ISSN: 2347-467X, Vol. 12, No. (3) 2024, Pg. 1248-1260

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Feasibility of Carotenoid Production by Novel Yeast, *Occultifur* sp. M2004, for Nutraceutical Applications

SARAN PROMSAI[1]*, YAOWANOOT PROMNUAN[1] and SUJINAN MEELAI[2]

[1]Faculty of Liberal Arts and Science, Department of Science and Bioinnovation, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand.
[2]Faculty of Science, Department of Microbiology, Silpakorn University, Sanam-Chandra Campus, Nakhon Pathom, Thailand.

Abstract

One of the most prominent pigments found in nature, carotenoids are employed extensively in the cosmeceuticals, pharmaceuticals, food industries, and feeding sectors in addition to phytomedicine. Recently, microbial synthesis of products has gained popularity in various industrial sectors. The economic and environmental aspects of microbial carotenoid production have attracted a lot of attention. The aims of this research were to investigate the potential of novel yeast, *Occultifur* sp. M2004 for carotenoids production, identify the pigment structure and evaluate the anti-collagenase and anti-elastase properties of pigments produced by a newly isolated yeast. Antibiotic susceptibility tests revealed that this strain was sensitive to colistin and gentamicin but resistant to co-trimoxazole, vancomycin, amoxiclav, doxycycline, ampicillin, and cefalexin. The optimal conditions for yeast growth and red pigmentation were achieved by cultivating in yeast extract-glucose (YG) medium at pH 6, 25 °C with agitation at 150 rpm for 7 d. Beta-carotene was identified as the produced pigment using Fourier transform infrared spectroscopy (FT-IR) technique and liquid chromatography-mass spectrometry (LC-MS) analysis. The half maximum inhibitory concentration (IC50) values for anti-collagenase and anti-elastase activities were 2.61 µg/mL and 100.16 µg/mL, respectively. This study highlights the potential of *Occultifur* sp. M2004 as a valuable and versatile resource for producing carotenoids, which could have potential applications in nutraceuticals.

Article History
Received: 04 September 2024
Accepted: 30 October 2024

Keywords
Antibiotic Susceptibility;
Beta Carotene;
Carotenoids;
FT-IR;
Red Yeast.

CONTACT Saran Promsai saranpromsai@hotmail.com Faculty of Liberal Arts and Science, Department of Science and Bioinnovation, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand.

Doi: https://dx.doi.org/10.12944/CRNFSJ.12.3.21

Introduction

Nutraceuticals are foods or dietary ingredients that give health or medicinal benefits, such as preventing sickness or treating it. They are available as tinctures, tablets, and capsules. A variety of items fall under the category of nutraceuticals, including functional drinks, genetically modified foods, herbal/protein/mineral/vitamin supplements, and other processed goods, food or a portion of it that is used to make functional foods and nutraceuticals. These may resemble traditional meals in appearance. However, nutraceuticals are typically thought of as single or purified food forms that are marketed as medicines. Many types of natural plant sources (fruits and vegetables, herbs, cereals), mal sources (dairy products, meat, eggs, animal foods), crustaceans and algae with biologically active substances may be used to fight against cancer, chronic disease, neurodegenerative diseases, and other health-related diseases in the development of novel nutraceuticals products and functional foods. Additionally, there is increasing consumer interest in natural and eco-friendly products, driving research and development of new product types. The use of natural herbs as raw materials in nutraceutical products is a rapidly growing trend.[1]

Carotenoids are important pigments in many industries. Commonly found in nature, such as in vegetables and fruits, their presence is indicated by their vibrant colors. The market for carotenoids is expected to grow steadily worldwide and reach about USD 1.4 billion in 2018.[2] There are two categories for carotenoids: carotenes, which are orange-red, and xanthophylls, which are yellow. Some carotenoids serve as precursors in vitamin synthesis and positively impact human health by acting as bioactive phytochemicals that reduce the chance of developing conditions like conjunctivitis, cancer, and cardiovascular disease. Carotenoids are derived from fat; they are soluble in fat solvents but insoluble in water. They also exhibit excellent solubility in non-polar solvents such as hexane, whereas xanthophylls dissolve well in polar solvents like alcohol. Carotenoids are resistant to both acidity and alkalinity but are sensitive to sunlight and heat, which accelerate oxidation reactions, particularly in the presence of metal peroxides.[3-5]

Carotenoids are gaining popularity due to increasing demand in the functional food, cosmetic, medicinal products, and feed industries. Research on fermentation-based carotenoid production has accelerated in the search for natural compounds applicable in food. The growing need for natural carotenoids can be met by microbial production, which is also more environmentally safe than chemical synthesis The demand from consumers for natural carotenoids is not met by commercially produced carotenoids, as most of them are obtained by chemical synthesis. Consequently, the present focus has changed to isolating carotenoids from biological sources rather than chemical production.[6] Carotenoids cannot be synthesized de novo by humans and can only be acquired through the dietary route. Only six carotenoids: α-carotene, β-carotene, lutein, zeaxanthin, lycopene, and cryptoxanthin are often present in the human diet and blood serum, out of the approximately 700 that have been identified. Furthermore, the typical human diet only includes about 40 carotenoids. Numerous studies demonstrate that giving the body dietary carotenoids is linked to a lower chance of contracting viral diseases like HIV infection as well as lifestyle diseases like cancer, osteoporosis, diabetes, or cataracts.[7-10] The benefits of carotenoids are numerous. Their primary characteristic is that they are powerful scavengers of reactive oxygen species (ROS), such as peroxide and single molecular oxygen.[8] Higher blood-carotene and lycopene levels are associated with a decreased risk of lung cancer, even in smokers, according to recent epidemiological studies.[11]

Interestingly, a growing amount of research suggests that carotenoids could be useful in the management of certain diseases, including neuroblastoma, cervical cancer, and prostate cancer.[12-14] Numerous studies conducted both in vivo and in vitro have demonstrated the impact of carotenoids on a range of immune-related and inflammatory response processes in the body. These substances have been shown to affect humoral immunity (cytokine synthesis and secretion) as well as cellular immunity (lymphocyte proliferation, phagocytosis, and NK cell cytotoxicity).[15] It has been discovered that β-carotene can prevent human skin fibroblast (FEK4) exposed to UV-A from having its production of heme oxygenase 1 upregulated.[7,16]

A substantial body of study has been conducted on the topic of aging skin because of scientists'

increased interest over the past several decades. Extrinsic and intrinsic aging are the two categories of skin aging. Extrinsic aging, on the other hand, is brought on by exposure to outside variables, most notably ultraviolet (UV) radiation or photoaging. Intrinsic aging is the ageing process that occurs naturally over time. The most prevalent protein in the human dermis layer, collagen, gives skin its tensile strength. The elastic recoil of the skin is caused by a network of fibers called elastin, which is present in connective tissue. The health and youth of the skin depend on collagen and elastin, which give the skin its plumpness, flexibility, integrity, and elasticity. However, reactive oxygen species (ROS) accumulate up as a result of exposure to photoaging stimuli, which has the ability gradually activate skin enzymes like collagenase and elastase. Collagen and elastin are broken down and degraded by these enzymes, accelerating the aging process of the skin, manifesting as sallowness, wrinkles, freckles, laxity, and a leathery skin.[17]

Yeast is a microorganism belonging to the kingdom Fungi. It is a single-celled eukaryote, typically round, oval, or lemon-shaped, larger than bacteria with an average diameter of about 5 microns. Yeast primarily reproduces asexually through budding. It is commonly found in nature, such as in soil, water, various parts of plants, and sometimes in association with insects and the stomachs of some animals. Yeast thrives in environments with high sugar concentrations, such as sweet fruit juices.[18] Research has shown that yeast contains up to 50% protein, making it an excellent protein source. It also contains almost all amino acids in balanced quantities, all B vitamins, and chromium, which helps maintain constant energy levels in the body. Yeast metabolites help reduce symptoms of fainting and dizziness, adjust blood sugar levels, and reduce fat blockages in arteries. Additionally, selenium from yeasts has cancer-prevention properties.[19]

Several groups of researchers have reported producing these important chemicals from algae, microbes, plants, and animals in recent years.[20, 21] Higher yields, reduced environmental effect, and season-independent production have made microbial cell factories for the manufacture of carotenoids a superior option than plant-based extraction techniques. A high-value carotenoid with anticancer, antioxidant, cardioprotective, and hepatoprotective properties, astaxanthin, is produced intracellularly by the yeast *Xanthophyllomyces dendrorhous*, one of the carotenoids microorganism producers.[22, 23]

Carotenoids production is limited by low yields and limited natural sources. One of the drawbacks to the commercialization of carotenoids from yeasts is the high production cost involved. Many researchers have focused on discovering novel microbial producers of carotenoids to meet the growing demand for carotenoids. In this context, the present work was aimed at study of an isolated pigment producing strain for pigment production and characterization. Fourier transform infrared spectroscopy (FT-IR) and Mass spectrophotometry were applied for characterization of the pigment. Our results describe this new astaxanthin producers and provided important insights into some pharmaceutical properties in *Occultifur* sp. M2004.

Materials and Methods

Yeast Strain

Occultifur sp. M2004 was isolated from the digestive tract of the red dwarf honeybee (*Apis florea*), collecting in Chiang Mai, Thailand. From the analysis of the D1/D2 domains of the large subunit ribosomal RNA gene (LSU D1/D2), this yeast was close to *Occultifur* externus IGC 4817T, with 98.55% similarity. Therefore, the strain M2004 could represent a novel *Occultifur* species. Its glycerol stock (30% v/v) was kept at -80°C in the yeast culture collection of the Department of Microbiology, Faculty of Science, Silpakorn University, Sanam-Chandra Campus, Nakhon Pathom. The animal study protocol was approved by the Institutional Animal Care and the Use Committee (IACUC), Silpakorn University (Ethic number: 8603.16/0328)

Antibiotic Susceptibility Assay

Antibiotic susceptibility was performed using an agar disc diffusion method. *Occultifur* sp. M2004 was inoculated in yeast extract-malt extract (YM) broth and incubated at 25 °C for 2 d. The culture broth was then swabbed on the surface of a YM agar plate. Antibiotic discs (Himedia, Mumbai, India), including co-trimoxazole (50 µg), vancomycin (30 µg), gentamicin (30 µg), amoxiclav (30 µg), colistin (25 µg), doxycycline (30 µg), ampicillin (10 µg) and cefalexin (30 µg), were placed on the surface of the culture plate. The inhibition zones were evaluated.

Optimization of Culture Condition

The yeast strain was cultured in various modified media under different conditions. The modified media, YM and yeast extract-glucose (YG) broth, were adjusted to pH 4, 6, 7, and 8. The yeast strain was inoculated in each modified medium and incubated at shaking incubator at 150 rpm, 25 °C for 7 d. Turbidity and orange-red color in the flasks were observed. The optimal condition, which exhibited high yeast cell density and orange-red color, was selected for further examination.

Yeast Cultivation in Flasks

The strain was inoculated in YM medium at 25 °C for 3 d. Ten percent inoculum (10^7 CFU/mL) of yeast culture was added to 1 L of YG medium at pH 6. The culture medium was then incubated at 25 °C for 7 d with agitation at 150 rpm.

Carotenoids Extraction

Carotenoids extraction was modified from Ribeiro.[24] The cell cultures were centrifuged at 2260×g for 10 min to obtain the pellets. The obtained cell pellets were frozen at -20 °C for 24 h. The frozen pellet was thawed and suspended in DMSO (Univar, Ingleburn, NSW, Australia). The glass beads were added in the solution, vortexed for 2 min, and put in water bath at 60 °C for 15 min. In that order, acetone, (Univar, Ingleburn, NSW, Australia), petroleum ether (Univar, Ingleburn, NSW, Australia), and 20% NaCl (Himedia, Mumbai, India) were added. The solution was rigorously mixed for 5 min and centrifuged at 2260×g for 10 min. The recovered carotenoids were extracted from the top layer of petroleum ether (PE), which was collected and then evaporated at 45 °C in an evaporator (Buchi, Switzerland).

Characterization of Red Pigment

The red pigment extracted from *Occultifur* sp. M2004 was subjected to FT-IR spectrophotometer (Spectrum Two, PerkinElmer, USA) with spectral range of 4000–400 cm^{-1} (4 cm^{-1} resolution). The supernatant from the culture broth and red pigment extract were also analyzed by LC-MS. LC analyses were carried out using a UHPLC Accela system (Thermo Fisher Scientific, Waltham, MA, USA). UHPLC chromatographic separations were performed on a reversed-phase column Agilent Zorbax C18 (4.6 × 150 mm, 5 µm). The gradient program is shown in Table 1 (mobile phase solvent A: acetonitrile:methanol (70:30, v/v), solvent B: H_2O 100%). The column was set at 32 °C, and the temperature was 25 °C. The solutions were filtered using 0.2 µm Membrane Nylon (Millipore, Bedford, MA, USA). Ten microliters were injected into column system.

Table 1: Gradient program for separation of carotenoids

No.	Time (min)	A (% v,v)	B (% v,v)	Flow rate (µL/min)
0	0.00	85.0	15.0	800
1	2.00	85.0	15.0	800
2	3.00	100	00.0	800
3	7.00	100	00.0	800
4	8.00	100	00.0	800
5	11.6	100	00.0	800
6	12.6	85.0	15.0	800
7	15	85.0	15.0	800

To attain the highest level of quantitative sensitivity, the most frequent API-MS/MS transition for every molecule was observed using the multiple-reaction monitoring mode. For each molecule, specific optimizations were made to cone voltages, energy collisions, and other instrument parameters in order to maximize signal intensity for the selected fragmentation products. The combined flow state mode was used for these investigations, and standard solutions were directly infused into acetonitrile (70:30, v/v). The conditions of mass spectrophotometer were as follows: APCI positive mode, probe temperature 400 °C, corona 10.0 A, and scan events time 0-15 min.

Anti-Collagenase Testing

The inhibitory activity of collagenase was tested using the method of Jiratchayamaethasakul.[17]

One milligram of collagen impregnated with azo dye was dropped into a well microplate. After adding 800 µl of 0.1 M Tris-HCl (pH 7) and 100 µl of carotenoid sample into each well, the mixture was homogenized. One hundred microliter of collagenase (200 units/mL) was then immediately mixed into the homogenized solution and incubated at 43 °C for 1 h. Subsequently, the solution was centrifuged at 3000 rpm for 10 min. After that, the absorbance at 550 nm was determined with a microplate reader (Spectrostar Nano, BMG Labtech, Ortenberg, Germany).

Anti-Elastase Test

Elastase inhibitory activity was tested using the method of Jiratchayamaethasakul.[17] A solution of AAAPVN elastase substrate in 0.1232 M Tris-HCl buffer (pH 8) was formulated to achieve a concentration of 1.015 mM. Ten microlitter of carotenoid extract sample was mixed with elastase substrate and incubated at 25 °C for 10 min. After that, 10 µl of elastase from porcine pancreas (7.5 units/mL) in Tris solution buffer was added. The absorbance at 410 nm determined with a microplate reader (Spectrostar Nano, BMG Labtech, Ortenberg, Germany).

Statistical Analysis

The experiment of yeast growth optimization was carried out at least three times, with means and standard deviations calculated from these values. Statistical analysis software (SSPS Inc., Chicago IL, USA, IBM®, Armonk, NY, USA) was used for data analysis. Analysis of variance was used to test significant differences between trials ($P < 0.05$).

Results and Discussion

Antibiotic Susceptibility

Among the eight types of antibiotics tested, *Occultifur* sp. M2004 was sensitive to colistin and gentamicin (28.7 ± 0.9 mm and 35.3 ± 0.5 mm of inhibition zones, respectively). Due to the antibacterial nature of most antibiotics, this yeast strain was resistant to many antibiotics in this study. The yeast strain was evaluated for virulent characteristics pertinent to human health using general antibiotics commonly applied in clinical studies. Although this yeast strain was isolated from a natural habitat, it is necessary to assess its safety for human use. In several cases, isolated microorganisms are primarily tested for antibiotic susceptibility.[25-28] Górzyńska[26] evaluated 55 isolates of *Saccharomyces cerevisiae* to ten antifungal agents (amphotericin B, flucytosine, fluconazole, voriconazole, posaconazole, micafungin, anidulafungin, caspofungin, and itraconazole) and manogepix (novel drug). Most strains of *S. cerevisiae* were sensitive to amphotericin B, flucytosine and echinocandins. Georgieva[27] evaluated the antibiotic susceptibility of *Lactobacillus* (23 strains) and *Bifidobacterium* (3 strains) isolated from several habitats. All isolated bacteria were sensitive to ampicillin, gentamicin, erythromycin, and tetracycline, but some *Lactobacillus* spp. were resistant to streptomycin, kanamycin, clindamycin, and chloramphenicol. Hence, a thorough reevaluation of the safety of cultures proposed for use as food additives is necessary, even though many strains of the *Lactobacillus* and *Bifidobacterium* spp. are classified as 'generally recognized as safe' (GRAS) cultures considering their proven health advantages and long-lasting history of safe consumption.

The current study aimed to select a new yeast strain for pharmaceutical applications related to humans. Therefore, assessing the safety of the yeast isolates was crucial. The habitat of the selected yeast strain is the digestive tract of a honeybee. The scientific research of *Occultifur* spp. in terms of harmful effects is scarce. Based on the characteristics of *O. tropicalis* and *O. externus*, closely related species that have been isolated from plants, there have been no reported pathogenic properties in humans.[29, 30] Additionally, red pigment-producing yeasts such as *Phaffia rhodozyma, Xanthophyllomyces dendrorhous,* and many species of *Rhodosporidium, Rhodotorula, Sporobolomyces,* and *Sporidiobolus* are prominent producers of carotenoid and have significant biotechnological potential in many sectors, including food, animal feed, cosmetics, pharmaceuticals, and agriculture.[2]

Optimization of Yeast Growth

The red pigment found in yeasts is an intracellular metabolite, necessitating efforts to achieve mass production of yeast cells. In the present study, the highest yield of yeast cells and red color in culture was achieved using the optimal media and pH, which was YG medium at an initial pH of 6 (Table 2). Due to its sustainability and affordability, microbial carotenoid production has attracted a lot of attention. To effectively utilize the potential of specific microbial strains, microbial fermentation requires optimization

of the fermentation medium and ambient conditions. Hence, to produce pigment, it is essential to optimize the strain's chemical and physical properties. Temperature, pH, osmolarity, and the nature and quantity of nutrient sources are some of the variables that affect and regulate pigment production.

Table 2: Yeast growth and pigment observation after cultivation in various conditions

Media	Initial pH	Temperature (°C)	Growth (A_{660})	Pigmentation
YM broth	4	25	2.50 ± 0.08 ^{b,c,d,e}*	+**
		37	2.72 ± 0.08 ^{a,b}	+
	6	25	2.43 ± 0.05 ^{c,d,e}	+
		37	2.45 ± 0.04 ^{c,d,e}	+
	8	25	2.33 ± 0.05 ^{e}	++
		37	2.40 ± 0.05 ^{d,e}	++
YG broth	4	25	2.63 ± 0.12 ^{a,b,c,d}	+++
		37	2.58 ± 0.12 ^{a,b,c,d,e}	+++
	6	25	2.83 ± 0.05 ^{a}	+++
		37	2.63 ± 0.05 ^{a,b,c,d}	+++
	8	25	2.78 ± 0.02 ^{a}	+++
		37	2.67 ± 0.05 ^{a,b,c}	+++

* Mean ± S.D.
A different lower case superscript letter in column represents a significantly different value in statistics using Tukey's test with a confidential level of 95%.
** +, low pigmentation; ++, moderate pigmentation; +++ high pigmentation

Nimsi[19] used a one-factor-at-a-time approach to optimize several physical parameters affecting pigment formation, such as temperature, pH, salinity, and incubation days. They determined the optimal medium parameter by investigating in YM broth, pH 5, 15% salinity at 28 °C, resulted in a pigment production of 194.78 µg/g and cell concentration of 9.2 ± 0.12 g/L. Ribeiro[24] assessed the viability of growing *Rhodotorula glutinis* on cassava wastewater as a low-cost material for synthesizing fatty acids and carotenoids. The high yeast growth (10.28 g/L) was obtained, along with substantial carotenoid (0.98 mg/L) and lipid (1.34 g/L) production. Silva[31] improved carotenoid production in a stirred-tank bioreactor with magnetic field application, finding that *Phaffia rhodozyma* produced sufficient carotenoids to support high carotenogenesis. Applying a magnetic field successfully enhanced astaxanthin and β-carotene production by 22.9% and 8.6%, respectively. Additionally, the astaxanthin and β-carotene recoveries of several protic ionic liquids (PILs) based on ammonium were enhanced by lengthening their anion alkyl chain length. Also, Jiang[32] demonstrated the potential of Jerusalem artichoke extract in *P. rhodozyma* cultivation to produce astaxanthin without the need for acidic or enzymatic inulin hydrolysis. The substrate-feedback fed-batch fermentation was conducted in submerge fermenter. The carotenoid concentration was 982.50 mg/L and yield was13.30 mg/g. The highest dry cell mass of 83.60 g/L was achieved with the optimized medium components.

In the present study, the physical elements worked together to help the cultural conditions reflect their surrounding environment. Various sources of carbon and nitrogen elements were optimized that resulted in the highest cell mass from *Occultifur* sp. M2004. The results indicate that yeast growth and metabolite accumulation in its cells may be supported by using YG medium as the sole nutrient source. However, to produce large amounts of carotenoids, future studies should examine whether new raw materials are suitable for use as a cultivation medium for red yeast. Successful utilization of such byproducts would enable the manufacture of these valuable compounds at a lower cost and with less environmental impact. Additionally, it is interesting

to investigate carotenoids involving genes found in red yeast genetic elements.

Carotenoids Characterization

The FT-IR spectrum of the pigment that was extracted from red yeast displayed similarities with beta-carotene and astaxanthin or another carotenoid in the peak patterns (Figure 1). The investigation of the sample solution illustrated the stretching of several functional groups with peaks at 969, 1051, 1377, 1462, 1738, 2853, and 2923 cm^{-1}. The spectrum of the pigment exhibited peaks at 2923 and 2853 cm^{-1} for the asymmetric and symmetric stretching vibrations of the CH_2, respectively, and 969 cm^{-1} for the trans conjugated alkene –CH=CH– out-of-plane deformation mode. The shift of the C=O peak from 1738 cm^{-1} to a lower frequency due to the conjugated system and the hydrogen bonds in the dimer form may be induced by a high peak of astaxanthin at 1650 cm^{-1}.[33] Regarding 1462 cm^{-1}, the bands correspond to the bending and vibration of methylene –CH_2 (scissoring), seen in the beta-carotene standard at a peak height of 1450.68 cm^{-1}.[34] A peak located at 1385 cm^{-1} related to the C-H bending.6 The red pigment's identification as a carotene in a trans form was validated by spectrum peaks. Operating in the mid-infrared spectrum (4000 to 400 cm^{-1}), FTIR is a useful tool for quantitative investigation of fats, oils, and palm carotene. In addition to detecting unique functional groups like C=C=C and "cross epoxides" that are difficult to identify by conventional techniques like 1H-NMR, it may assign bands to functional groups including CH3, CH2, C=C, C=O, and OH.[34]

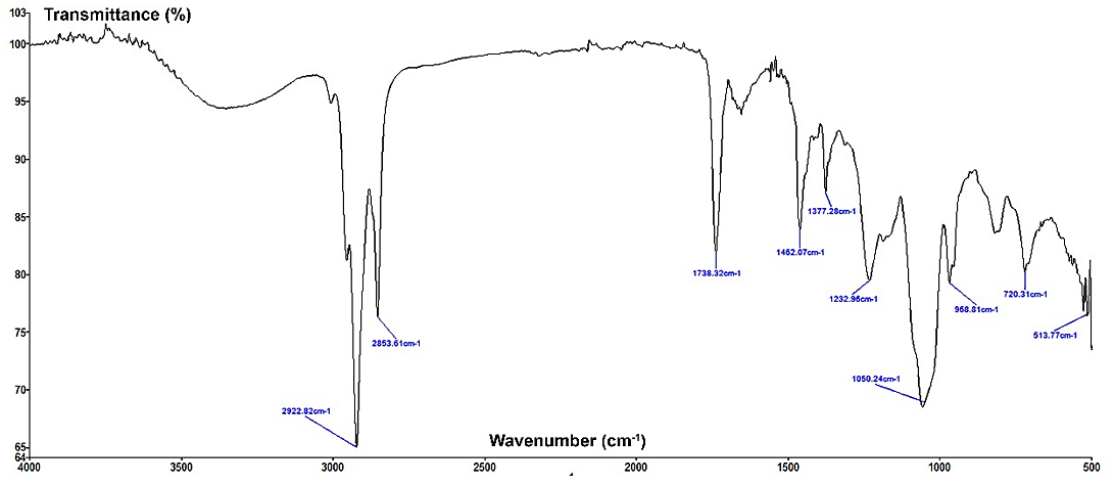

Fig.1: FT-IR spectrum of crude carotenoids extracted from *Occultifur* sp. M2004

In the chromatographic circumstances that the UHPLC analysis employed, it was found that the supernatant extracted from yeast culture and red-pigment extract were identified as β-carotene. The mass spectra of the yeast supernatant and red-pigment extract correspond to signals of β-carotene (537.7 m/z) and astaxanthin (597.6 m/z), but the confirmation of target analytes was based on the retention time (Figure 2,3). By comparing the mass spectra with fragment-ion abundances or publicly available mass spectra databases, carotenoid identification by LC-MS can be easily confirmed. It is possible to clearly identify even unknown carotenoids and isoprenoid quinones with LC-MS, in contrast to UV-Vis spectrophotometry. When combined with online photodiode-array or UV-Vis detectors, information from LC-MS/MS can be useful in identifying unknown peaks. Additionally, due to the numerous isomers, structurally related molecules, and metabolites, chromatograms are typically quite complex.[2] To increase carotenoid productivity, it is crucial to develop rapid carotenoid detection technologies that can provide accurate results in the shortest amount of time. This will allow for the adjustment of operational growth conditions. Each of these carotenoids should have transitions that are unique to it so that it can be distinguished and measured.

All photosynthetic organisms, as well as many non-photosynthetic bacteria and fungi, produced carotenoids, which are naturally occurring colors found in many fruits and vegetables. These are liposoluble tetraterpenes that are produced by condensation of isoprenyl units, which combine to create conjugated double bonds that make up a system of chromophores. The two main classes of naturally occurring carotenoids are (I) xanthophylls, which are oxygenated derivatives of carotenes (violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, and lutein), and (II) carotenes, which are hydrocarbons that are either linear or cyclized at one or both ends of the molecule (α-carotene and β-carotene).

According to the research of Saha[6], β-carotene was identified as the produced pigment using FTIR analysis. This carotene was produced by *Sporidiobolus pararoseus*, a newly isolated yeast. The spectrum of the carotenoid extract exhibited transmission peaks at 965, 1,385, 1,635, and 2,920 cm^{-1} in agreement with peaks exhibited by the β-carotene standard. Peaks from these spectra confirmed the identification of the extracted carotenoid as β-carotene in a trans configuration. Nimsi[19] revealed that the red pigment yeast, *Rhodotorula mucilaginosa* PV 8 produced β-carotene that was characterized by thin layer chromatography (TLC) and FT-IR. This β-carotene demonstrated strong antioxidant activity. Moreover, the carotenoid displayed promising antibacterial activity against multidrug-resistant organisms, including *Aeromonas* sp. and *Vibrio* sp. The β-carotene from PV 8 exhibited the ability to transfer its vibrant color to various food products, maintaining color stability even under varied conditions.

In this present study, the identification of the extracted pigment revealed that the promising yeast isolate M2004, which produced pigment, was able to synthesize β-carotene. This seemed to suggest that, similar to prior research, this pigment could be used in food and medicine. In order to increase the possibility of using the extract from the isolate M2004 as dietary supplements or in any food product, its capacity to inhibit collagenase and elastase was evaluated.

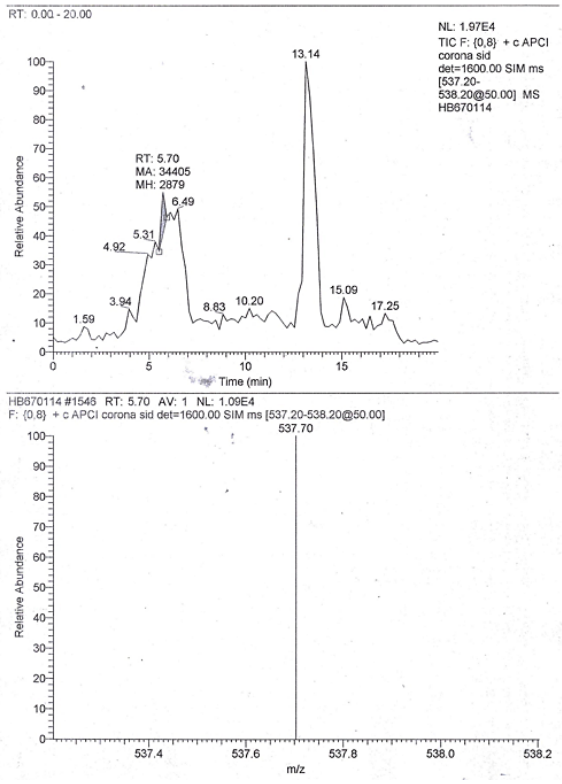

Fig.2: Chromatogram of LC-MS separation of carotenoids extracted from *Occultifur* sp. M2004.

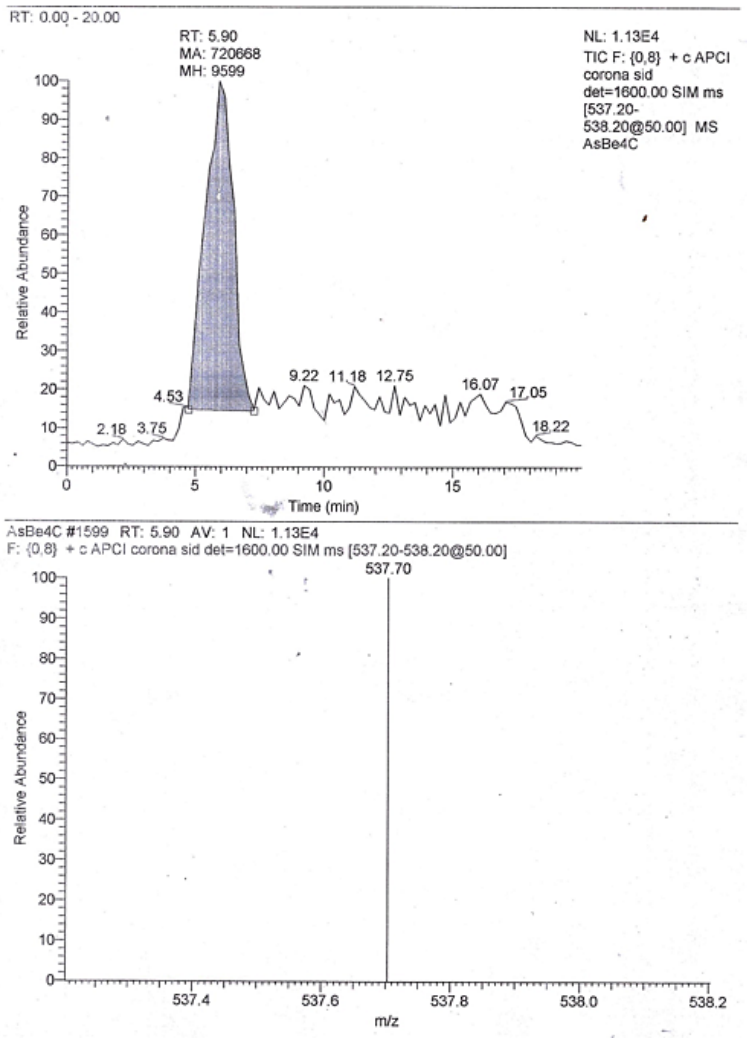

Fig.3: Chromatogram of LC-MS separation of β-carotene standard showing the mass spectra obtained from a total ion chromatogram for β-carotene

Collagenase and Elastase Inhibitory Activity

The inhibitory activities of carotenoid extracts against enzymes involved in wrinkle formation, specifically collagenase and elastase, were reported as the half maximal inhibitory concentration (IC50). Carotenoids extracts showed inhibitory effect against both collagenase and elastase, with IC50 values of 2.61 and 100.16 µg/mL, respectively.

Skin aging is an inevitable process that is of special concern, particularly for facial skin. Sunlight exposure is believed to be the primary cause of photoaging, which leads to the premature appearance of aging on the skin. Photoaging triggers extrinsic ROS generation in cells, and excessive ROS production can lead to lipid peroxidation, DNA damage, and eventually cell damage and death. Antioxidant activity that mitigates ROS production may help delay the onset of skin aging issues.[17]

Several reports have indicated that various plant extracts exhibit anti-wrinkle activities. Examples include *Clitoria ternatea, Ixora coccinea, Triglochin maritimum, Argusia sibirica, Artemisia princeps, Rosa rugosa, Chenopodium glaucum, Peucedanum japonicum,* and *Curculigo latifolia.*[35,36,37] However, limited information is publicly available about collagenase and elastase inhibitors derived from carotenoids. Most studies frequently demonstrate the antioxidant properties of carotenoids, including

beta-carotene and astaxanthin. Interestingly, the associations between the antioxidant activity of each *C. latifolia* extract and elastase inhibition were discovered using Pearson correlation statistical analysis. The findings demonstrate a correlation between elastase inhibition and each extract's antioxidant activity.[37]

The current study revealed that carotenoids extracted from yeast can inhibit collagenase and elastase. Further research should be conducted, including advanced tests such as cell culture testing and in silico studies using molecular docking, to validate these activities.

Conclusion

The research concludes that the pigments extract from *Occultifur* sp. M2004 have potential applications as pharmaceuticals due to their anti-collagenase and anti-elastase properties. The optimum conditions for yeast growth and carotenoid production were microbial cultivation in YG medium at pH 6, at 25 °C with shaking150 rpm for 7 d. Carotenoids obtained from red yeast were identified as beta-carotene using FT-IR and LC-MS analysis. This newly isolated red yeast strain shows promise for carotenoid production and has potential uses in emerging worldwide industries, including pharmaceuticals, medicals, functional food, pet food, and cosmeceuticals. Furthermore, the antibiotic susceptibility test was acknowledged for its demonstrated health benefits and extensive history of safe use. This yeast produced pigments that could be useful in industry, from natural colors to possible pharmaceuticals. Research and investigations will surely uncover new environmentally friendly solutions as we explore the potential of using yeast pigments obtained from native Thai bees. This will ultimately lead to the sustainable advancement of biotechnological applications. Further research should be performed on a number of aspects, including antioxidant activity, antibacterial activity, anti-tyrosinase activity, eco-friendly pigment extraction, and food formulations that include yeast pigment in order to develop carotenoids for nutraceuticals and added-value functional food applications.

Acknowledgement

The author would like to thank Kasetsart University, Kamphaeng Saen Campus and the Research Promotion and Technology Transfer Center (RPTTC), Faculty of Liberal Arts and Science, Kasetsart University for granting the research work.

Funding Sources

The research was funded by Kasetsart University, Kamphaeng Saen Campus (Grant number: KPS-RDI 2021-011) and the Research Promotion and Technology Transfer Center (Grant year 2023, Grant number: RPTTC.213/2566), Faculty of Liberal Arts and Science, Kasetsart University.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

The animal study protocol was approved by the Animal Care and Use Committee, Silpakorn University, Thailand (Project approval number 8603.16/0328).

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Permission to reproduce material from other

Not applicable.

Clinical Trial Registration

This research does not involve any clinical trials.

Author Contributions

- **Saran Promsai:** Conceptualization, Methodology, Formal Analysis, Validation, Supervision, Project Administration, Writing-Original Draft, Review and Editing.
- **Yaowanoot Promnuan:** Methodology, Formal Analysis, Writing-Review and Editing.
- **Sujinan Meelai:** Methodology, Formal analysis, Writing-Review and Editing.

References

1. Jalgaonkar K., Mahawar M. K., Bibwe B., Nath P., Girjal S. Neutraceuticals and Functional Foods. In: Chakraborty I., Paul P. K., Mani A., Tiwary A. K., Prasad K. Trends and Prospects in Processing of Horticultural Crops. Today & Tomorrow's Printers and Publishers, New Delhi; 2019; 231-250. https://krishi.icar.gov.in/jspui/bitstream/123456789/71744/1/FINAL-231-250.pdf. Accessed on August 15, 2024.
2. Mannazzu I., Landolfo S., da Silva T. L., Buzzini P. Red Yeasts and Carotenoid Production: Outlining a Future for Non-conventional Yeasts of Biotechnological Interest. *World J Microbiol Biotechnol.* 2015; DOI: https://dx.doi.org/10.1007/s11274-015-1927-x
3. Sadighara P., Saghafi M., Erfanmanesh A., Mahdaviyekta M. Antioxidant Activity and Properties of Outer Shell Pistachios in Different Temperature of Cooking. *Der Pharmacia Lettre.* 2016; 8(12):263-266.
4. Tapeiro H., Townsend D. M., Tew K. D. The Role of Carotenoids in the Prevention of Human Pathologies. *Biomed Pharmacother.* 2004; 58:100-110. DOI: https://dx.doi.org/10.1016/j.biopha.2003.12.006
5. Tanumihardjo S. A. Factor Influencing the Conversion of Carotenoids to Retinol: Bioavailability to Bioconversion to Bioefficacy. *Int J Vitam Nutr Res.* 2002; 72:40-45. DOI: https://dx.doi.org/10.1024/0300-9831.72.1.40
6. Saha N., Samanta A. K., Chaudhuri S., Dutta D. Characterization and Antioxidant Potential of a Carotenoid from a Newly Isolated Yeast. *Food Sci Biotechnol*. 2015; 24(1):117-124. DOI: https://dx.doi.org/10.1007/s10068-015-0017-z
7. Rasmus P., Kozłowska E. Antioxidant and Anti-Inflammatory Effects of Carotenoids in Mood Disorders: An Overview. *Antioxidants.* 2023; 12:676. DOI: https://doi.org/10.3390/antiox12030676
8. Jomova K., Valko M. Health Protective Effects of Carotenoids and Their Interactions with Other Biological Antioxidants. *Eur J Med Chem.* 2013; 70:102-110. DOI: https://doi.org/10.1016/j.ejmech.2013.09.054
9. Saini R. K., Nile S. H., Park W. Carotenoids from Fruits and Vegetables: Chemistry, Analysis, Occurrence, Bioavailability and Biological Activities. *Food Res Int*. 2015; 76,735-750. DOI: https://doi.org/10.1016/j.foodres.2015.07.047
10. Pechinskii S. V., Kuregyan A. G. Molecular-biological Problems of Drug Design and Mechanism of Drug Action. *Pharmaceut Chem J.* 2014: 47:509-513.
11. Heber D., Lu Q. Y. Overview of Mechanisms of Action of Lycopene. *Exp Biol Med.* 2002; 227, 920-923. DOI: https://doi.org/10.1177/153537020222701013
12. Kim Y. S., Lee H. A., Lim J. Y., Kim Y., Jung C. H., Yoo S. H., Kim Y. Carotene Inhibits Neuroblastoma Cell Invasion and Metastasis In Vitro and In Vivo by Decreasing Level of Hypoxia-inducible Factor-1. *J Nutr Biochem.* 2014; 25:655-664. DOI: https://doi.org/10.1016/j.jnutbio.2014.02.006
13. Guo L., Zhu H., Lin C., Che J., Tian X., Han S., Zhao H., Zhu Y., Mao D. Associations Between Antioxidant Vitamins and the Risk of Invasive Cervical Cancer in Chinese Women: A Case-Control Study. *Sci Rep.* 2015; 5:13607. DOI: https://doi.org/10.1038/srep13607
14. Karppi J., Kurl S., Laukkanen J. A., Kauhanen J. Serum Betacarotene in Relation to Risk of Prostate Cancer: The Kuopio Ischaemic Heart Disease Risk Factor Study. *Nutr Cancer.* 2012; 64:361-367. DOI: https://doi.org/ 10.1080/01635581.2012.658949
15. Park J. S., Chyun J. H., Kim Y. K., Line L. L., Chew B. P. Astaxanthin Decreased Oxidative Stress and Inflammation and Enhanced Immune Response in Humans. *Nutr Metab.* 2010; 7:18. DOI: https://doi.org/10.1186/1743-7075-7-18
16. El-Agamey A., Lowe G. M., McGarvey D. J., Mortensen A., Phillip D. M., Truscott T. G., Young A. J. Carotenoid Radical Chemistry and Antioxidant/pro-oxidant Properties. *Arch Biochem Biophys.* 2004; 430:37-48. DOI: https://doi.org/10.1016/j.abb.2004.03.007

17. Jiratchayamaethasakul C., Ding Y., Hwang O., Im S. -T., Jang Y., Myung S. -W., Lee J. M., Kim H. -S., Ko S. -C., Lee S. -H. In vitro Screening of Elastase, Collagenase, Hyaluronidase, and Tyrosinase Inhibitory and Antioxidant Activities of 22 Halophyte Plant Extracts for Novel Cosmeceuticals. Fish *Aquatic Sci.* 2020; 23:6. DOI: https://dx.doi.org/10.1186/s41240-020-00149-8
18. Kurtzman C. P., Fell J. W., Boekhout T. Definition, Classification and Nomenclature of the Yeasts. In: The Yeasts: a taxonomic study. 5th edition. Elsevier; 2011.
19. Nimsi K. A., Manjusha K., Farzana M. N. Diversity, Distribution, and Bioprospecting Potentials of Carotenogenic Yeast from Mangrove Ecosystem. *Arch Microbiol.* 2024; 206:189. DOI: https://dx.doi.org/10.1007/s00203-024-03879-8
20. Villegas-Mèndez M. A., Papadaki A., Pateraki C., Balagurusamy N., Montanez J., Koutinas A. A., Morales-Oyervides L. Fed-batch Bioprocess Development for Astaxanthin Production by Xanthophyllomyces dendrorhous Based on the Utilization of Prosopis sp. *Pods Extract.* 2021; 166:107844. DOI: https://doi.org/10.1016/j.bej.2020.107844
21. Saini R. K., Keum Y. S. Microbial Platforms to Produce Commercially Vital Carotenoids at Industrial Scale: an Updated Review of Critical Issues. *J Ind Microbiol Biotechnol.* 2018; 49:657-674. DOI: https://doi.org/10.1007/s10295-018-2104-7
22. Xiao A., Jiang X., Ni H., Yang Q., Cai H. Study on the Relationship Between Intracellular Metabolites and Astaxanthin Accumulation During Phaffia rhodozyma Fermentation. *Electron J Biotechnol.* 2015; 18:148-153. DOI: https://doi.org/10.1016/j.ejbt.2015.02.002
23. Fakhri S., Abbaszadeh F., Dargahi L., Jorjani M. Astaxanthin: a Mechanistic Review on its Biological Activities and Health Benefits, *Pharmacol Res.* 2018; 136:1-20. DOI: https://doi.org/10.1016/j.phrs.2018.08.012
24. Ribeiro J. E. S., da Silva Sant'Ana A. M., Martini M., Sorce C., Andreucci A., de Melo D. J. N., da Silva F. L. H. Rhodotorula glutinis Cultivation on Cassava Wastewater for Carotenoids and Fatty Acids Generation. *Biocatal Agric Biotechnol.* 2019; 22:101419.
25. Khumdee P., Hematulin P., Yansombat J., Mungkornkaew N., Kabir S. M. L., Samosornsuk W., Samosornsuk S. Species Characterization and Antifungal Susceptibility Profile of Yeast Isolates from Blood Cultures of Fungemic Patients in Thammasat University Hospital, Thailand. *J Assoc Med Sci.* 2022; 55(3):44-50.
26. Górzyńska A., Kondracka K., Korzeniowska-Kowal A., Nawrot U. Antifungal Susceptibility of Saccharomyces cerevisiae Isolated from Clinical Specimens. *Pathogens.* 2024; 13:248. DOI: https://dx.doi.org/10.3390/pathogens13030248
27. Georgieva R., Yocheva L., Tserovska L., Zhelezova G., Stefanova N., Atanasova A., Danguleva A., Ivanova G., Karapetkov N., Rumyan N., Karaivanov E. Antimicrobial Activity and Antibiotic Susceptibility of Lactobacillus and Bifidobacterium spp. Intended for Use as Starter and Probiotic Cultures. *Biotechnol Biotechnol Equip.* 2015; 9(1):84-91. DOI: https://dx.doi.org/10.1080/13102818.2014.987450
28. de Freitas A. R., Baeza L. C., Faria M. G. I., Dota K. F. D., Martínez P. G., Svidzinski T. I. E. Yeasts Isolated from Nosocomial Urinary Infections: Antifungal Susceptibility and Biofilm Production. *Rev Iberoam Micol.* 2014; 31(2):104-108. DOI: https://dx.doi.org/10.1016/j.riam.2013.06.004
29. Khunnamwong P., Surussawadee J., Jindamorakot S., Ribeiro J. R. A., Hagler A. N., Limtong S. *Occultifur* tropicalis f.a., sp. nov., a Novel Cystobasidiomycetous Yeast Species Isolated from Tropical Regions. *Int J Syst Evol Microbiol.* 2015; 65:1578-1582. DOI: https://dx.doi.org/10.1099/ijs.0.000140
30. Sampaio J. P., Oberwinkler F. *Occultifur* Oberwinkler (1990). In: Kurtzman C. P., Fell J. W., Boekhout T. (Ed.). The Yeasts, a Taxonomic Study, 5th edition. Elsevier, Amsterdam, 2011; 1515-1518.
31. Silva P. G. P., Mussagy C. U., de Azevedo Lima C., Santos-Ebinuma V. C., de Medeiros Burkert J. F., Santos L. O. Sustainable Approach to Recover β-carotene and Astaxanthin from Phaffia rhodozyma Grown in a Stirred-tank Bioreactor under the Influence of Magnetic Fields. *Bioresour Technol.* 2023; 390:129906. DOI: https://doi.

org/10.1016/j.biortech.2023.129906

32. Jiang G. -L., Zhou L. -Y., Wang Y. -T., Zhu M. -J. Astaxanthin from Jerusalem Artichoke: Production by Fed-batch Fermentation Using Phaffia rhodozyma and Application in Cosmetics. *Process Biochem.* 2017; 63:16-25. DOI: https://dx.doi.org/10.1016/j.procbio.2017.08.013
33. Liu J., Chen X., Yang X., Chen J., Qi Z. Astaxanthin Accumulation in Haematococcus pluvialis Observed Through Fourier-transform Infrared Microspectroscopy Imaging. *J Mol Struct*. 2019; 1182:119-122.
34. Trivedi N., Tandon S., Dubey A. Fourier Transform Infrared Spectroscopy (FTIR) Profiling of Red Pigment Produced by Bacillus subtilis PD5. *Afr J Biotechnol.* 2017; 16(27):1507-1512. DOI: https://dx.doi.org/10.5897/AJB2017.15959
35. Lertkanchanasak S., Khanaassanarom T., Boonsitte P., Chaikraveephand R., Keebut S., Thawai C., Tadtong S. Collagenase, Elastase, Hyaluronidase Inhibitory and Antioxidant Activities of Yellow Ixora coccinea L. Flowers Extract. *Thai Pharm Health Sci J.* 2024; 19(1):20-27.
36. Suksomboon T., Sornchaithawatwong C., Tadtong T. Phytochemical Study and Anti-wrinkle Activity of Blue Clitoria ternatea L. Petal Extract. *Thai Pharm Health Sci J.* 2021; 16(3):234-238.
37. Nur S., Setiawan H., Hanafi M., Elya B. Phytochemical Composition, Antioxidant, *In vitro* and In silico Studies of Active Compounds of Curculigo latifolia Extracts as Promising Elastase Inhibitor. *Saudi J Biol Sci* 2023; 30:103716. DOI: https://dx.doi.org/10.1016/j.sjbs.2023.103716