Evaluation of Fucoxanthin Content in Popular Weight Loss Supplements: The Case for Stricter Regulation of Dietary Supplements

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Abstract
Misbranded and counterfeit dietary supplements have been an issue on which the US Food and Drug Administration has been vigilantly regulating. The ubiquity of online-shoppable weight-loss supplements and their unrestricted consumption by obese people are serious matters of concern. Fucoxanthin, a brown-seaweed-extracted carotenoid has exhibited anti-obesity property in some clinical trials through its ability to over express uncoupling protein (UCP1) in the white adipose tissue, which leads to fat burning. However, since the clinical trials apply pure fucoxanthin instead of fucoxanthin-bearing dietary supplements, a critical analysis is warranted to quantify fucoxanthin in brown seaweeds to validate their rationale in weight loss mechanism. This study examined ten randomly chosen online-sourced brands of fucoxanthin-containing dietary supplements and analyse the content using High-Performance Liquid Chromatography (HPLC) equipped with UV-Vis and photodiode array detector. Our exploration revealed that, of these 10 products, 3 (30%) did not have any detectable quantity of fucoxanthin, 5 (50%) contained only trace amount of it, ranging from 0.001-0.01 mg per capsule, and only 2 (20%) products contained 0.4 mg or 2 mg of fucoxanthin, meeting their label claim. This worrying finding may be interpreted that the existing ease-of-access and pervasive nature of online-sourced dietary supplements require more stringent regulatory screening.

Keywords
Fucoxanthin, Weight loss, Dietary supplements, Misbranding, Quality assurance

Abbreviations
UCP1: Uncoupling Protein; HPLC: High-Performance Liquid Chromatography; NIH: National Institutes of Health; BMI: Body Mass Index; DSHEA: Dietary Supplement of Health and Education Act of 1994; cGMP: Current Good Manufacturing Practice; DSF: Dietary Supplement Fraud; LOD: Limit of Detection

Introduction
Obesity is common, serious and costly. The prevalence of obesity was 39.8% and affected about 93.3 million US adults and 17% of teenagers in 2015-2016 [1]. Obesity related conditions include heart disease, stroke, type 2 diabetes, osteoarthritis and certain types of cancers (endometrial, breast, colon, kidney, gallbladder and liver). Obesity in addition to its serious health consequences, obesity has an estimated annual health care costs of obesity related illness are a staggering $190.2 billion or nearly 21% of annual medical spending in the United States. Obesity as defined by National Institutes of Health (NIH) as a BMI (Body Mass Index) of 30 and above. Waist Circumference has also become another screening tool for determining excess abdominal fat, the independent risk factor for disease, the evaluation of waist circumference is to assess the risk associated with obesity in normal or overweight individuals [2]. Obesity is a serious concern because it is associated with poorer mental outcomes, reduced quality of life and the leading cause of death in the US and worldwide [3]. Obesity has been heavily studied over the past decade to determine the causes and more importantly ways to effectively manage the disease. Weight loss is the sole identified treatment for obesity. however, different ways to lose weight, main-
tain the sustained lost weight without gaining weight has proved to be difficult for most patients. A significant number of people who lose weight actually eventually gain back all the weight back after 3 years [4].

Different strategies have been employed by an individual to reduce weight, some of which include behaviour therapy, dietary therapy, physical activity, supplements & pharmacologic agents and surgery [5,6]. Dieting is the most popular and common strategy in which people lose weight, however NIH has made some recommendation regarding the use of drugs and supplements in aiding weight loss especially in individuals with a BMI ≥ 30 and for patients with a BMI between 25 and 29.9 or High-risk waist circumference, and two or more risk factors [7]. The most effective way is the combination of diet modification, increase physical activity and behaviour therapy. Weight loss surgery is an option for patients with extreme obesity/clinically severe obesity BMI ≥ 40 OR a BMI ≥ 35 and serious comorbid conditions. However, there are more risks associated with surgery, it is usually more expensive, and the resources may not be available [8,9]. Moreover, since most prescription FDA approved medications carries serious adverse effects, require frequent monitoring by some skilled paid personnel, expensive or classified as controlled medication (schedule 4), most people rely heavily on the use of inexpensive over the counter supplements to assist in losing weight [10].

Fucoxanthin is one of the most abundant marine carotenoids which can be found in marine brown seaweeds, macroalgae, microalgae, and diatoms. Fucoxanthin is an orange-colored pigment, along with chlorophyll a, c and b carotene present in Brown Seaweeds and Diatoms [11]. Recent studies have reported that fucoxanthin has many positive properties, one of which being anti-obesity. Results indicated that Fucoxanthin affects the plasma and hepatic lipid profile, fecal lipids, and body fat mass, and alters hepatic cholesterol metabolism, FA synthesis and lipid absorption [11,12]. Food supplements are frequently used in weight loss for one of two reasons: a) Providing nutrients that may be lacking in calorie restricted diets, and b) Potential benefits in enhancing weight loss. There are many botanical products that claim to enhance weight reduction, although the evidence of their efficacy is often inconclusive [13].

Dietary supplements defined as a product taken by mouth that contains “dietary ingredient”. The FDA regulations for dietary supplements are different from drugs. Dietary supplements do not require premarket approval by the FDA whereas all the drug products must be approved by the FDA before they can be marketed. Under the dietary supplement of health and education act of 1994 (DSHEA): Manufacturers and distributors of dietary supplements are prohibited from marketing adulterated and misbranded products. According to DSHEA law, the supplement manufacturer is responsible for ensuring the safety and labelling of their products before they are marketed but they do not have to provide that evidence to the FDA. FDA has the authority to oversee any misbranded or adulterated dietary supplements and take action. FDA does not review for safety and efficacy of dietary products before they are marketed [14]. Also, in 2007 Current Good Manufacturing Practice (cGMP) was enforced and it provides systems that assure proper design, monitoring and control of manufacturing process and facilities [15].

Quality control testing is essential for numerous reasons. It provides a competitive advantage along with ensuring the safety of its consumers and providing a company with credibility. Every member of a company is responsible for assuring quality starting at the top management to provide training and education, and including all employees to follow quality control procedures [16]. Dietary supplements today due to willful economic gain have resulted to the manufacturing of products which are misbranded and sometimes adulterated [17]. Most supplements companies advertise a certain active ingredient or compound that has been associated with significant positive health benefits but almost ending up to never include that agent in the final product. Sometimes sufficient quantity is not provided, but the label carries entirely different specified quantity [18]. Adverse health events resulting from dietary supplement fraud (DSF)-fraud conducted for economic gain using dietary supplements-have received increased recognition from agencies and industry. There is a growing awareness that this issue represents a significant public health threat. With increasing consumption of supplements, there are increasing consequences [19,20]. Americans spent about $36.7 billion dollars on dietary supplements (vitamins, minerals, amino acids, and herbs) [21]. However, the majority of this was spent on three major categories weight loss, bodybuilding, and sexual function. Weight loss due to the prevalence of obesity in the United States has led many people to choose a dietary approach to lose weight, as such is using different supplements as a meal replacement. However, due to the overwhelming proportion of misleading information regarding their use, many people find it difficult to choose the right supplements [22]. Many consumers attribute higher price supplements to mean they are more efficacious and of higher quality, which may not necessarily be true. Many companies make claims to consumers about the effectiveness of a product, which cannot be substantiated due to their lack of compliance with what they put in their product. The main objective of the study was to determine the content of Fucoxanthin in dietary supplements available in US market.

Materials

All solvents used in this study were HPLC grade and...
procured from commercial supplier. Acetonitrile was purchased from EMD Millipore, MA, USA and the purified water was obtained through the in-house water distillation system, model SZ-93A, manufactured by Huanyu, China. Fucoxanthin (CAS Number 3351-86-8) was purchased from Sigma Aldrich and the fucoxanthin supplements were purchased from online retailer in the USA such as Amazon.com.

**Methods**

The sample preparation and chromatographic methods used in this study were adopted and modified from GL Sciences [23]. In brief, the powder sample (equivalent to 2.0 mg of Fucoxanthin) was placed in 25 mL of Acetonitrile in a 50 mL volumetric flask and sonicated for 5 min. Then, 25 mL of diluent (the mixture of acetonitrile and purified water in the ratio of 1:1) was added to the preparation and again sonicated for 5 min. Then, the contents were allowed to cool at room temperature and adjusted the volume up to 50 mL using diluent (if necessary). The solution was then filtered through 0.45 µm membrane filter. After which, 1.0 mL of the filtered solution and 1.0 mL of the diluent was transferred to an Eppendorf tube and vortexed. Ten microliters of this solution were injected into the HPLC system to quantify the fucoxanthin content.

**Chromatographic conditions**

Liquid chromatography is one of the most powerful and most commonly used separation techniques, which has been using in food and pharmaceutical industry for its excellent sensitivity and specificity over Ultraviolet-visible spectroscopy technique. Generally, the content of organic compounds present in food and pharmaceutical products are determine by RP-HPLC integrated with UV-Vis Detector during quality testing. The chromatographic separation of the fucoxanthin was estimated by using the Shimadzu prominence HPLC system; using a gradient elution mode on a Waters X-Select CSH C-18 column (4.6 mm × 150 mm, 5.0 µm particle size, Part no. 186005290). The HPLC system consisted of a quaternary pump (LC-20AD), a UV-Vis detector (SPD-20AC, having exceptional level of sensitivity and stability). Mobile phase consisted of 850 mL of Acetonitrile and 150 mL of purified water. The instrumental setup included the flow rate at 1.0 mL/min and the temperature of the column oven was at 30 °C. The injection volume was set to 10 µL with a run time of 8 minutes. The effluent was detected at 447 nm based on the lambda max (Figure 1a) obtained using alliance 2695 separation module (Waters, MA, USA) connected to a Photodiode Array detector (Waters, MA, USA) to get maximum detector response and sensitivity. A 1.0 mg/mL of fucoxanthin stock standard solution was prepared in Acetonitrile and then 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL and 25 µg/mL of fucoxanthin solutions were prepared from the stock solution using the diluting solution to quantify fucoxanthin present in the test solution (fucoxanthin dietary supplements). All weights were measured using a pre-calibrated analytical balance Mettler Toledo PB303-S/FACT (Mettler Toledo, Switzerland).

The reliability of the methodology used for the determination of fucoxanthin in the dietary supplements was confirmed by evaluating the specificity, sensitivity, linearity, limit of detection (LOD), reproducibility and solution stability [24,25]. Specificity is one of the most important parameter for an analytical method particularly for natural products because natural products contain a lot of unknown compounds. The specificity for the chromatographic method used in this study was confirmed by checking the blank interference, resolution between the adjacent peaks, and peak purity (spectral analysis) [26]. To evaluate the peak purity of the chromatogram the spectral analysis was achieved using vector analysis algorithms using Photodiode Array detector (Figure 1b). The purity angles (0.734) is found lower than the purity thresholds (0.740) for the fucoxanthin peak, which indicates the peak is spectrally homogeneous and contains only one compound. HPLC chromatogram of the diluting solution clearly indicates that diluent and mobile phase has no interference in the studies because no peak is co-eluting at the retention time of 5.9 min (Figure 1f). Both of the test sample chromatograms (Figure 1g and Figure 1h) visibly indicate that peaks are well separated from each other, the resolution between the neighbouring peaks is more than 1.5. The responses for the proposed method was found to be linear (y = 84727370x + 25246, R² = 0.9995) from 5 µg/mL to 25 µg/mL (Figure 2). Detection limit of the methods was calculated based on the visual evolution as per ICH guidelines and FDA guidelines. LOD was found to be 0.67 µg/mL (Figure 1d). A known concentration of standard solution (100 µg) was spiked to the sample powder to evaluate the recovery and impact of the filter during filtration process. The recovery was found to be 102.3% and there was no interference of sonication and filtration was observed (Figure 1f). The solution was also found to be stable at room temperature for more than 48 hours.

**Results and Discussion**

The brown seaweed dietary supplements purchased from the online retailer were analyzed immediately to quantify the content of fucoxanthin present in the products. Table 1 represents the testing results of the ten products. All the products were analyzed twice at different time points. Most of the products failed to meet their specifications as per their product label claim. The Results showed that 3 out of 10 products (DS05, DS06 & DS08) did not have any Fucoxanthin and 5 out of 10 products (DS01, DS03, DS04, DS09 & DS10) have trace amounts of Fucoxanthin ranging from 0.001 - 0.01 mg per capsule and only 2 out of 10 products...
Anthin raw materials failed to meet their specifications analysed by HPLC. Other carotenoids plant pigments may present in the raw materials or the fucoxanthin raw materials may be contaminated with other chemicals (such as yellow/orange dye to the raw materials may make it appear to be compliant but contains little to none of the specified ingredient) that absorb at the same wavelength and give falsely UV absorbance. It is not a good practice to use UV-vis spectrophotometer to identify and quantify any ingredients in herbal products without purification. As, HPLC technique is more

\textbf{Figure 1:} HPLC chromatograms obtained in assay test, a) UV-Vis Spectrum of Fucoxanthin; b) Peak purity plot; c) Diluting solvent; d) LOD solution; e) Identification and reference standards; f) Test sample spiked with reference standard; g) Test solution (DS02); h) Test solution (DS07).

(DS02 & DS07) contains 0.4 mg and 2 mg of Fucoxanthin which conformed with the amount stated on their supplement facts.

In general, the colour of carotenoids plant pigments is deeply yellow or orange, because they absorb wavelengths ranging from 400-550 nanometres. This is what gives brown seaweed raw material its characteristic yellow or orange colour. We have analysed some Fucoxanthin raw materials (Fucoxanthin 1%, 5%, 35%, & 50%, extracted from Brown Seaweed) to evaluate the root cause of the substandard products. Most of the fucoxanthin raw materials failed to meet their specifications analysed by HPLC. Other carotenoids plant pigments may present in the raw materials or the fucoxanthin raw materials may be contaminated with other chemicals (such as yellow/orange dye to the raw materials may make it appear to be compliant but contains little to none of the specified ingredient) that absorb at the same wavelength and give falsely UV absorbance. It is not a good practice to use UV-vis spectrophotometer to identify and quantify any ingredients in herbal products without purification. As, HPLC technique is more
The system of regulating over-the-counter dietary supplements without FDA approval or a system in place to ensure the quality and safety of the final product before being sold. The health of patients is placed at an increased risk with the current model in place for regulating these products. The current model unfortunately places the authority to ensure quality and safety of a product that makes it over-the-counter in the hands of the manufacturers themselves. This self-regulation has led to mislabelled and/or misbranded products on the market for consumption. In short, the systems and rules to ensure quality and safety exist. If companies of supplements were held to the same good manufacturing practices already in place it would significantly reduce the problems existing in the market today. These practices need to be applied to all over-the-counter dietary supplement products and then enforced to maintain the integrity of all products that reach consumers. The results of this study highlight the issue faced when trying to make informed decisions about over-the-counter products and the need for a better system.

Specific, accurate and precise than UV method, companies need to move away from using only UV-Spectrometry to determine the content of Fucoxanthin in brown seaweed raw material. HPLC analysis was able to show that there was very trace amount of Fucoxanthin in the products and moving forward the use of HPLC during quality testing should be the gold standard in the manufacturing of dietary supplements. In summary, using more sophisticated testing practices such as HPLC would further reduce the error in products being sent to the market.

The practice of using over-the-counter dietary supplements to help aide with weight loss and other ailments will continue to be a trend as these products are easily accessible and available without a prescription or consult from a healthcare provider. Manufacturers have been able to market their products on a large scale and patients have come to readily trust products sold online or on their local pharmacy or grocery shelf. These products have little documented evidence to support efficacy or even the quality of the product being sold direct to consumers. This has led to a multitude of over-the-counter dietary supplements without FDA approval or a system in place to ensure the quality and safety of the final product before being sold. The health of patients is placed at an increased risk with the current model in place for regulating these products. The current model unfortunately places the authority to ensure quality and safety of a product that makes it over-the-counter in the hands of the manufacturers themselves. This self-regulation has led to mislabelled and/or misbranded products on the market for consumption. In short, the systems and rules to ensure quality and safety exist. If companies of supplements were held to the same good manufacturing practices already in place it would significantly reduce the problems existing in the market today. These practices need to be applied to all over-the-counter dietary supplement products and then enforced to maintain the integrity of all products that reach consumers. The results of this study highlight the issue faced when trying to make informed decisions about over-the-counter products and the need for a better system.

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Label Claim (Fucoxanthin/content/Capsule)</th>
<th>Results (Fucoxanthin content/Capsule)</th>
<th>Comments</th>
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<tbody>
<tr>
<td>DS01</td>
<td>5% Fucoxanthin (25 mg)</td>
<td>Detected (0.002 mg)</td>
<td>Non-compliance</td>
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<tr>
<td>DS02</td>
<td>2 mg</td>
<td>Detected (2.0 mg)</td>
<td>Compliance</td>
</tr>
<tr>
<td>DS03</td>
<td>2.67 MG</td>
<td>Detected (0.001 mg)</td>
<td>Non-compliance</td>
</tr>
<tr>
<td>DS04</td>
<td>5% Fucoxanthin (25 mg)</td>
<td>Detected (0.006 mg)</td>
<td>Non-compliance</td>
</tr>
<tr>
<td>DS05</td>
<td>5 mg</td>
<td>Not Detected</td>
<td>Non-compliance</td>
</tr>
<tr>
<td>DS06</td>
<td>5 mg</td>
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<td>Non-compliance</td>
</tr>
<tr>
<td>DS07</td>
<td>Fucoxanthin Concentrate</td>
<td>Detected (0.4 mg)</td>
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<td>DS08</td>
<td>200 mg</td>
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<td>5% Fucoxanthin</td>
<td>Detected (0.007 mg)</td>
<td>Non-Compliance</td>
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</table>

Figure 2: Linear curve (0.005 mg/mL to 0.025 mg/mL of Fucoxanthin).

Table 1: Content of Fucoxanthin in dietary supplements.
Conclusion

In summary, our analysis revealed that most weight loss products containing Fucoxanthin in the market may be either misbranded or mislabelled and there may be a need for more oversight of dietary supplements by the FDA to protect consumers who are taking these products. This would protect the everyday consumer from potentially harmful products or falling prey to false claims made by manufacturers.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Statement of Equal Authors’ Contribution

We have evaluated the Fucoxanthin content in popular weight loss supplements to support the following research project “Fucoxanthin for Diabetes Prevention and Control”. All the authors are working on this project. Drs. Hossain, Rashid, Justice, and Abdelfattah are supervising this project and contributed to the final version of the manuscript. Drs. Burniston and Ahmed performed the analysis and helped in writing the manuscript. Drs. Wu, Kataye, and Sidhu discussed the results and contributed to the manuscript writing.

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