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**ORIGINAL RESEARCH PAPER**

# PIGMENT CONTENTS OF *Sargassum polycistum* MACROALGAE LIPID FROM SAYANG HEULANG BEACH, INDONESIA

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**Abstract:** Macroalgae has a rich source of potential bioactive compounds. This study aims to identify the pigment profiles of *Sargassum polycistum* lipid collected from Sayang Heulang Beach, West Java, Indonesia. Lipid was isolated from *Sargassum polycistum* using chloroform: methanol (2:1, v/v), based on Folch method, which is the initial part of pigment extraction process. Pigment extract was identified by using Thin Layer Chromatography (TLC) with hexane: acetone (7:3; v/v) as eluent. The results showed six spots which successfully identified. Moreover, the spots were separated by using coloumn chromatography and followed characterization by UV-Vis spectroscopy in range 400-900 nm. The experimental results revealed that *Sargassum polycistum* lipid contained four pigments namely phaeophytin a, *α*-carotene, *β*-carotene and fucoxanthin.

**Keywords:** *alpha-carotene, beta-carotene, fucoxanthin, phaeophytin-a, Sargassum polycistum*

## INTRODUCTION

Macroalgae or well known as seaweed is important sources of bio-active natural substances and offers a wide range of food and health purposes (namely pharmaceutical industry) [1]. Further, it is known as valuable sources of protein, elements, dietary fibers, vitamins, essential amino acids and lipid (essential fatty acids) and secondary metabolites [1, 2]. In addition, macroalgae or seaweeds are commonly grouped as brown, green or red according to the color of their photosynthetic pigments [3]. The photosynthetic pigments including chlorophyll, carotenoids and xanthophylls are present in seaweeds as bioactive compounds [4]. This pigment has been isolated and identified from edible brown seaweeds such as *Sargassum siliquastrum, Hizikia fusiformis,* and *Undaria pinnatifida* and play an important role in health maintenance and have traditionally attracted the attention of the pharmaceutical and food industry [5].

One of the well known brown seaweeds species is *Sargassum* sp. which spreads in all Indonesia waters [6]. Some studies have been carried out on the extracts from seaweeds (*Sargassum ilicifolium* [7], *Sargassum wightii* [8], and *Sargassum vulgare* [9] and their extracts were reported to exhibit antibacterial activities from their secondary metabolite. Moreover, Heriyanto et al., have been successfully identified 21 pigments from *Sargassum crassifolium* [6]. In addition, a study, which conducted by Panjaitan & Warganegara, has demonstrated antibacterial activity from lipid of *Sargassum polycistum* [10].

The present study was undertaken to identify the pigment contents from *Sargassum polycistum* lipid, which have been isolated in previous study by Panjaitan & Warganegara [10], using UV-Vis spectroscopy method.

## MATERIALS AND METHODS

### Materials

*Sargassum polycistum* (seaweed or macroalgae) which collected from Sayang Heulang Beach in Indonesia, organic solvents (such as methanol (Merck, p.a), chloroform (Merck, p.a), hexane (Merck, p.a), and acetone (Merck, p.a)), distilled water, silica gel G60 7733, thin layer chromatography (TLC) plate.

### Methods

#### *Macroalgae or seaweed sampling*

*Sargassum polycistum* was collected from Sayang Heulang Beach, Mancagahar Village, Pameung peuk District, West Java, Indonesia without considering the age of plant. This seaweed sampling was done in the morning and along the seashore. Then, the seaweed samples were kept in the cooler box in transportation process from the beach to the laboratory to get fresh seaweed. In the laboratory, the seaweed samples were rinsed with sterile water remove sands and any associated debris. Moreover, the samples were kept in the freezer for further experiment.

***Determination of Seaweed Species***

Seaweed sample which collected from Sayang Heulang Beach has determined to figure out its species or its classification in the Research Center for Oceanography of LIPI (Indonesian Institute of Sciences), Jakarta, Indonesia.

***Lipid Extraction***

Lipid extraction of seaweed was done using Soxhlet apparatus with chloroform solvent (p.a.) and methanol (p.a.) with a ratio of 2:1 (v/v), based on Folch method [10]. This process took for 16-18 hours where there was a color changing in Soxhlet chamber to be colorless. Moreover, this extraction process results two layers or phases where upper-layer/phase (which is predicted to contain methanol) and bottom-layer/phase (which is predicted to contain chloroform) because there is a different of specific weight between both solvents. These two layers/phases are separated by using separating funnel and each phase is purified by using rotary evaporator to get lipid extract. Then, lipid extracts were kept in dark bottle in freezer to avoid oxidation. The whole lipid extraction process followed previous study which conducted by Panjaitan & Warganegara [10].

***Identification of Pigments from Lipid Crude Extract using Thin Layer Chromatography (TLC)***

Bottom-layer/phase of lipid crude extract was carried out on Thin Layer Chromatography (TLC) plates (silica gel G60 7733). An aliquot of bottom-layer/phase of lipid was spotted onto the silica gel plate and allowed to dry for a few minutes. Then, the plate was developed with hexane: acetone (7:3, v/v) as mobile phase in a previously saturated glass chamber with eluting solvents at dark condition and room temperature. The developed plate was dried and the spots were visualized.

***Fractionation of Sargassum polycistum Lipid with Column Chromatography***

After identifying how many pigments which contained in lipid by Thin Layer Chromatography (TLC), the pigments were separated by silica gel-coloumn chromatography. 7 grams of G60 7733 silica powder (200 – 500 µm particle size) were dissolved with hexane/acetone (7:3, v/v) eluent and then put in the coloumn (37 cm in height and its diameter is 1 cm). Afterwards, sample was added with 0.7 g of silica powder and this mixture was put into the column chromatography which is contained with hexane/acetone (7:3, v/v) for 14-16 hours in dark condition and flow rate was 6 drops/minute. The crude pigments were collected, concentrated by evaporation and stored in a sterile vial.

***Characterization of Pigments with UV-Visible Spectroscopy***

Pigment compounds which obtained from column chromatography process were characterized with UV-Visible spectroscopy (*Biochrom*) and the spectrum of purified pigment compounds were recorded from 400-900 nm.

## RESULTS AND DISCUSSION

### *Result of Determination of Seaweed Species*

Brown seaweed or macroalgae used in this research was collected from Sayang Heulang Beach, which is located in Mancagahar Village, Pameung peuk District, Garut Regency, West Java Province, Indonesia. In this beach, seaweed or macroalgae grows naturally in hard substrates such as rock or coral.

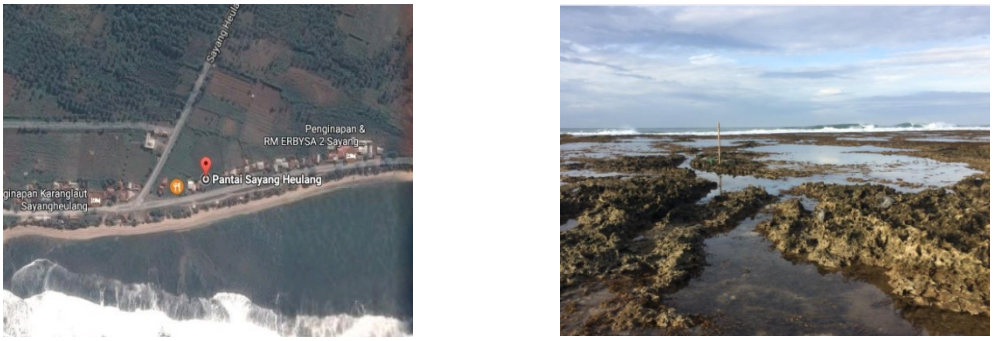



***Figure 1.*** *Map (left) and Image (right) of Sayang Heulang Beach*

In visual observation, brown seaweed or macroalgae which collected from Sayang Heulang Beach comes from species of *Sargassum* where have some physical properties such as brown thallus and about 28 cm in length, cylindrical, its leaves shape is oval and its leaves diameter is about 1 cm.

***Figure 2.*** *Sargassum polycistum collected from Sayang Heulang Beach (West Java, Indonesia)*

The determination of brown macroalgae species was conducted in the Research Center for Oceanography of LIPI (Indonesian Institute of Sciences), Jakarta, Indonesia where the result is shown below:

| | | |
|---|---|---|
| Division | : | *Phaeophyta* |
| Class | : | *Phaeophyceae* |
| Order | : | *Fucales* |
| Family | : | *Sargassaceae* |
| Genus | : | *Sargassum* |
| Species | : | *Sargassum polycistum* |

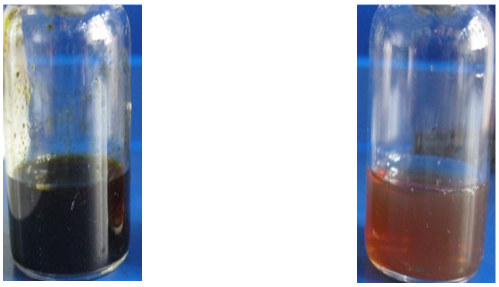
***Lipid Content of Sargassum polycistum***

Briefly, lipid extraction process resulted two phases (upper and bottom) and each phase has purified (according to the result of previous study [10]). The lipid content is shown in Table 1.

***Table 1.*** *Lipid Content of Sargassum polycistum [10]*

| Layer/Phase | Lipid Content [% volume/mass] |
|---|---|
| Upper | 3.8 % |
| Bottom | 11.2 % |

According to the Table 1, it can conclude that lipid content of seaweed (*Sargassum polycistum)* is relatively low. In agreement with our results, Kumari *et al* noticed that the range of algal lipid content in seaweed is from 0.12 % to 6.73 % (dry weight) [11]. They are composed mainly of phospholipids, glycolipids and non-polar glycerolipids (neutral lipids).



Bottom Phase Upper Phase

***Figure 3.*** *Lipid of Sargassum polycistum*

***Identification and Fractionation of Pigment Compounds***

The pigments which contained of *Sargassum polycistum* lipid were identified by Thin Layer Chromatography (TLC). There are 6 spots which formed from *Sargassum polycistum* lipid.

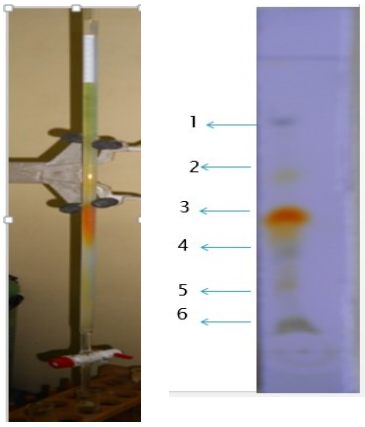



***Figure 4.*** *Identification and Separation of Pigment Compounds*

Furthermore, the pigments compounds (spots) were separated by column chromatography with silica gel G60 7733 as stationary phase and hexane/acetone (7:3,

v/v) eluent as mobile phase where the length of stationary phase was 37 cm. This separation results some pigment fractions which will characterized by UV-Vis spectroscopy (400-900 nm). There are four pigments which successfully identified such as phaeophytin a, $\alpha$-carotene, $\beta$-carotene and fucoxanthin.

Brown seaweed contains pigments which come from chlorophyll group (and its derivative) and carotenoid one. Chlorophyll a (blue-green) is a main pigment which roles on photosynthesis process while carotenoid is as the complementary pigment. Moreover, phaeophytin is chlorophyll's derivative which loses magnesium ion in its porphyrin ring because of heating and storing processes (especially in the presence of acid), as described in Figure 5 [12].

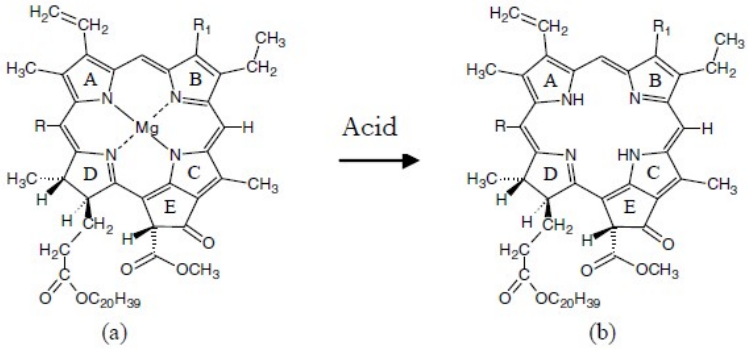



***Figure 5.*** *Phaeophytin forming reaction from chlorophyll a (a) to phaeophytin a (b) due to acid effect*

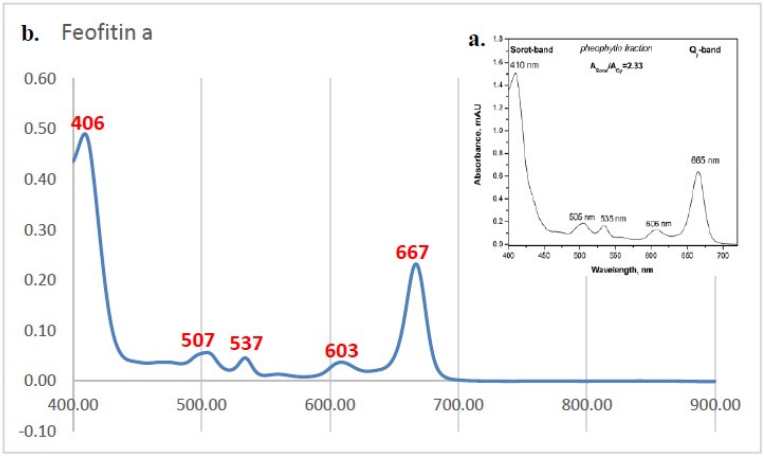



***Figure 6.*** *Spectra of standard (a) [13] and characteristic UV-Vis (b) (Phaeophytin a) from Sargassum polycistum lipid*

The pattern of the spectrum is shown in Figure 6 (a). Further, the result showed the pattern and the maximum wavelength at 406 nm, 507 nm, 537 nm, 603 nm and 667 nm is the same as the literature of phaeophytin a (Figure 6 (b)) [13]. Compare to the chlorophylls which absorb light at the wavelength around 660 nm [14]. Different pigments absorb light at different wavelengths.

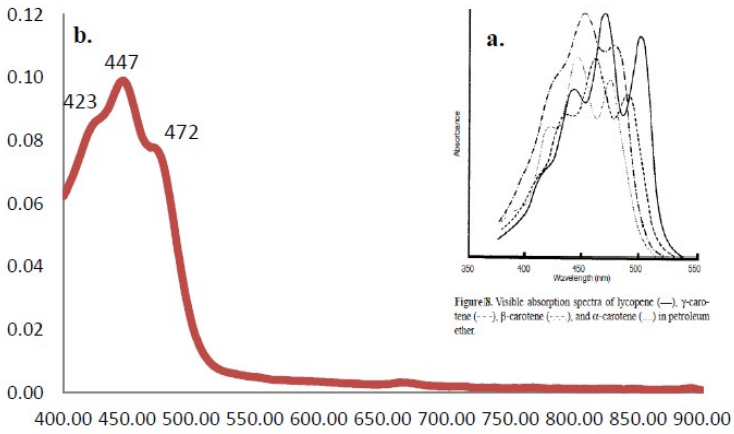



***Figure 7.*** *Spectra of standard (a) [13] and characteristic UV-Vis (b) (α-carotene) from Sargassum polycistum*

In this study, the UV-Vis spectrum of α-carotene from lipid of *Sargassum polycistum* was recorded (Figure 7) and its absorption maximum ($\lambda_{max}$) was compared with the literature. The results in Figure 7 exhibited the pattern and the maximum wavelength at 423 nm, 447 nm and 472 nm is very similar to the literature, according to Rodriguez and Amaya, which are at 422 nm, 445 nm and 473 nm in hexane solvent [15]. Most carotenoids absorb light in the range between 400-500 nm [16].

Carotenoids are the pigments responsible for yellow, orange, red and purple color distributed throughout nature which have been identified from plants, algae, bacteria and animals. They came with green chlorophyll in leaves and herbs which are present in many flowers, fruits, seeds or roots and synthesized only in plants. Carotenoids are only slightly soluble hydrophobic molecules, or no soluble in water [17]. There are two major groups of carotenoid such as carotenes and xanthophylls. Carotenes or hydrocarbon carotenoids consist of only carbon and hydrogen atoms (about 40 carbon atoms, formula $C_{40}H_{56}$) without oxygen and hydrophobic, while xanthophylls are their oxygenated derivatives and contain, such as hydroxyl, keto, epoxy and methoxy groups [18]. The most important of carotenes are lycopene (red carotenoid pigment), *α*-carotene (a precursor to retinoic acid), *β*-carotene and *γ*-carotene. Moreover, *α*-carotene is antioxidant substance that gives color and flavor, orange-colored and red-one fruits and vegetables. By heating, *α*-carotene may convert to *β*-carotene. The quantities of α-carotene in plants are smaller than *β*-carotene [17].

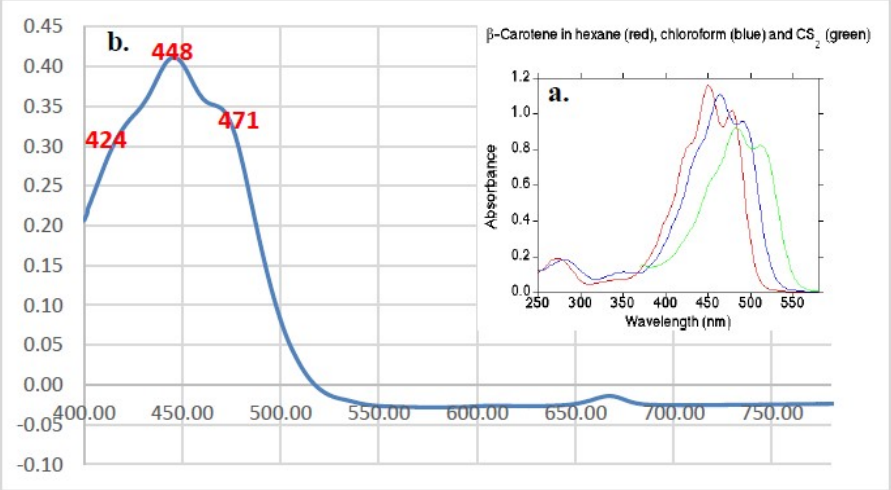



***Figure 8.*** *Spectra of standard (a) [15] and characteristic UV-Vis (b) (β-carotene) from Sargassum polycistum*

Seaweeds (macroalga) are an important source of carotenoids such as fucoxanthin, lutein, carotene and siphonaxanthin. Carotenoids produced by algae are generally localized in the chloroplast or accumulated in vesicles, cytoplasmic matrix or bound to membranes and other macromolecules in the intracellular space [19]. Further, fucoxanthin, chlorophyll a, and *β*-carotene were detected as the main pigments, while other minor pigments, such as antheraxanthin, zeaxanthin, violaxanthin, chlorophyll and degradation products including *cis*-fucoxanthin, phaeophytin a, epimer chlorophyll-a, and chlorophyllide a were also observed [20].

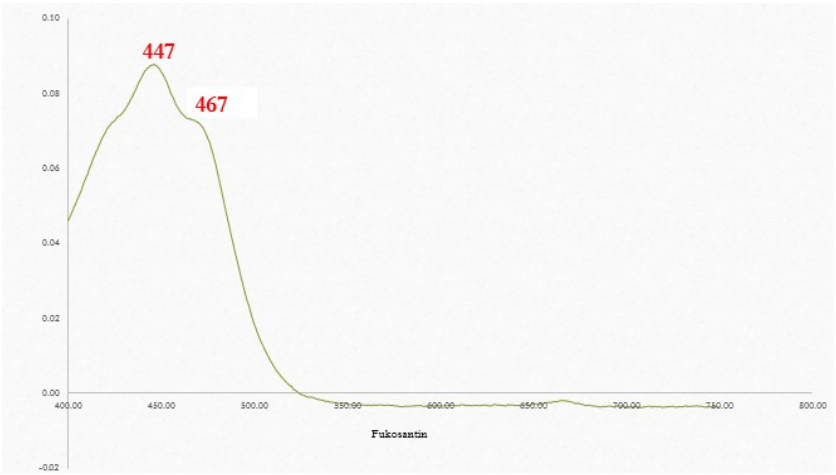
Moreover, *β*-carotene is a red/orange-colored fat-soluble terpenoid with antioxidant properties [17]. Heriyanto *et.al* reported that four brown seaweeds (namely, *Dictyota dentata, Padina australis, Sargassum crassifolium,* and *Turbinaria conoides)* which collected from Panjang Island, Central Java, Indonesia, had the same pigment composition and fucoxanthin, chlorophyll a and *β*-carotene were the main pigments [6]. In addition, the UV-Vis spectra (in Fig. 8) showed the absorption peaks at 424 nm, 448 nm, 471 nm is very similar to the literature, according to Takaichi, which are at 425 nm, 450 nm, 478 nm in hexane solvent [18].

Xanthophylls are hydroxyl derivatives of carotenoid hydrocarbons, which comprise a diverse group of oxygenated carotenoids with varied structures and complexes functions such as lutein, zeaxanthin and fucoxanthin. Fucoxanthin, with formula $C_{42}H_{58}O_{6,}$ is commonly found in brown seaweed like *Sargassum* sp. [17]. Moreover, it is a fat soluble carotenoid compound and sensitive to light, temperature, *p*H and strong acidic & alkaline conditions [6]. Furthermore, it has an orange-colored pigment, found in brown seaweeds along with chlorophyll, to give a brown or olive-green color.

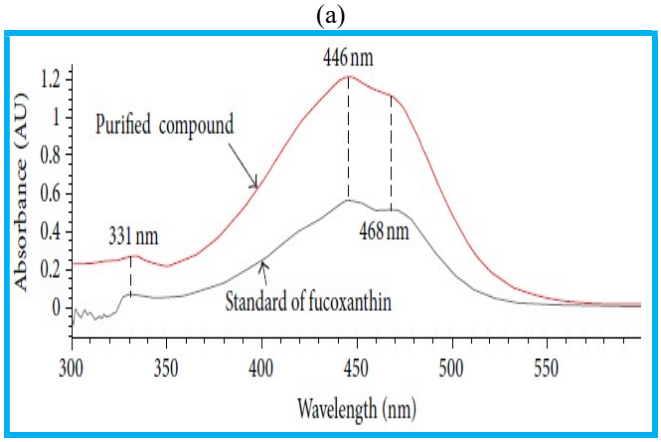
In this research, fucoxanthin has been successfully isolated from *Sargassum polycistum* lipid and its UV-Vis spectra was recorded and shown in Fig. 9. The spectra in Fig. 9 exhibited the pattern and the maximum wavelength at 421 nm, 447 nm and 471 nm is very similar to the literature, according to Rodriguez and Amaya, which are at 420 nm, 444 nm and 467 nm in hexane solvent [15].

Sudhakar *et.al* has reported about five brown seaweeds such as *Sargassum wightii, Sargassum ilicifolium, Sargassum longifolium, Padina* sp. and *Turbinaria* sp., from Mandapam, Gulf of Mannar coast, Rameswaram, India, contained fucoxanthin pigment

[4]. In addition, Yip *et.al* stated that *Sargassum binderi,* from Semporna, Sabah, Malaysia, contained pigments such as fucoxanthin, carotenoid, and chlorophyll where fucoxanthin is a pigment from brown seaweeds that has promising health enhancing properties and is suitable to be applied as bioingredient and functional food [21]. Among the pigments reported, fucoxanthin is one of the most abundant carotenoids, especially in brown seaweeds, and contributes >10 % of the total estimated production of carotenoids in nature [5]. Moreover, based on Papagiannakis *et. al*, the absorbance of fucoxanthin ranges from 420-470 nm [22]. Since the maximum absorption wavelengths of both the standard [8, 22] and sample were similar, this indicates that the spectra in Figure 9 in the *Sargassum polycistum* lipid was fucoxanthin.




(a)




(b)

***Figure 9.*** *Characteristic UV-visible (a) (fucoxanthin) from Sargassum polycistum and spectra of standard (b) [5]*

The difference of maximum absorption wavelengths between our study and previous study happened since the specific photosynthetic pigments and their concentrations in brown seaweeds varied depend on various environmental factors and morphological structure. However, comparative study in closely related species might give useful information concerning differences among habitats [19].

## CONCLUSIONS

Algal lipid has been successfully isolated from *Sargassum polycistum* (namely 3.8 % for top layer and 11.2 % for bottom layer (volume/mass)). Then, pigments from *Sargassum polycistum* lipid were clearly separated by column chromatography and characterized by UV-Vis spectrophotometry. There are four pigments which identified successfully such as phaeophytin a, $\alpha$-carotene, $\beta$-carotene and fucoxanthin where each pigment has different UV-Vis spectra.

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