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SUSTAINABLE PRODUCTION OF LUTEIN FROM MICROALGAE *C. vulgaris*: ISOLATION, CHARACTERIZATION, AND ANTIOXIDANT POTENTIAL

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Abstract

Lutein is a carotenoid pigment with significant antioxidant potential and health benefits, including the prevention of eye diseases, protection of the skin from UV damage, and reduction of cancer risk. Currently, marigold flowers are the primary source of lutein production. However, its production is limited by seasonal and climatic dependencies, high labor costs and extensive land use. *Chlorella vulgaris* is a viable alternative with higher growth rates, reduced land and water requirements, and year-round availability. This study aimed to isolate lutein from *C. vulgaris* and evaluate its characteristics and antioxidant properties. The method used was a descriptive experimental design consisting of several stages: (1) maceration, (2) saponification, (3) identification, (4) isolation, (5) characterization of lutein isolation results, and (6) antioxidant activity assessment. The results showed that maceration yielded 8.01% lutein, whereas saponification yielded 24.37%. Column chromatography identified the third fraction as lutein, which was confirmed by FTIR analysis, revealing alkenyl, alkyl, alkene, aromatic (C=C), (C−H), and hydroxyl (−OH) groups. The isolate exhibited a yellow value of 66.79 and a hue angle of 88.65, which is consistent with the characteristic color of lutein. Antioxidant testing revealed an IC_{50} value of 62.54 ppm, indicating strong antioxidant activity. In conclusion, *C. vulgaris* is a promising alternative source of lutein with potent antioxidant properties.

Keywords: bioactive compounds, carotenoids, FTIR spectroscopy, IC_{50} value, nutraceuticals

Produksi Lutein Berkelanjutan dari Mikroalga *C. vulgaris*: Isolasi, Karakterisasi, dan Potensi Antioksidan

Abstrak

Lutein merupakan pigmen karotenoid yang memiliki potensi antioksidan tinggi serta manfaat kesehatan, yaitu mencegah penyakit mata, melindungi kulit dari kerusakan akibat sinar UV, dan menurunkan risiko kanker. Selama ini, bunga gemitir menjadi sumber utama lutein, namun produksinya masih terbatas oleh faktor musiman, iklim, biaya tenaga kerja yang tinggi, dan kebutuhan lahan yang luas. *Chlorella vulgaris* menawarkan alternatif yang lebih unggul dengan laju pertumbuhan tinggi, kebutuhan lahan dan air yang lebih rendah, serta ketersediaan sepanjang tahun. Penelitian ini bertujuan untuk mengisolasi lutein dari *C. vulgaris*, serta mengevaluasi karakterisasi dan aktivitas antioksidan dari senyawa hasil isolasi. Penelitian dilakukan menggunakan metode eksperimental deskriptif melalui tahap (1) maserasi, (2) saponifikasi, (3) identifikasi, (4) isolasi, (5) karakterisasi hasil isolasi lutein, dan (6) uji aktivitas antioksidan. Hasil penelitian menunjukkan bahwa proses maserasi menghasilkan rendemen lutein sebesar 8,01%, sedangkan tahap saponifikasi menghasilkan kandungan lutein sebesar 24,37%. Berdasarkan kromatografi kolom, fraksi ketiga teridentifikasi sebagai lutein yang dikonfirmasi melalui analisis FTIR, dengan ditemukannya gugus fungsi alkenil, alkil, alkena, aromatik (C=C), (C−H), dan hidroksil (−OH). Isolat lutein menunjukkan nilai warna kuning sebesar 66,79 dan hue angle sebesar 88,65, sesuai dengan karakteristik warna lutein. Uji antioksidan menunjukkan nilai IC_{50} sebesar 62,54 ppm, yang mengindikasikan aktivitas antioksidan yang

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kuat. Dengan demikian, *C. vulgaris* berpotensi sebagai sumber alternatif lutein yang menjanjikan dengan aktivitas antioksidan yang tinggi.

Kata kunci: bioaktivitas, maserasi, mikroalga, *ultrasound-assisted extraction*

INTRODUCTION

The global demand for natural antioxidants is rapidly increasing, driven by growing consumer awareness regarding health and wellness, as well as concerns about the potential adverse effects of synthetic additives in food products. Natural antioxidants, such as carotenoids, phenolics, and flavonoids, are increasingly valued not only for their role in food preservation but also for their health benefits, including protection against oxidative stress and reduction of chronic disease risk (Atta *et al*., 2017; Berkani *et al*., 2021; Chamidah *et al*., 2024; Rahman *et al*., 2025). Among these compounds, lutein, a carotenoid pigment belonging to the xanthophyll group, has attracted considerable attention because of its unique biological functions. Lutein is a carotenoid pigment with significant antioxidant potential and health benefits, including the prevention of eye diseases, protection of the skin from UV damage, and reduction of cancer risk. One of its key features is its selective accumulation in the macula and lens of the human eye, which is associated with the prevention of age-related macular degeneration (AMD), cataracts, and other oxidative stress-related disorders (Serra *et al*., 2021; Muszyńska *et al*., 2017).

Lutein cannot be synthesized endogenously by the human body and must be obtained from dietary sources. Although animal-derived foods, such as fish and egg yolk, contain lutein, plant sources, including carrots, peppers, and corn, are more common. Additionally, photosynthetic organisms, such as macroalgae and microalgae, are natural producers of lutein (Alotaibi *et al*., 2021; Iwamoto *et al*., 2024). The largest industrial source of lutein is marigold flowers (*Tagetes erecta*); however, their commercial production is challenged by seasonal fluctuations, land and water requirements, and high extraction and purification costs, often yielding lutein in oleoresin forms requiring further processing (Molino *et al*., 2018; Fábryová *et al*., 2021; Wu *et al*., 2024).

In contrast, microalgae, particularly *Chlorella vulgaris*, offer compelling advantages as sustainable lutein producers, including faster growth rates, lower resource consumption, absence of seasonal limitations, and year-round cultivation potential (Ru *et al*., 2020; Lin *et al*., 2015). Studies have reported that *Chorella vulgaris* can accumulate significant concentrations of lutein up to 1.2% of dry weight, with carotenoid profiles dominated by lutein, positioning it as a promising alternative to traditional sources (Zheng *et al*., 2022; Bazarnova *et al*., 2022). Moreover, preliminary evidence suggests that lutein from *Chlorella vulgaris* may demonstrate comparable or superior antioxidant activity to that of lutein derived from marigold flowers (Serra *et al*., 2021).

Despite the promising attributes of *Chlorella vulgaris* as a lutein source, a substantial research gap remains in developing efficient and scalable extraction and purification methodologies tailored to this microalga. Most existing studies still emphasize plant-based lutein extraction or provide only limited characterization of microalgal lutein, leaving questions about purity, spectral profile, and antioxidant efficacy largely unanswered, which are factors that impede industrial and commercial uptake. Compounding this gap, there is currently no commercial production of lutein derived from *Chlorella vulgaris*, and investigations into its lutein content and functionality are still scarce (Fábryová *et al*., 2021). Consequently, rigorous investigations are required to refine extraction techniques and ensure that lutein obtained from *Chlorella vulgaris* meets the highest quality standards. This study aimed to isolate lutein from *Chlorella vulgaris* and evaluate its characteristics and antioxidant potential.

MATERIALS AND METHODS

The materials used in this study included *Chlorella vulgaris* microalgae powder obtained from the Freshwater Fisheries Center of Situbondo, East Java. Other materials

included: n-hexane, acetone, chloroform, isopropanol, NaOH, label paper, Whatman filter paper no. 42, silica gel plate 60 F254 (all analytical grade, Merck Darmstadt, Germany), filter paper (Whatman), silica gel powder 1.07734 (Merck), DPPH = 2,2-diphenyl-1-picrylhydrazyl (Merck), seawater, aluminum foil, salt, plastic wrap, glass wool, and cotton.

The extraction and isolation of lutein in this study were conducted through a series of sequential steps, including (1) maceration, (2) saponification, (3) identification, and (4) isolation, each of which is described in detail below.

Maceration

The extraction method followed the method described by Lasmarito *et al*. (2022), with slight modifications. The *Chlorella vulgaris* powder used in this study was obtained from a commercial supplier to ensure the consistency and quality of the biomass. Extraction was performed using a biomass-to-solvent ratio of 1:10. Thirty grams of commercial *Chlorella vulgaris* powder was dissolved in 300 mL of acetone. The mixture was then macerated for 24 h at room temperature, with stirring every 6 h using a glass spatula to facilitate pigment extraction. After 24 h, the extract was filtered through Whatman filter paper no. 42. The maceration process was repeated up to three times or until the filtrate became clear, indicating maximal pigment removal. The combined filtrates were concentrated using a rotary vacuum evaporator (IKA RV 10, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 100 rpm and 40°C until a viscous crude extract was obtained. The resulting crude extract was immediately transferred to amber glass vials, flushed with nitrogen to minimize oxidation, tightly sealed with PTFE-lined caps, labelled, and stored in the dark at 4°C until further analyses were performed.

Saponification

The crude extract obtained from the maceration process was saponified as described by Susanti *et al*. (2018). Briefly, 15 mL of isopropanol was added to the crude extract, and the mixture was heated at 50 °C for 1 h under continuous stirring with a magnetic stirrer. Subsequently, 22.5 mL of 50% NaOH solution was added at the same temperature and time until a semisolid solution formed. The mixture was then cooled for 4 h, followed by the addition of 37.5 mL of distilled water while stirring with a magnetic stirrer for 1 h until a yellow fraction formed on the surface, indicating the separation of lutein and other carotenoids. The yellow fraction was collected by centrifugation at 5000 rpm for 15 min (Eppendorf 5810 R, Hamburg, Germany). The filtrate was discarded, and the viscous yellow extract adhering to the walls of the centrifuge tube was collected to obtain a concentrated lutein extract. Immediately after recovery, the lutein-rich fraction was transferred to amber glass vials, flushed with high-purity nitrogen to minimize oxidative degradation, sealed with PTFE-lined screw caps, labelled, and stored in the dark at 4°C until further chromatographic purification and analytical assays were conducted.

Identification

Lutein compound identification was confirmed using thin-layer chromatography (TLC) (Migas *et al*., 2020). Crude lutein extract (saponification product) was used to determine the presence of carotenoids. Thin-layer chromatography was performed using silica gel 60 GF254 plates (Merck, Darmstadt, Germany). The solvent used is a mixture of n-hexane: chloroform: acetone (6:2:2). The initial step in the TLC process involved drawing lines on a plate measuring 1.5 cm in width and 6 cm in length, using a 2B pencil. Borderlines of 0.5 cm were marked at the top and bottom of the plates. Thin-layer chromatography analysis was considered successful when distinct spots were obtained, indicating the separation of compounds. The presence of lutein was indicated by a yellow spot on the silica gel plate, and the retention factor (Rf) value of the spot was compared with the reference lutein Rf value. The Rf value can be calculated using the following formula:

$$\text{Rf} = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

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Isolation

Lutein was isolated using the method described by Permatasari (2013). Lutein was isolated using column chromatography with silica gel 60 GF254 as the stationary phase and a mixture of non-polar and polar solvents (n-hexane: chloroform: acetone (6:2:2)) as the mobile phase. Prior to isolation, silica gel 1.07734 was prepared by weighing 40 g of silica gel and drying it for 4 h in an oven at 105°C. The silica gel was then mixed with a solvent mixture of n-hexane: chloroform: acetone (6:2:2) until it formed a slurry. The chromatography column was prepared by inserting glass wool and filter paper at the bottom of the column until compact. The silica gel slurry was then slowly added along the column walls until it filled ¾ of the column height, while tapping the column walls to remove air pockets, and left overnight. The crude lutein extract was dissolved in 1 mL of acetone and slowly introduced into the column. After the extract was absorbed, 10 mL of acetone was gradually added until the compound components were separated. During the separation process, lutein elution was visually detected through the gel, facilitating its collection. The resulting fractions were collected in several vials according to their color.

Characterization of Lutein
Yield calculation

The yield was obtained based on AOAC (2005) from the ratio of the final product weight to the initial material weight. Yield can be calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Final product weight}}{\text{Initial material weight}} \times 100$$

Purity test

Purity tests were conducted following the method described by Kusmiati *et al.* (2018). The obtained fractions were analyzed using TLC to determine the purity of the isolated lutein compound. Thin-layer chromatography was performed according to a previously described identification method. The presence of pure lutein was indicated by a single yellow spot on the silica gel plate. The Retention factor (Rf) value calculation was conducted using the same method as previously described.

Spectral pattern test

The spectral pattern test was conducted following the method described by Limantara *et al.* (2010) using a UV-Visible spectrophotometer. This analysis was performed to determine the light absorption characteristics of the isolated pigment (lutein). The analyzed sample consisted of concentrated pigment obtained from the isolation process and dissolved in acetone. Approximately 30 mL of the pigment solution was transferred to a cuvette and placed in a UV-Visible spectrophotometer for analysis. The results were recorded as maximum absorption peaks formed by the isolated pigment.

Color test

The color test followed Caliskan and Dirim (2016) and was carried out using a colorimeter with the Hunter color system: L* (brightness), a* (redness), and b* (yellowness). The lutein sample was measured using a colorimeter instrument then the L*, a*, and b* values were read 3 times and the average value was calculated.

FTIR test

The structure of the lutein compound was identified following the method of Wei *et al.* (2021) using Fourier Transform Infrared Spectroscopy (FTIR). The sample analyzed was obtained by isolating lutein from the microalgae *Chlorella vulgaris*. The sample used in this analysis was the result of lutein isolation in the form of a concentrated extract that had been dried using nitrogen gas.

Antioxidant test

The antioxidant activity of the lutein extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Cai *et al.*, 2015). The stock solution was prepared by dissolving the isolated lutein sample in ethanol at a concentration of 1000 ppm. Serial solutions were prepared at concentrations of 25, 50, 75, and 100 ppm. Furthermore, 0.2 mL of lutein extract at each concentration was added to 3.8 mL of DPPH solution. The mixture was homogenized and incubated at 37 °C for 30 min. The absorbance of each solution was measured using a UV-Vis

spectrophotometer at wavelengths of 517 nm for lutein and 480 nm for ascorbic acid. The percentage of DPPH free radical inhibition by the sample solution was determined using the following formula:

$$\%\text{Inhibition} = \frac{\text{Blank abs} - \text{Sample abs}}{\text{Blank abs}} \times 100\%$$

After obtaining the % inhibition, the antioxidant activity was expressed as the inhibition concentration (IC_{50}), which is the concentration of the sample that can capture 50% of the DPPH radicals. The smaller the IC_{50} value, the better the antioxidant activity.

Data Analysis

All experimental data were analyzed using Microsoft Excel. The results are expressed as mean ± standard deviation from three independent replicates. Antioxidant activity was assessed based on the IC_{50} value, calculated from the linear regression of DPPH inhibition percentages.

RESULTS AND DISCUSSION

Maceration Results

Maceration aims to extract the active substances and chemical compounds contained in *Chlorella vulgaris*, producing a crude extract as the initial stage of lutein extraction. In this study, acetone was used as the solvent for maceration because of its effectiveness in extracting semi-polar compounds (Kashyap *et al*., 2022). The maceration yield was defined as the percentage ratio of the crude extract weight obtained to the initial dry weight of the *Chlorella vulgaris* powder used in the extraction process. This yield represents the efficiency of the maceration step in recovering the extractable compounds from the biomass.

Subsequently, saponification was performed on the crude extract to isolate lutein by breaking down the esterified compounds. The saponification yield was calculated as the percentage ratio of the weight of the final lutein-containing extract to the weight of the initial crude extract obtained from maceration. This reflects the efficiency of the saponification step in purifying and concentrating lutein from crude extracts. The average yield values for both the maceration and saponification stages were calculated from three replicates to assess the extraction and purification efficiency. The summarized yield results from both stages are listed in Table 1.

The data in Table1 shows that the average crude extract yield using acetone was 8.01%+0.251. This result is higher than that reported by Susanti *et al.* (2018), who obtained a crude lutein extract yield of 5.26% using *Tagetes erecta* L. samples. The yield in this study was also higher than that reported by Noviantari *et al.* (2017), where macroalgae *Sargassum polycystum* macerated using acetone produced a maximum yield of 1.41%. In the maceration process, the use of acetone as a solvent offers advantages in extracting photosynthetic pigments, including lutein, with better efficiency than other solvents (Izanlou *et al*., 2023; Amaro *et al*., 2015). Significant differences in crude extract yields between *Chlorella vulgaris* and other microalgal species can be influenced by various factors, including the type of solvent used, extraction method, and the characteristics of the species itself. Studies have shown that

Table 1 Crude lutein yield from *C. vulgaris* using maceration and saponification methods

Methods	Replication	Final weight (g)	Yield (%)	Average yield (%)	Standard deviation
Alkaloid	1	2.19	7.30	8.01	0.251
	2	2.68	8.93		
	3	2.34	7.80		
Triterpenoid	1	0.450	20.54	24.37	0.126
	2	0.695	25.93		
	3	0.624	26.66		

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more advanced extraction methods, such as ultrasound-assisted extraction, significantly increase lutein yield from *Tagetes erecta*. However, *Chlorella vulgaris* continues to demonstrate strong potential using the conventional maceration method (Prueser *et al*., 2024). Additionally, research also indicates that the cell characteristics and cell wall structure of *Chlorella vulgaris* can affect extraction efficiency, where a more rigid cell wall can reduce yield if not addressed with the appropriate method (Kriechbaum *et al*., 2024).

Saponification Results

The saponification of the crude acetone extract using 50% NaOH aimed to separate the chlorophyll components, fatty acids, and wax esters in the crude extract (Bazarnova *et al*., 2022). Chlorophyll in the crude acetone extract is converted into its derivative, chlorophyllin, which is soluble in the aqueous phase. During saponification, the phytyl ester group is cleaved from the chlorophyll structure, resulting in water-insoluble chlorophyll derivatives (Derrien *et al*., 2019). Water-insoluble lutein accumulates in the organic phase, separating it from chlorophyll (Low *et al*., 2020). The yield results of the saponification stage are presented in Table 1.

Based on the data in Table 1, the average crude lutein yield from the saponification process of *Chlorella vulgaris* reached 24.37%+0.126, equivalent to ± 19.6 mg of lutein per gram of dry *Chlorella vulgaris* microalgal biomass (19.6 mg/g). This result indicates the high efficiency of lutein extraction via saponification. This value is considerably higher than the saponification of *Chlorella vulgaris* microalgal extract reported by Kashyap *et al*. (2022), which was 8.02%. Similarly, Low *et al*. (2020) reported a yield of 11.92 mg/g lutein. The saponification process involves the hydrolysis of carotenoid esters using bases such as NaOH or KOH to separate free lutein from the lipid matrix and other components within microalgae cells. The efficiency of this process is greatly influenced by the concentration of the base used; a base concentration that is too low may not be sufficient to completely break down the carotenoid esters, whereas a concentration that is too high can lead to the degradation of the lutein pigment itself. The high yield obtained reinforces the position of *Chlorella vulgaris* as an efficient alternative source of lutein compared to other sources, especially when combined with appropriate extraction and purification techniques (Wu *et al*., 2024).

Identification Results

Thin-layer chromatography was conducted to identify the compounds in the crude extract after saponification. The results of the identification of the crude lutein extract using the TLC method are shown in Figure 1.

Thin-layer chromatography analysis was performed to identify the compounds present in the crude extract after saponification. The results, depicted in Figure 1, revealed the presence of two distinct yellow spots on

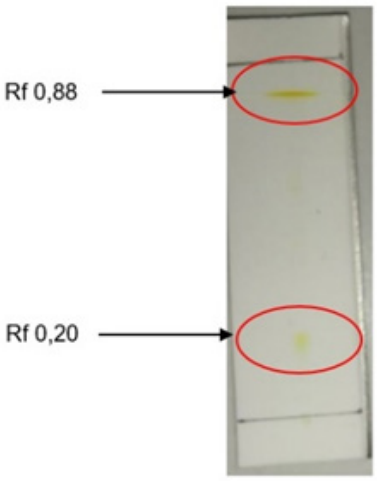

Figure 1 Results of lutein compound identification using the TLC method

the silica gel plate. The first spot exhibited an Rf value of 0.20, whereas the second spot exhibited an Rf value of 0.88. The Rf value of the first yellow spot was identical to that reported by Jaime *et al.* (2019), who reported an Rf value of 0.2 for lutein from the microalgae *Spirulina* sp. Similarly, Iyer *et al.* (2015) showed an Rf value of 0.23 for lutein from the microalgae Coleastrella sp.. These consistent Rf values suggest that the first spot corresponds to lutein. Kusmiati *et al.* (2018) stated that the Rf range of lutein is between 0.2 and 0.8, which is the optimal separation range. Therefore, the Rf values of the resulting spots were in accordance with those of similar studies.

The second spot, with an Rf value of 0.88, was suspected to be β-carotene. This is supported by the findings of Hynstova *et al.* (2018), who identified beta-carotene in the microalgae *Chlorella* sp. at an Rf of 0.86. Kusbandari *et al.* (2017), also identified beta-carotene at an Rf value of 0.86-0.87. Kondororik *et al.* (2016), identified beta-carotene compounds in the Rf range of 0.87-0.93. Thus, it can be concluded that the crude lutein extract after saponification still contains more than one pigment, thus requiring separation using column chromatography.

Isolation Results

Lutein was isolated using thin-layer chromatography (TLC). This study used silica gel as the stationary phase and n-hexane:chloroform:acetone as the mobile phase with a ratio of 6:2:2. This is supported by research Kusmiati *et al.* (2018), where the solvent that can be used to isolate lutein is n-hexane:chloroform:acetone with a ratio of 6:2:2. The isolation of lutein compounds using column chromatography is shown in Figure 2.

Column chromatography produces separation results with the formation of colored bands based on polarity. Based on the research conducted, three different color groups were obtained, as shown in Figure 3: fraction 1 has a bright yellow color, fraction 2 is clear, and fraction 3 is yellow. The fractions obtained need to be tested for spectral patterns and thin layer chromatography to prove the presence of lutein compounds and to determine the purity of the lutein compounds produced.

Spectral Pattern Results

Spectral pattern analysis was conducted to identify lutein and confirm that the obtained compound was indeed lutein. The spectral pattern of fraction 3 from the isolation results

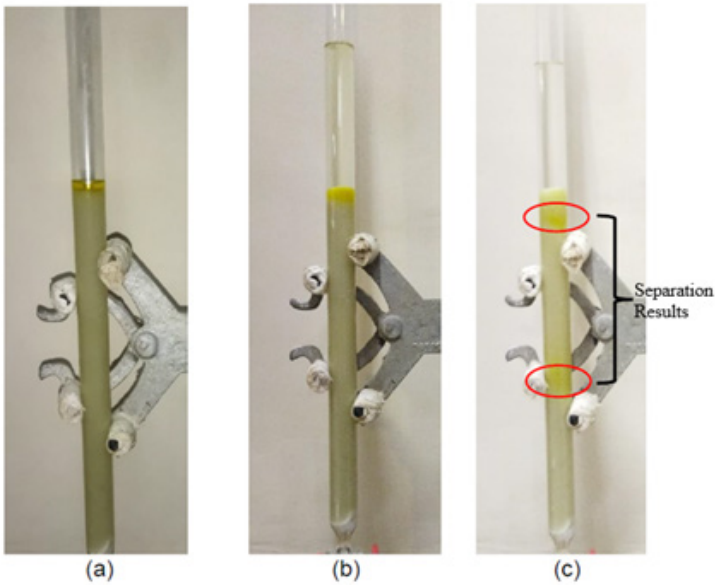

Figure 2 (a) Crude lutein extract is loaded into the chromatography column; (b) The solvent mobile phase is loaded into the chromatography column; (c) Results of compound separation with chromatography column

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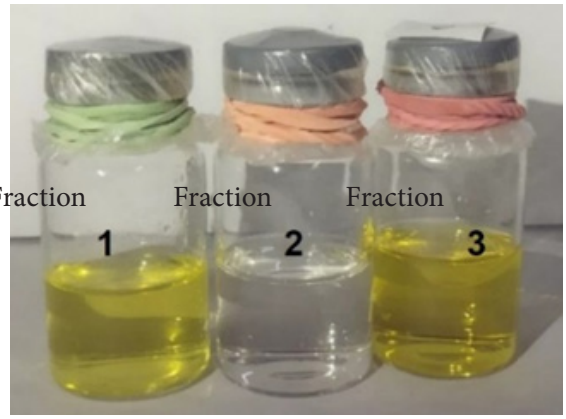

Figure 3 Results of isolation by column chromatography method

was identified using UV-Vis spectroscopy. The spectral pattern results of fraction 3 are shown in Figure 4 and were compared with the spectral patterns from the literature.

The spectral pattern of fraction 3 showed a maximum absorption at 445 nm with an absorbance value of 0.885, along with absorptions at the left and right shoulders at 423 nm and 473 nm, respectively. These results indicate that fraction 3 contains lutein. This is supported by Bernstein *et al.* (2016), who stated that lutein has maximum absorption at 445 nm. These results are similar to those of Kurniawan *et al.* (2019), who obtained lutein with three peak absorptions: a maximum absorption at 445 nm and absorptions at the right and left shoulders at 423 and 473 nm, respectively. These findings are consistent with those of Gayathri *et al.* (2016), who reported that lutein pigment isolates exhibit an absorbance peak at 445 nm. Based on the above analysis, fraction 3 contains lutein compound.

Purity Results

The purity of the isolated lutein was further assessed using TLC. The fraction targeted for purity testing was fraction 3, which was identified as containing lutein based on the spectral pattern test. The stationary and mobile phases used were the same as those in the first TLC test. The results of the TLC test are shown in Figure 5.

The results of the purity test using TLC showed that only one yellow spot pattern was formed on the silica gel plate. This is in accordance with Kusmiati *et al.* (2018), who stated that the spots produced by lutein pigments are yellow. Lutein spots can be directly identified visually on silica gel plates. The presence of a single spot on the silica gel plate indicated that the isolate was qualitatively pure (Boonnoun *et al.*, 2012). These results indicate that the isolated lutein was qualitatively pure. The Rf value obtained for the lutein isolate in this study was 0.2. These results are consistent with those of Jaime *et al.* (2019), who reported a Rf value of 0.2 for

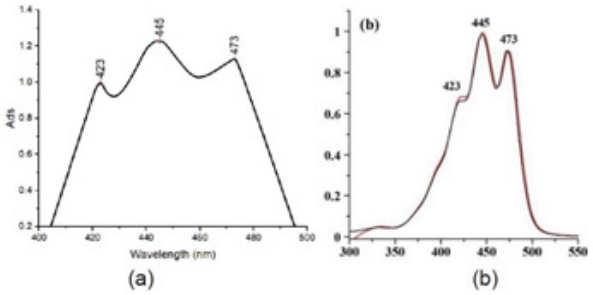

Figure 4 (a) Spectral pattern of fraction 3 (b) Spectral pattern of isolated lutein with chromatography column according to Kurniawan *et al.* (2019)

Figure 5 Identification of lutein compound purity with TLC

lutein extracted from the microalgae *Spirulina* sp. The Rf range of lutein ranges from 0.2-0.8 where this range is the maximum separation range.

Color Test Results

The color quality test of lutein isolate using a colorimeter yielded average values of brightness (L*) of 62.67, (a*) value indicating red tendency of -1.57, and (b*) value indicating yellow tendency of 66.79. These data show that the (a*) value was lower than the (b*) value, indicating that the sample tended to be bright yellow in color (Chen *et al*., 2016). Based on the obtained (a*) and (b*) values, hue angle calculations were performed to determine the color range of the samples. The hue angle obtained for the lutein isolate was 88.65°. This value falls within the chromaticity color range of 54°–90°, which is classified as yellow-red. Lutein is a pigment that, in its pure state, has a color ranging from yellow to orange, depending on its concentration in a sample (Niu *et al*., 2020). Therefore, it can be concluded that the obtained isolate indeed contains lutein, as indicated by the yellow-red color. Colorimetric analysis offers a reliable approach for quantifying lutein concentrations, enabling researchers to evaluate the efficacy of different extraction techniques and optimize microalgal growth conditions (Yin *et al*., 2023; Patel *et al*., 2022). This methodology not only promotes sustainable lutein production but also deepens the understanding of carotenoid biosynthesis pathways in microalgae (Patel *et al*., 2022, Fuentes *et al*., 2020).

FTIR Test Results

Functional group analysis was performed using a spectrophotometer as a follow-up analysis of the lutein pigment isolation results from *Chlorella vulgaris* to determine the characteristic groups present in the sample. The FTIR results of lutein compared with those in the literature are shown in Figure 6.

The blue pattern shows the FTIR results of lutein isolated from *Chlorella vulgaris* microalgae, while the orange pattern shows the FTIR results of lutein isolated from Prabhu *et al*. (2015). Based on the results above, the spectrum pattern of the lutein compound isolated from the microalgae *Chlorella vulgaris* has absorption patterns similar to the lutein compound isolation results in the study by Prabhu *et al*. (2015). The lutein standard has functional groups, namely alkenyl, alkyl, alkene, aromatic, and hydroxyl groups, which correspond to the lutein formula. In the lutein isolation results from *Chlorella vulgaris* microalgae, hydroxyl groups (–OH) were detected at wavelength 3293.12 cm^{-1}; C–H groups at 1384.85 cm^{-1}, 1466.15 cm^{-1}, 2979.36 cm^{-1}, and 2939.42 cm^{-1}, which belong to Alkene 2800-3000 cm-1 and Alkyl 1340-1470 cm^{-1}; C=C groups at absorption 1640.14 cm^{-1}; alkenyl groups at absorption 811.51 cm^{-1} and 944.15 cm^{-1} which fall within the alkenyl group range of 675-995 cm^{-1}.

Antioxidant Test Results

In this antioxidant activity test, lutein was compared with ascorbic acid. The antioxidant activity data of lutein compounds

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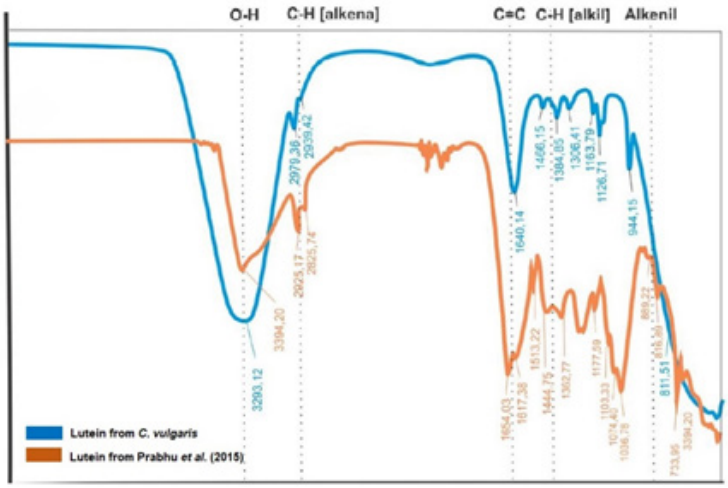

Figure 6 FTIR test results of lutein from *C. vulgaris* compared with literature

from *Chlorella vulgaris* microalgae are presented in Table 2.

Based on Table 2, the antioxidant activity of lutein extracted from *Chlorella vulgaris* was evaluated and compared with that of a reference antioxidant, ascorbic acid, across a concentration range of 25–100 ppm. At each concentration level, both compounds exhibited increasing inhibition percentages, indicating a dose-dependent antioxidant response. At 25 ppm, lutein demonstrated a higher inhibition percentage (21.08±0.76%) than ascorbic acid (15.96±1.97%), suggesting higher antioxidant activity at this low concentration. However, at higher concentrations (50–100 ppm), ascorbic acid consistently showed stronger inhibition values (55.97–92.88%) than lutein (45.55–72.25%), highlighting the superior antioxidant activity of ascorbic acid at elevated doses.

Based on the IC_{50} values, which represent the concentration of a compound required to inhibit 50% of free radicals, lutein showed an IC_{50} of 62.54±1.78 ppm, while ascorbic acid had a lower value of 51.89±1.68 ppm. As a lower IC_{50} indicates a stronger antioxidant potential, this result suggests that ascorbic acid has a higher overall antioxidant activity than lutein, even though lutein showed a slightly higher inhibition percentage at the lowest tested concentration (25 ppm). According to Bahriul *et al.* (2014), IC_{50} values ranging from 50–100 ppm are classified as strong antioxidants. Based on this classification, both lutein and ascorbic acid were categorized as strong antioxidants. However, the difference in their IC_{50} values helps to compare their actual effectiveness. These findings suggest that although lutein

Table 2 Antioxidant activity data of *Chlorella vulgaris* lutein compound

Sample	Concentration (ppm)	%Inhibition	IC_{50} (ppm)	Category
Lutein	25	21.08%±0.76	62.54±1.78	Strong*
	50	45.55%±4.81		
	75	57.34%±3.08		
	100	72.25%±3.97		
Ascorbic acid	25	15.96%±1.97	51.89±1.68	Strong*
	50	55.97%±3.74		
	75	78.08%±1.12		
	100	92.88%±2.21		

*Bahriul *et al.* (2014)

from *Chlorella vulgaris* shows potential as a natural antioxidant, its overall effectiveness is lower than that of ascorbic acid across various concentrations.

Compared to lutein from other microalgae, the IC_{50} value of lutein extracted from *Chlorella vulgaris* appears relatively higher, suggesting lower antioxidant potency. For instance, Andrade *et al*. (2018) reported that lutein from *C. ellipsoidea* had an IC_{50} value of 40.73 ppm, whereas *Scenedesmus* sp. exhibited even stronger activity with IC_{50} values ranging from 2.54 to 5.05 ppm (Lumba *et al*., 2013). Similarly, Dinh *et al*. (2022) showed that lutein from *C. sorokiniana* had IC_{50} values ranging from 58.07 to 81.69 ppm. These variations in antioxidant activity are likely influenced by species-specific characteristics and environmental conditions, such as water quality and cultivation parameters.

CONCLUSION

The research results showed that the crude lutein extract yield from the maceration process was 8.01% and from the saponification process was 24.37% (19.6 mg/g) or equivalent to ± 19.6 mg of lutein per gram of dry *Chlorella vulgaris* microalgal biomass. The identification and isolation process using column chromatography yielded three fractions, of which fraction 3 was identified as lutein. Spectral analysis of fraction 3 showed a maximum absorption at 445 nm, and FTIR confirmed that the functional groups were consistent with the lutein structure. The color test results indicated that the lutein isolate tended to be yellow, with a value of 66.79 and a hue angle of 88.650, which falls within the lutein color range. The antioxidant test results of lutein isolated from *Chlorella vulgaris* showed an IC_{50} value of 62.54 ppm, which classifies it as a strong antioxidant. Thus, *Chlorella vulgaris* is a sustainable and efficient lutein source with strong antioxidant potential.

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