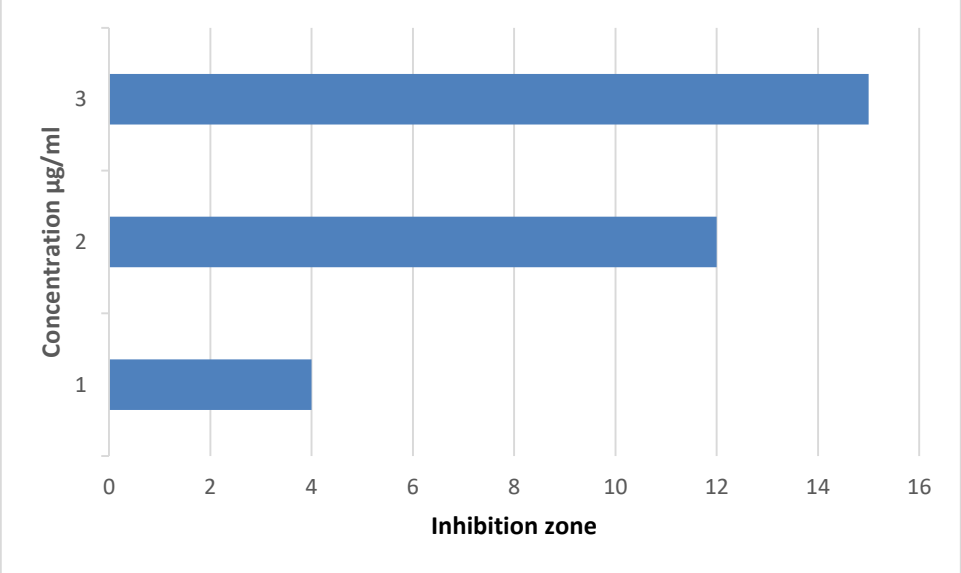

Figure (4-15) Diameter of inhibition zone of *S. pyogenes* treated with β-carotene

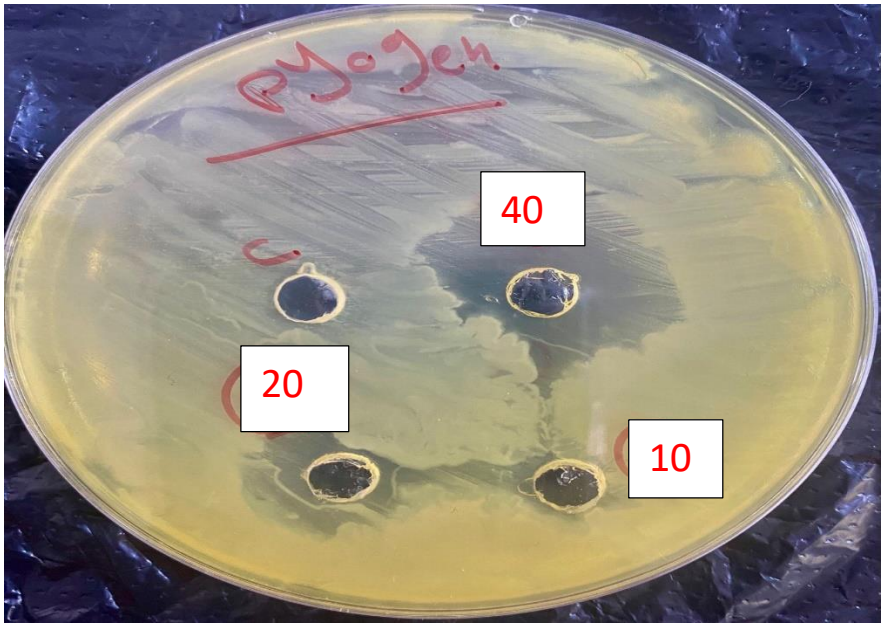

Figure (4-16) Inhibition zone of *S. pyogenes* treated with β-carotene

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. (Singh *et al.*, 2007) indicated that, the difference in cell wall structures makes the antibacterial efficacy to be selective. Ushakumari and Ramanujam (2013) 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. aeroginosa*, *B. subtilis* and *S. aureus*. Geweely (2011) reported that the extracellular pigment of *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, Sanjay (2009) 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.

4-5- Anticancer characters of β-carotene pigment

4.5.1. Colon cancer Cell Line

To demonstrate the cytotoxicity of β-carotene purified from *Rhodoturella*. Each cell lines of colon cancer (LS174T) were exposed to different concentrations of β-carotene (0.3215, 0.625, 1.25, 2.5, 5, 10) µg/ml, and estimated the cytotoxicity by finding the percentage of growth inhibition compared to the control group considering their growth rate 100.0%.

The present results show that the purified β-carotene have variant cytotoxic activity against colon cancer cells with different concentration of β-carotene, where showed higher cytotoxic activity (88.5%) in concentration 10 µg/ml against colon cancer cells. While the lowest inhibition percentage (-3.43%) was at the highest concentration used (0.3125 µg/ml) in cell line of colon cancer (LS174T).

The current results also showed the highest viability at concentration 0.3125 µg/ml which is 103.44% after the absorbance reading with the MTT technique and lowest viability rate, at 10 µg/ml and it reached 11.5% as in figure (4-16). The mean concentration of IC50 of purified β-carotene was 4.83, figure (4-17).

Table (4-6): Efficacy of β-carotene extract from *Rhodoturella sp* on Colon cancer

LS174T	**Concentration µg/ml**					
	0.3125	**0.625**	**1.25**	**2.5**	**5**	**10**
Mean ±SD	**45 ±** 2.0^{A}	41 ± 1.5^{B}	39 ± 2.0^{C}	37 ± 1.5^{D}	34 ± 3.2^{E}	5 ± 1.3^{I}
P value	**0.001 † HS**					
Different latters denote to the significant differences at $p < 0.05$						

SD: standard deviation; †: one way ANOVA; **HS**: Highly significant at $P < 0.001$

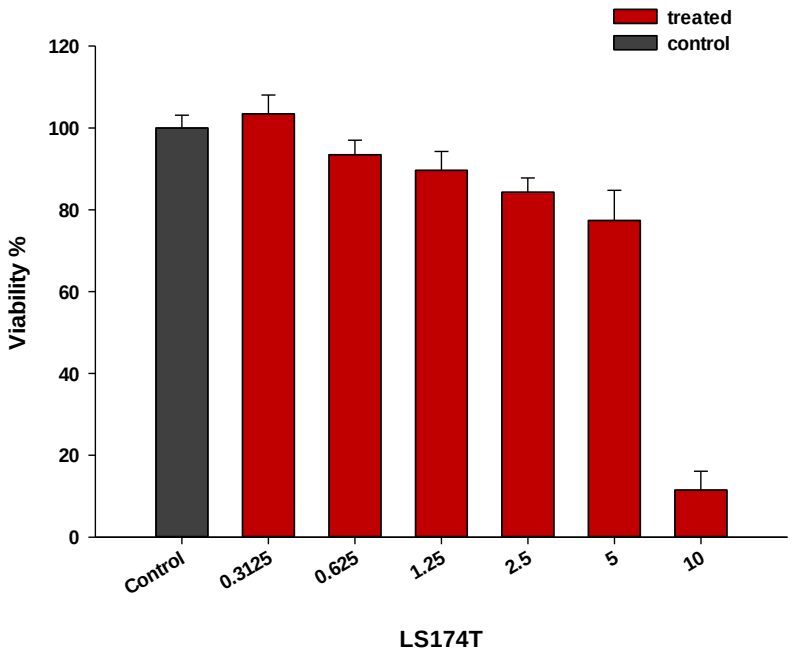

Figure (4-17): The viability of colon cancer cell Line (LS174T) after treated with β-carotene**.**

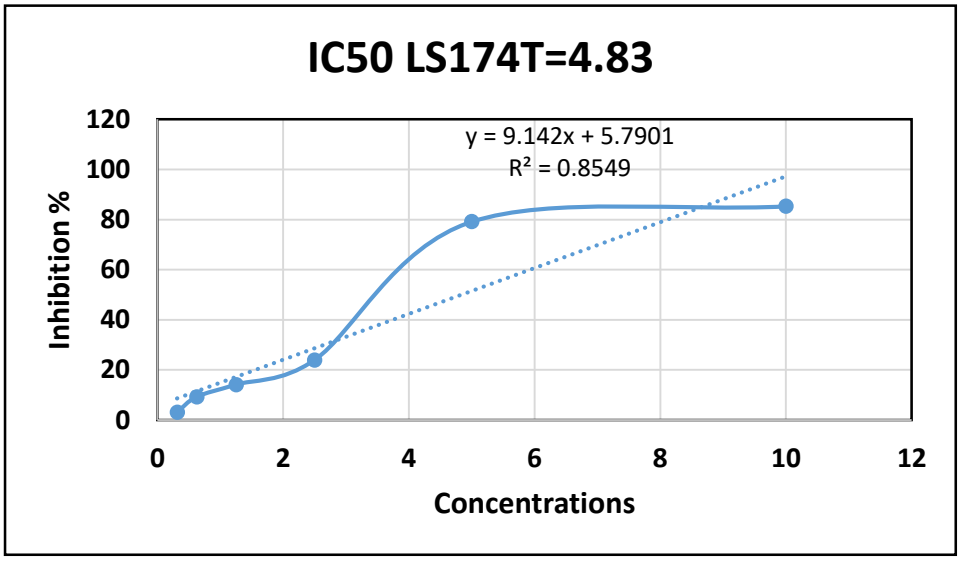

Figure (4-18): The effectiveness of β-carotene isolate on LS174T colon cancer line cells.

4.5.2. Bladder cancer Cell Line (EJ138)

To demonstrate the cytotoxicity of β-carotene. Each cell lines of bladder cancer (EJ138) were exposed to different concentrations of β-carotene (0.3215, 0.625, 1.25, 2.5, 5, 10) µg/ml, and estimated the cytotoxicity by finding the percentage of growth inhibition compared to the control group considering their growth rate 100.0%.

The present results show that the purified β-carotene have variant cytotoxic activity against bladder cancer cells with different concentration of β-carotene, where showed higher cytotoxic activity (85.3%) in concentration 10 µg/ml against bladder cancer cells. While the lowest inhibition percentage (3.06%) was at the Lowest concentration used (0.3125 µg/ml) in cell lines of bladder cancer (EJ138).

The current results also showed the highest viability at concentration 0.3125 µg/ml which is 96.93% after the absorbance reading with the MTT technique and lowest viability rate, at 10 µg/ml and it reached 14.72% as in figure (4-17). The mean concentration of IC50 of purified β-carotene was 5.21, figure (4-18).

Table (4-7): Efficacy of β-carotene extracted from *Rhodoturella sp* on Bladder cancer

EJ138	**Concentration µg/ml**					
	0.3125	**0.625**	**1.25**	**2.5**	**5**	**10**
Mean ±SD	**26 ± 1.5^{A}**	25± 0.56^{A}	23± 1.5^{B}	21± 1.5^{C}	6 ± 1.1^{D}	4 ± 1.0^{E}
P value	**0.001† HS**					
Different letters denote to the significant differences at p < 0.05						

SD: standard deviation; †: one-way ANOVA; **HS**: Highly significant at $P < 0.001$

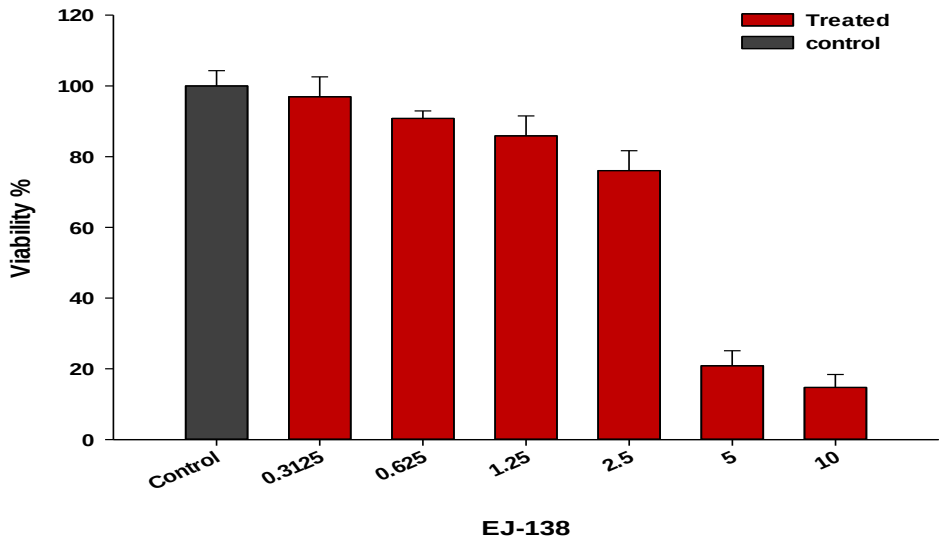

Figure (4-19): The viability of bladder cancer (EJ138) after treated with β-carotene.

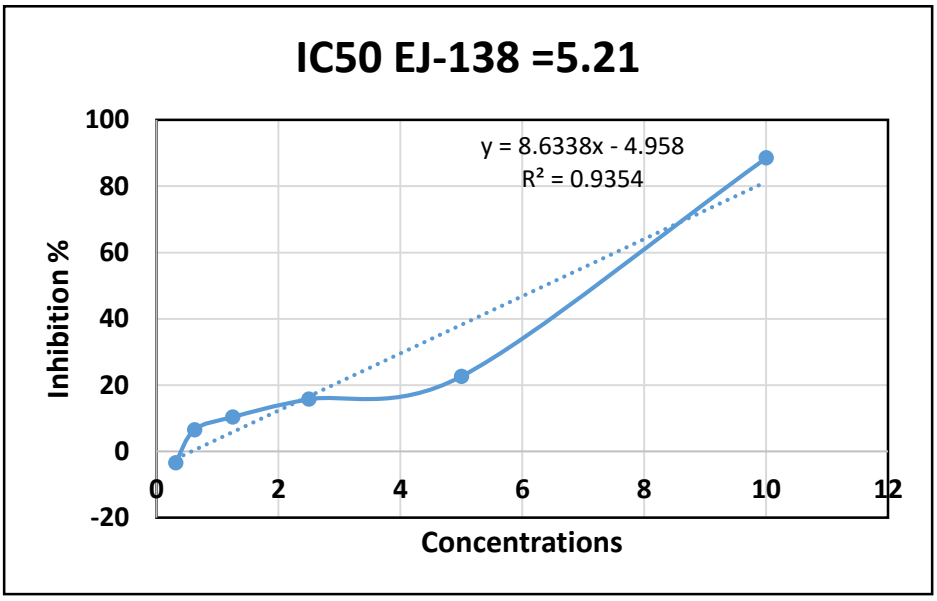

Figure (4-20): The effectiveness of β-carotene on EJ138 bladder cancer line cells.

4.6. Cytopathic Study

Cell cultures of both cancer cell line were stained after being treated with the half-lethal concentration (IC50) of β-carotene for 72 hours with H&E double dye and examined under a light microscope. The results of the microscopic examination showed that β-carotene causes obvious pathological and cytological changes.

4.6.1. Colon cancer cell line (LS174T).

Untreated colon cancer cell line and cultured on a slide, appear as a monolayer after a 72 hour of cultivation in a serum-free medium. The cells appear as elongated, with ovoid nuclei, the cytoplasm is stained in violet color and the nucleus in dark color. While the microscopic examination of colon cancer cells treated with *β-carotene* showed dissociation of cell culture, separation of cells from each other, and the appearance of large areas devoid of cells. Fig (4-19)to(4-25).

This finding is consistent with (Palozzas et *al.*, 2001) found that there is a special mechanism by which β-carotene regulates cell growth, that β-carotene at high concentrations can act as a changer of intracellular ROS (Reactive Oxygen Species) production and that this changer can modulate cell growth by affecting molecular pathways involved in apoptosis.

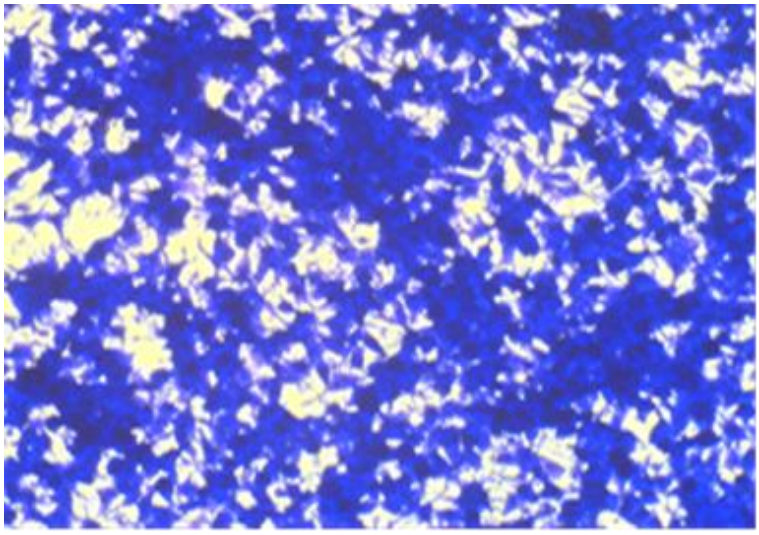

Figure (4-21): Cell culture without treatment

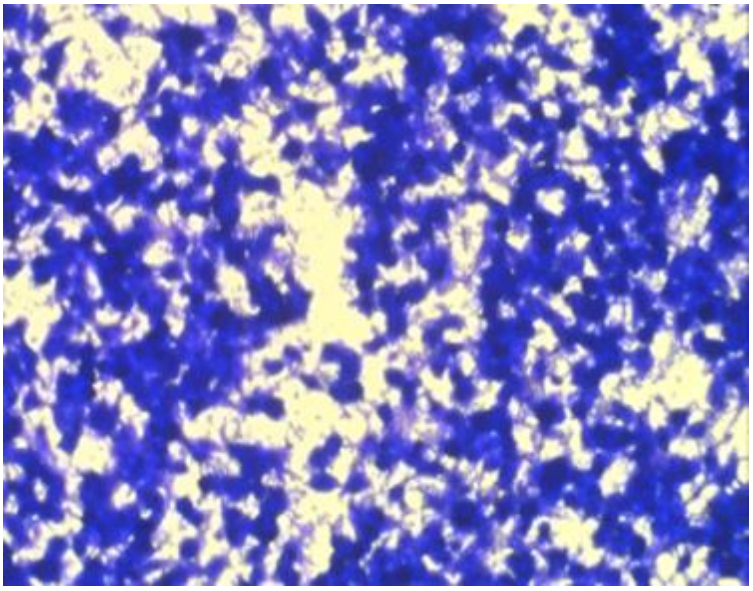

Figure (4-22) Cell culture with treatment (0.3125 µg/ml)

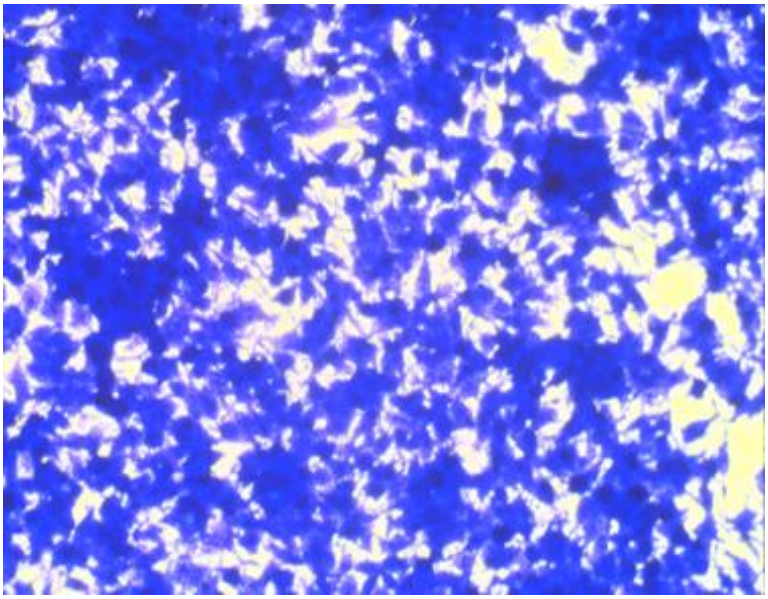

Figure (4-23) Cell culture with treatment (0.625 µg/ml)

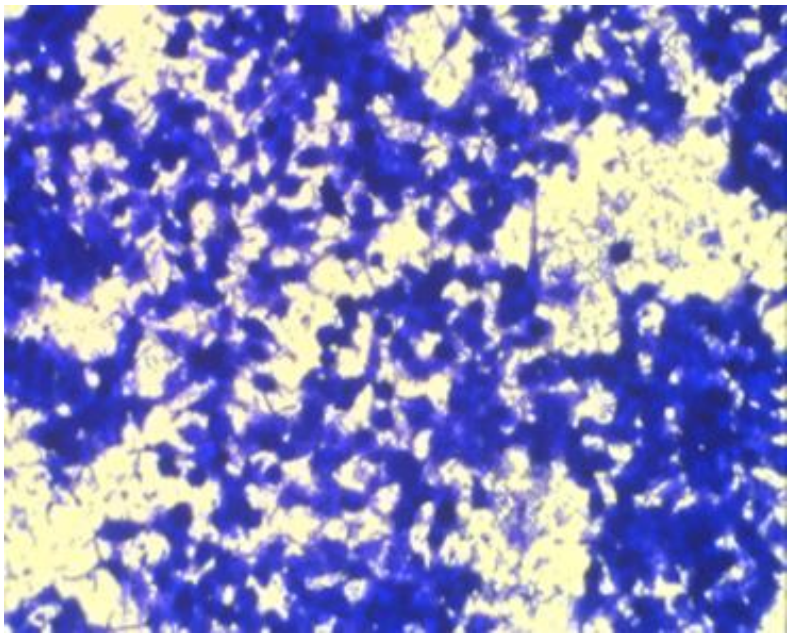

Figure (4-24) Cell culture with treatment (1.25µg/m)

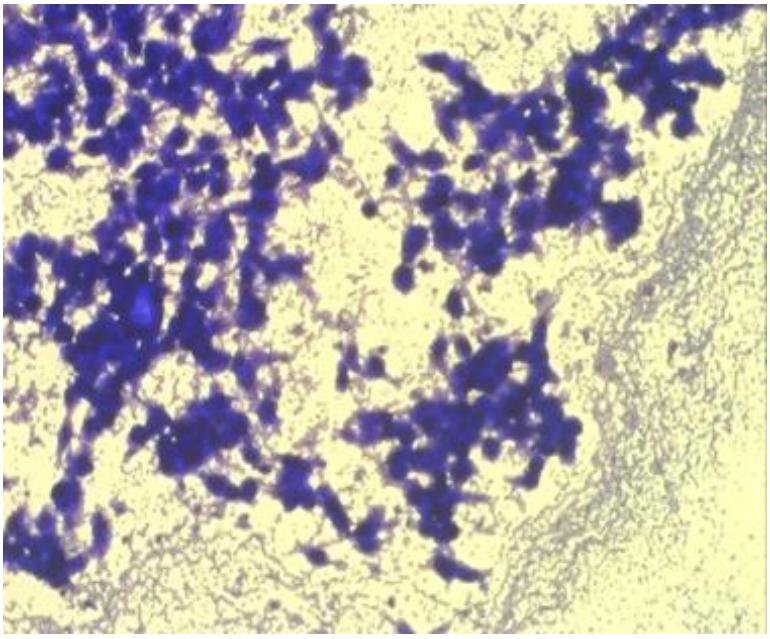

Figure (4-25): Cell culture with treatment (2.5µg/ml)

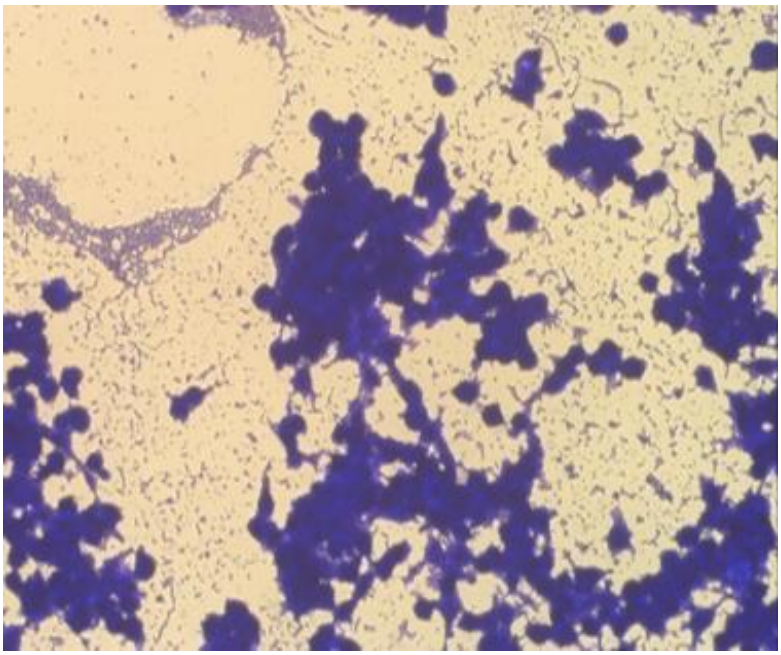

Figure (4-26): Cell culture with treatment (5µg/ml)

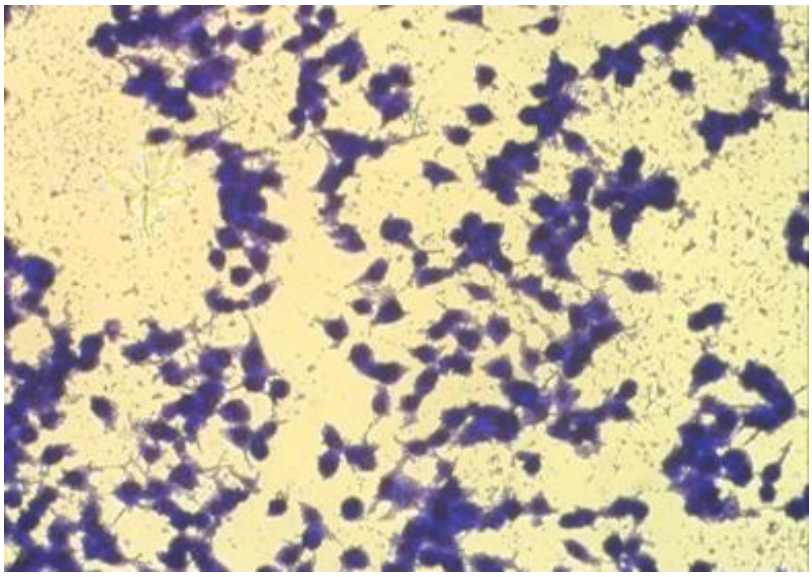

Figure (4-27): Cell culture with treatment (10µg/ml)

4.6.2. Bladder cancer Cell Line (EJ138)

Untreated colon cancer cell line and cultured on a slide, appear as a monolayer after a 72 hour of cultivation in a serum-free medium. The cells appear as elongated, with ovoid nuclei, the cytoplasm is stained in violet color and the nucleus in dark color. While the microscopic examination of colon cancer cells treated with *β-carotene* showed dissociation of cell culture, separation of cells from each other, and the appearance of large areas devoid of cells. Fig. (4-26) to (4-2. Naz *et.al*,2017, confirmed that *β-carotene* has a role in cell proliferation and apoptosis, during his study by using cancer cells (HeLa, HuH7 and MCF-7) and normal cell lines (HEK-293-T). An impressive anti-cancer activity of β-carotene was observed

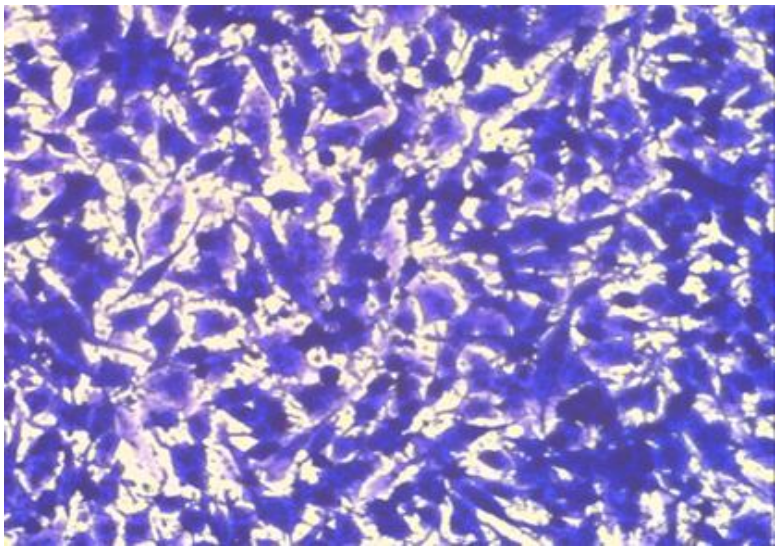

Figure (4-28): Cell culture without treatment

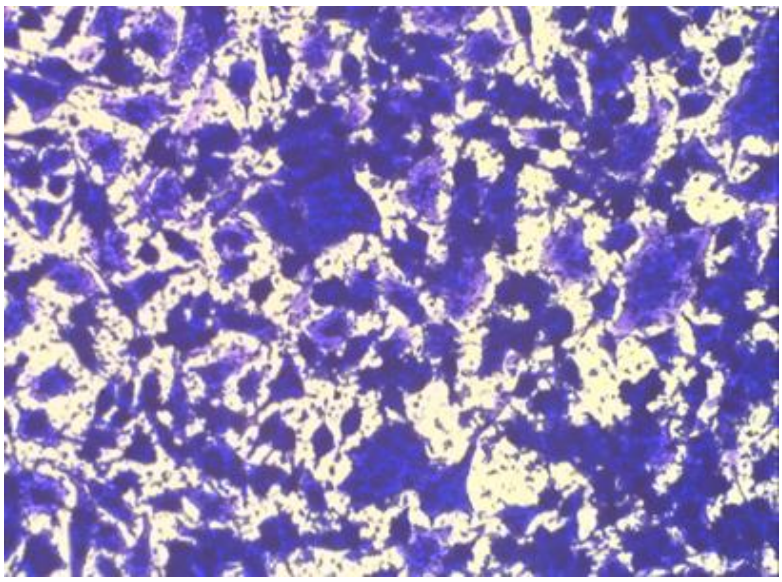

Figure (4-29): Cell culture with treatment (0.3125 µg/ml)

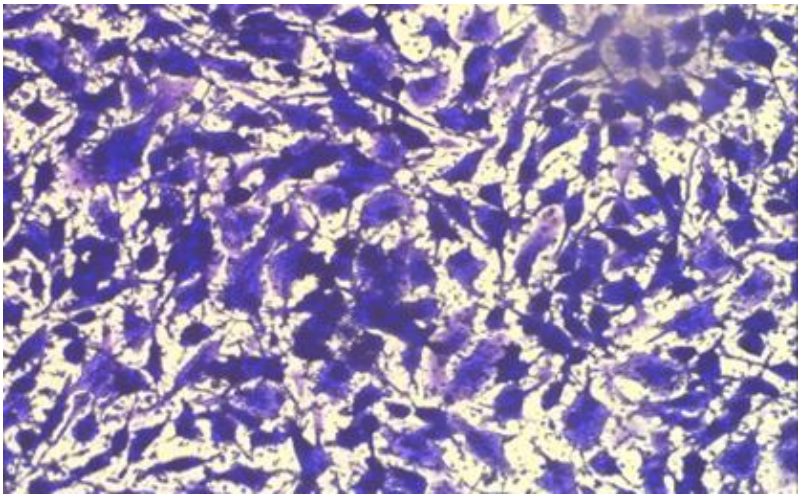

Figure (4-30): Cell culture with treatment (0.625 µg/ml)

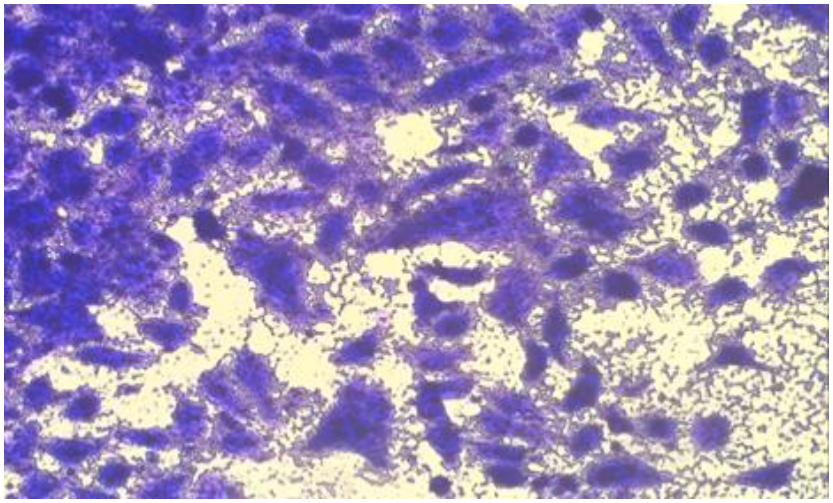

Figure (4-31): Cell culture with treatment (1.25µg/ml)

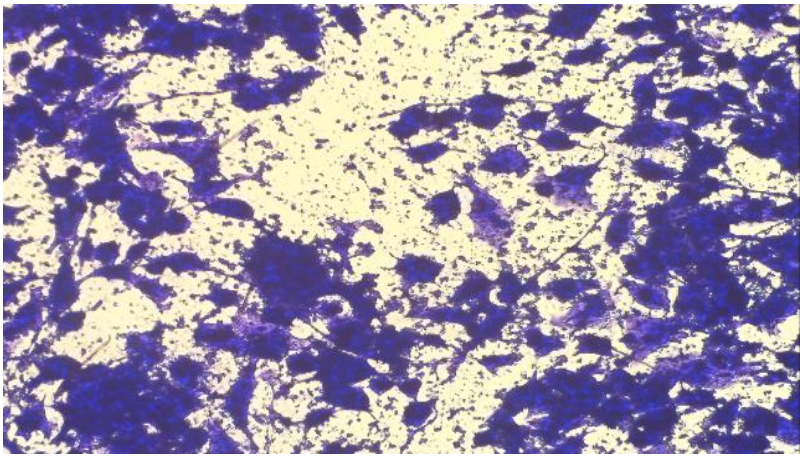

Figure (4-32): Cell culture with treatment (2.5µg/ml)

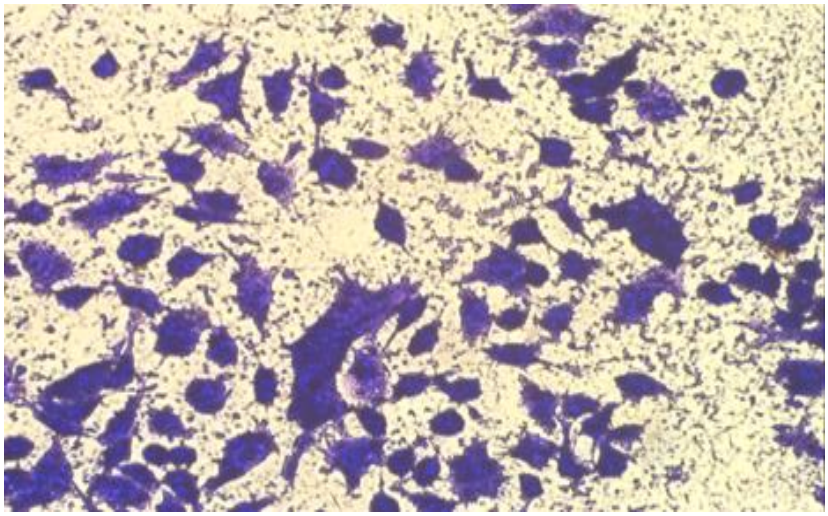

Figure (4-33): Cell culture with treatment (5µg/ml)

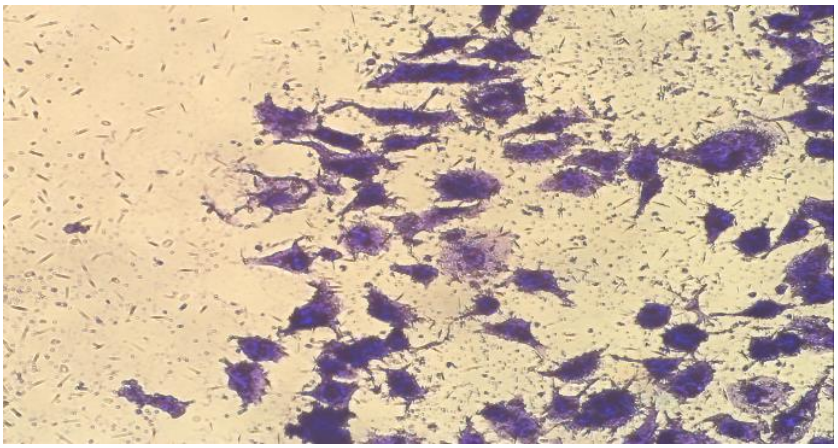

Figure (4-34): Cell culture with treatment (10µg/m)

Due to the fact that their cells are significantly smaller and grow at greater rates in a cheap culture media, microorganisms offer several advantages over plants and animals for the creation of pigments (Lebeau,*et.al*,2017). Additionally, microbes are highly productive and can continuously create a product. (Sen,*et.al*.,2019).

Moreover, a variety of microorganisms, including bacteria, fungi, and protozoa, create natural pigments including carotenoids, the greatest array of pigments can be produced by fungi on a range of substrates. Fungi create these pigments as secondary metabolites. (Suwannarach,*et.al*.,2019).

In the current study extraction carotene from *Rhodoturella* yeast by using acetone as solvent. results show the efficiency of the method used for extraction. And this result is consistent with (Naghavi, *et.al*,2015) concluded that using each of ether and methanol in extracting dyes, including β-carotene. Also with (Malla Obaeda,2017) which used this method in extraction β-carotene.

Studies on animal models and human populations reveal that p-carotene, or vitamin A, and its metabolites may affect cancer risk in humans. This fact confirmed in our study, Where the results appear, the carotene's cytotoxicity. Different amounts of the pigment carotene (0.3215, 0.625, 1.25, 2.5, 5, and 10) g/ml were applied to each cell line of bladder and colon cancer. and demonstrated that beta-carotene clearly causes pathological and cytological changes

This result is consistent with (Palozza, et.al,2001; Palozza,*et.al*,2004; Gloria, *et.al*,2014) findings that -carotene regulates cell growth through a unique mechanism, and carotene at high concentrations can act as a regulator of intracellular reactive oxygen species(ROS) production, and that this

regulator can modulate cell growth by influencing apoptotic molecular pathways. Jayappriyan *et al*(2013) reported that a good anticancer treatment should kill or dysfunction cancer cells without significantly harming healthy cells, and this is accomplished by inducing apoptosis.

Recent research has shown that beta-carotene slows the growth of esophageal squamous cell carcinoma (ESCC) cells and causes apoptosis, suggesting that diets high in carotenoids may give some degree of protection against cancer.(Zhang,et.al.2016).

Our research demonstrates beta-carotene ability to reduce cell growth, stop the cell cycle at various points, and stimulate apoptosis. And suggest that carotenoids may act as biologically active cancer-interfering agents

Conclusions:

1- possibility of isolating *Rhodotorula* yeast from dental samples of diabetic patients

2- *Rhodotorula* yeast is a natural source of beta-carotene

3- The use of acetone with alcohol in the process of extracting the beta-carotene pigment was efficient in obtaining a concentrated pigment

4- The results indicate that an antifungal activity of beta-carotene in the laboratory against *Candida krusie*, this activity increased with increasing the concentrations used, and the most effective concentration was 40 µg / ml.

5- The beta-carotene pigment showed an inhibitory effect against growth of *S. pyogenes*, especially at a concentration of 40 µg/ml.

6- The antibacterial effect of beta-carotene was more effective in the case of *S. pyogenes* than in the case of *Candida krusie*.

7- The ability of beta-carotene to reduce the growth of cancer cells for both Colon cancer cell line (LS174T) and Bladder Cancer Cell Line (EJ138), especially at high concentrations, where the concentration was 5µg/ml.

Recommendations:

1- Utilizing Rhodotorula yeast as a natural beta-carotene source and looking for other natural sources

2-Searching for solvents and other extraction methods that give more quantity and concentration in the beta-carotene extraction process

3- Conducting further studies on antifungal activity of beta-carotene on other fungi, especially pathogenic filamentous fungi such as dermatophytes and systemic fungi.

4- Conducting further studies on antibacterial activity of beta-carotene on other bacteria , especially pathogenic bacteria

5- Conducting further studies on anticancer activity of beta-carotene on other types of cancer such as Prostate cancer, lung cancer and Breast cancer.

6- Conducting further studies on all activities of beta-carotene invivo using animals lab.

7- Conducting studies on other activities of beta-carotene such as antioxidant and immunological studies

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Appendix

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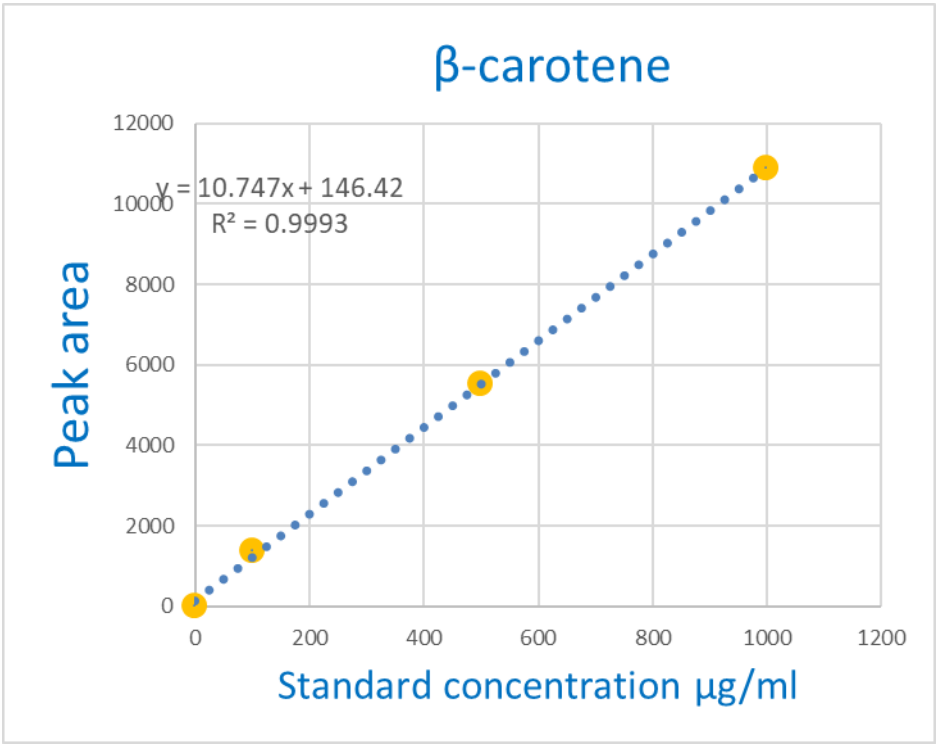
Appendix (1)



Appendix (2)

Table (4-2): Stander of β-carotene

Std	Peak area	concentration µg/ml
β-carotene	10880.7	1000
β-carotene	1388.09	100
β-carotene	0	0
β-carotene	5512.28	500

الخلاصة

الكائنات الدقيقة التي تنتج الصبغ هي من بين المصادر الطبيعية التي لديها القدرة على المساعدة في حل مشاكل اليوم. بالإضافة إلى ذلك ، تتمتع الألوان الطبيعية بفوائد إضافية مثل مضادات الأكسدة ومقاومة السرطان. في هذه الدراسة ، تم تحليل الخصائص البيولوجية والسريرية للأصباغ الميكروبية ، مثل قدراتها المضادة للسرطان. لذلك تم استخلاص بيتا-كاروتين من خميرة الرودوتوريلا باستخدام الأسيتون كمذيب للصبغة في هذه الدراسة وبحثت الإمكانات العلاجية للبيتا كاروتين كعامل مضاد للميكروبات ومضاد للسرطان.

اشتملت الدراسة على عزل وتشخيص الخمائر والبكتيريا من تجويف الفم التي استمرت من فترة 1 أكتوبر 2022 إلى 28 فبراير 2023. 150 عزلة من خميرة الرودوتورولا بنسبة 83.3٪ ، عزلتان من خميرة الكانديدا بنسبة مئوية 1.11٪ و 3 عزلات من *Streptococcus pyogenes* بنسبة 60.0٪. تم تشخيص العزلات بناءً على الاختبارات المظهرية الزرعية والكيموحيوية.

أظهرت نتائج استخلاص صبغة بيتا كاروتين أن خميرة الرودوتورولا كانت منتجًا طبيعيًا جيدًا للصبغة ، وأظهرت كفاءة الطريقة المستخدمة في الاستخلاص للحصول على صبغة مركزة. أظهر HPLC أيضًا أن الكاروتين الرئيسي في المستخلص هو بيتا كاروتين

اظهرت نتائج النشاط المضاد للميكروبات ، الفعالية المضادة للفطريات لبيتا كاروتين ضد *C.Krusei*بتركيزات مختلفة (10،20،40) ميكروغرام / مل ، قطر مناطق التثبيط (6،9،13) ملم على التوالي. كما اظهرت الفعالية المضادة للجراثيم ضد *S. pyogenes* قطر مناطق التثبيط (4،12،15) ملم على التوالي. ويزداد هذا النشاط بتركيزات عالية.

تم تطبيق كميات مختلفة من صبغة الكاروتين (0.3215 ، 0.625 ، 1.25 ، 2.5 ، 5 ، و 10) جم / مل على كل خط خلوي من سرطان المثانة والقولون. وأثبت أن بيتا كاروتين يسبب بوضوح تغيرات مرضية وخلوية.

أظهرت النتائج أن بيتا كاروتين المنقى له نشاط سام للخلايا مختلف ضد خلايا سرطان القولون بتركيزات مختلفة ، حيث أظهر نشاط سام للخلايا أعلى (88.5٪) بتركيز 10 ميكروغرام / مل ضد خلايا سرطان القولون. بينما أقل نسبة تثبيط (-3.43٪) كانت عند التركيز المستخدم (0.3125 ميكروغرام / مل)

في خط الخلايا لسرطان القولون (LS174T). كما أظهرت النتائج أعلى قابلية للبقاء عند التركيز 0.3125 ميكروغرام / مل أي 103.44٪ بعد قراءة الامتصاص بتقنية MTT (اختبار قياس لوني لتقييم النشاط الأيضي للخلية) وأدنى معدل بقاء عند 10 ميكروغرام / مل ووصل إلى 11.5. ٪. كان متوسط تركيز IC50 للبيتا كاروتين المنقى 4.83 .

كما أظهرت النتائج أن بيتا كاروتين المنقى له نشاط سام للخلايا مختلف ضد خلايا سرطان المثانة بتركيز مختلف من بيتا كاروتين ، حيث أظهر نشاط سام للخلايا أعلى (85.3٪) بتركيز 10 ميكروغرام / مل ضد خلايا سرطان المثانة. بينما كانت أقل نسبة تثبيط (3.06٪) عند أعلى تركيز مستخدم (0.3125 ميكروغرام / مل) في سلالات خلايا سرطان المثانة (EJ138). أظهرت النتائج الحالية أعلى قابلية للبقاء عند التركيز 0.3125 ميكروغرام / مل أي 96.93٪ بعد قراءة الامتصاص باستخدام تقنية MTT ولإثبات السمية الخلوية لـبيتا كاروتين. كان متوسط تركيز IC للكاروتين المنقى هو 5.21.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة القادسية

كلية العلوم / قسم علوم الحياة

الخصائص المضادة للميكروبات والسرطان لصبغة البيتا-كاروتين المستخلصة من *Rhodoturella sp.*

رسالة مقدمة الى

مجلس كلية العلوم / جامعة القادسية / قسم علوم الحياة

وهي جزء من متطلبات نيل شهادة الماجستير في علوم الحياة / علم الأحياء المجهرية

تقدمت بها

غصون غسان كريم الجبوري

(بكالوريوس احياء مجهرية / كلية العلوم للبنات/ جامعة بابل / 2019)

بأشراف

أ.د. نيران عبيد جاسم

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2023 م