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Institute of Advanced Technology , Universiti Putra Malaysia, Malaysia

Prof. Gao Zhiqiang
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ORAL PAPERS

Potential Synergism of Astaxanthin from Haematococcus pluvialis as an Antioxidant Supplements

Yuyun Yuniati [a], Renny Indrawati[a, b], Tatas H. P. Brotosudarmo[a, b], Wynona Agatha Nimpoeno[a], Leenawaty Limantara[b,c]

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ABSTRACT

Nowadays the epidemiology of degenerative diseases have reached a point of particular concern, since degenerative desease becoming a major mortality cause in almost all over the world. The imbalance of free radicals and antioxidants in the body whether due to normal bodily process or modern lifestyle preferences leads to degenerative cell alterations, which then disrupts the function of tissues or organs. Dietary intake of antioxidants is in someways of importance to prevent degenerative diseases. Haematococcus pluvialis, a rich source of astaxanthin, which is known to have good antioxidant properties, is already applied in most antioxidant supplements. This research aims to discover the potential synergism of astaxanthin. As a such, the research is to encourage future researchers on developing the most effective formula of astaxanthin with other pigments.

Keywords: antioxidant, astaxanthin, Haematococcus pluvialis, supplement, synergism

DEGENERATIVE DISEASES AS A MAJOR MORTALITY CAUSE

Degenerative disease is the most common death cause, even more so in modern countries. Some examples of degenerative diseases are heart disease, stroke, diabetes mellitus, and hypertension. Degenerative diseases are resulted from a continuous process based on degenerative cell changes, affecting tissues or organs, which increasingly deteriorates over time, whether due to normal bodily wear or lifestyle choices such as lack of exercise or bad eating habits. Modern lifestyles can be a trigger for degenerative diseases. Despite being caused by normal occurrences such as normal bodily wear, exercise, and eating habits, degenerative diseases have become a major mortality cause. Human death percentage caused by these diseases are reported to become more and more significant each year.

Figure 1 shown the most apparent death cause by around 13% is ischaemic heart disease, followed by stroke at 12% and both chronic obstructive pulmonary disease and lower respiratory infections following at 6% [1]. In that figure, it is shown that major death causes are causes are caused by degenerative diseases, which are heart disease, stroke, obstructive pulmonary disease, cancer, diabetes mellitus, and hypertension, which altogether makes 39% of world death cause in 2012.

These diseases are caused by imbalance of radicals and antioxidants in the body. Free radicals come from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. Free radicals are formed continuously in cells as a consequence of enzymatic process such as those involved in the respiratory chain, in phagocytosis, or in prostaglandin synthesis and non-enzymatic reactions of oxygen with organic compounds as well ionizing reactions [2]. Industrial chemicals and air pollutants are two things almost impossible to erase from our life these days. They exist in fast foods, packaged foods, even room freshener, instant beverages, snacks, everything. Air pollutants come from motorized vehicle residues, industrial waste, cooking, etc.

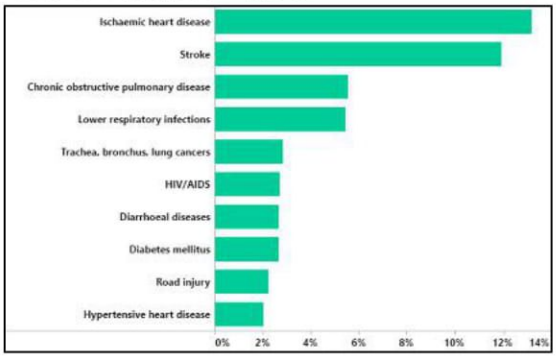

Fig 1. Percentages of Death Causes in 2012 based on GHO report [1]

Human body usually has a natural process in scavenging the free radicals we retrieve from our daily life, but since the amount of pollution, added by other lifestyles, is much higher, the neutralization process becomes slower, and thus, this ends up damaging our body more than it should have.

The desire to have a higher life quality and to keep our body healthy has significantly prompted to society to consume supplements to balance bodies' nutritional needs. To ward off free radicals, antioxidant is needed. Antioxidant will scavenge free radicals by inhibiting them from attacking body tissues and cells. Some of well-known dietary antioxidants are ascorbates, tocopherols and carotenoids. There are also a lot of natural antioxidants sources such as fruits and vegetables, seeds, cereals, berries, wine, tea, onion bulbs, olive oil and aromatic plants [3].

HAEMATOCOCCUS PLUVIALIS AS NATURAL SOURCE OF ASTAXANTHIN

Haematococcus pluvialis is the richest source of natural astaxanthin and it has already been cultivated at industrial scale [4]. Astaxanthin belongs to xanthophyll group which has the main function as antioxidants. The antioxidant properties of astaxanthin is ten times stronger than those of β-carotene. Astaxanthin consists of 40 carbons joined together with both single and double bonds (3,3'-dihydroxy- β, β -carotene-4,4'-dione) [5]. Fig 2 shows the chemical structure of astaxanthin.

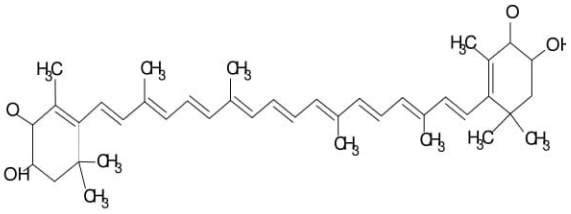

Fig 2. Chemical structure of astaxanthin

Astaxanthin has been widely used in food industry, medicine, health supplements, and aquaculture [6]. Most dietary supplements employ *H.pluvialis* as the source of astaxanthin whereas aquaculture industries prefer to cultivate *P. rhodozyma* [7].This pigment has a bright red color from the long, conjugated double chains at the center of the compound. This structure allows astaxanthin to have stronger antioxidant properties than other carotenoids. [5] The mechanism of its antioxidant properties is by protecting cells from oxidation by scavenging singlet oxygens, then releasing the excess energy in the form of heat. Astaxanthin can stop oxidation since it has free radical neutralizing properties. With this structure, electron decentralization – that can reduce the amount of reactive oxygens – happens [6]. Due to these properties, it is highly potential to use *H. pluvialis* as a natural source of antioxidant. The selection of *H.pluvialis* as the astaxanthin source is from the high astaxanthin concentration comparing to the other sources i.e. *Salmonidae* with the concentration ranging from 0-37 mg/kg, and the Crustacean family ranging from 10-1160 while the concentration of astaxanthin in *H. pluvialis* ranges around 10,000-30,000 mg/kg. [6]

TRACING THE ANTIOXIDANT-RICH WITH ASTAXANTHIN PIGMENT

To trace the distribution of product containing astaxanthin that is sold in market, a survey was carried out by browsing through 5 online shops that sell supplement.

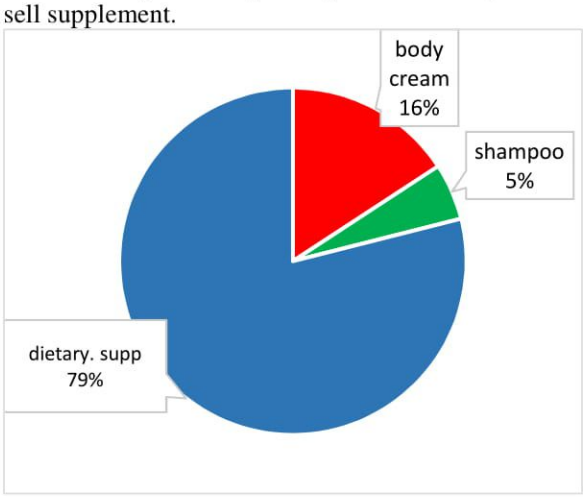

Fig 3. Product types containing astaxanthin

In the market, there hasn't been much use of astaxanthin, especially in the form of processed foods or beverages. The main usage is as an antioxidant supplement (79%) to tackle free radicals due to UV radiation and as bioactive compound in anti-aging cream, with one product using astaxanthin to stimulate hair growth. All the producers are found to be originated in USA. Fig 3. shows the percentages of each products containing asthaxanthin found in online market.

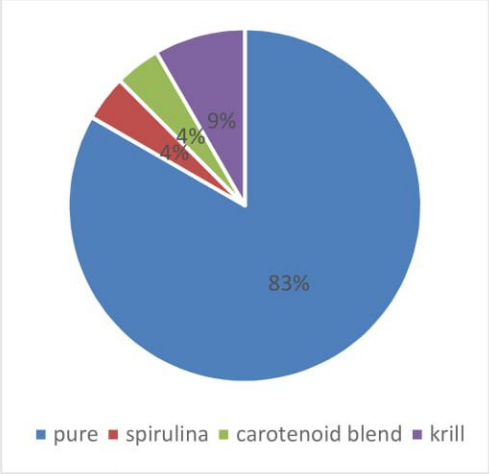

Fig 4. Supplements composition in market

Fig 4. shows the *composition* of commercial products containing astaxanthin. The supplements containing pure astaxanthin compose as much as 83% of the total products, leaving only 17% for supplements with combinations. The combinations are krill (9%) as the highest number followed by spirulina, carotenoid blend (containing zeaxanthin, lutein, cyanidin 3-glucoside, and meso-zeaxanthin) taking 4% of the total products count.

SYNERGISTIC POTENTIAL OF PIGMENT COMBINATION

The antioxidant activity of astaxanthin is ten times stronger compared to β-carotene. Based on market data, we observe that the use of astaxanthin is not only applied as a lone antioxidant, but it combined with spirulina, carotenoid, and krill. The synergistic effect of pigments has been researched on and reported by scientists. A research shows that mixtures of carotenoids were more effective than the single compounds, with the synergism effect most pronounced when lycopene or lutein was present. The superior protection of mixtures may be related to specific positioning of different carotenoids in membranes [9]. In another research, binary and ternary combination of quercetin, lutein, caffeic acid, chlorogenic acid, gallic acid and rosmarinic acid was found to able to influence the antioxidant ability [10]. It is also proven that a combination of β-carotene and α-tocopherol results in an inhibition of lipid peroxidation significantly greater than the sum of the individual inhibitions [11].

ASTAXANTHIN EXTRACTION FROM *H. PLUVIALIS*

The equipment used in astaxanthin extraction is beaker glass for extraction, Whatman 0,2 µm NYLON filter membrane, Rotary Evaporator IKA RV 10 Basic D, Shimadzu (Japan)'s, UV-VIS Spectrophotometer UV-1700 PharmaSpec, HPLC-20 AD (Shimadzu, Japan), and ultrasonic from Mosonic USA. The main materials used were *Haematococcus pluvialis* Flotow microalgae. The biomass of *H. pluvialis* was obtained from PT. Setia Kawan Abadi in the form of dried cells. Ethanol, acetone, methanol, and acetonitrile, KH_2PO_4, K_2HPO_4, nitrogen gas, and purified water were used for extraction procedure. The extraction was accomplised in a dark room to minimalize the degradation of pigment. Extraction method by sonication at the frequency of 32 kHz and bulk temperature range of 30-40°C is safe enough for the pigment. Variables set are the variation of solvents: 100% ethanol, ethanol-water (50:50 v/v), and ethanol-water (25:75) v/v

First, 1 gram of *H. pluvalis* powder was weighed in watch glasses, then poured into a beaker glass and is added with 5 ml of solvent. The sonication process was done for 1 minute. The sonication method results in the formation of micro hot spots in the solution, so every one minute, the sonication process was interspersed with cooling for one minute. This process was repeated for three times. After that, the supernatant was separated from the pellets and was strained by using Whatman 0,2 µm filter membrane. The pellets were then saluted again in 5 ml of solvents and the process is repeated. The strained supernatant was then kept in a flask to be evaporated with a Rotary Evaporator (IKA RV 10 Basic D). The astaxanthin pigment produced was then dried with nitrogen gas and kept in a freezer of -20°C in temperature. The crude pigment extract was then subjected to antioxidant assay. Free radical scavenging activity of different solvent in extraction were measured by 1,1-diphenyl-2 picrylhydrazyl (DPPH).

RESULTS AND DISCUSSION

Astaxanthin identification using Spectrophotometry produces an absorption spectra with its main peak corresponding to astaxanthin at 482 nm and one small peak at 670 nm. The peak at 670 nm wavelength shows the presence of chlorophyll in the pigment extract [12]. The spectra profile in Fig. 5 shows that the absorbance value increases in the presence of pure ethanol, ethanol-water (50:50, v/v), and ethanol-water (25:75, v/v) as solvent proportional to the amount of extracted astaxanthin. The value of absorbance in different types of solvent were 0.1523, 0.1031 and 0.1001 respective to pure ethanol, ethanol-water (50:50, v/v), and ethanol-water (25:75, v/v).

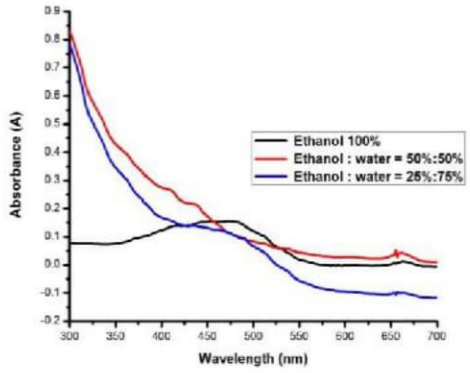

Fig. 5. Spectra profile extract of *H.* pluvialis in various solvent

The antioxidant activity of astaxanthin extracted from *H.* pluvialis microalgae in ethanolic solution was determined using DPPH method. This assay provides an easy method to rapidly determine the antioxidant activity of astaxanthin. The color change in the astaxanthin-DPPH solution from purple to yellow causes a decrease in absorbance due to the reduction of the antioxidant compounds in astaxanthin. The percentage of antioxidant activity (%AA) also gives different results based on solvents used. The value of astaxanthin's antioxidant activity in different types of solvent were 21.51%, 18.8%, and 15.65% respective to pure ethanol, ethanol-water (50:50, v/v), and ethanol-water (25:75, v/v). Studies has also reported that xanthopylles (astaxanthin, lutein, and xeazanthin) have antioxidant effect to prevent oxidative stress [13,14].

CONCLUSION

Astaxanthin is a potential antioxidative compound that can be isolated from *H.* pluvialis. The highest value of antioxidant activity was obtained in pure ethanol solvent. It has been known that astaxanthin show antioxidant activity and has good effect on human health. Astaxanthin itself also has a synergistic effect with spirulina, carotenoid, and krill. The innovation of astaxanthin use as a natural source of antioxidant still needs to be researched further, especially related to the most effective formula of astaxanthin in combination with other antioxidative pigments. Finding the combination and formula that has the optimal synergistic effect is extremely important in order to obtain the highest antioxidant activity

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