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REVIEW

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Astaxanthin: A super antioxidant from microalgae and its therapeutic potential

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Abstract

Astaxanthin is a ketocarotenoid, super antioxidant molecule. It has higher antioxidant activity than a range of carotenoids, thus has applications in cosmetics, aquaculture, nutraceuticals, therapeutics, and pharmaceuticals. Naturally, it is derived from *Haematococcus pluvialis* via a one-stage process or two-stage process. Natural astaxanthin significantly reduces oxidative and free-radical stress as compared to synthetic astaxanthin. The present review summarizes all the aspects of astaxanthin, including its structure, chemistry, bioavailability, and current production technology. Also, this paper gives a detailed mechanism for the potential role of astaxanthin as nutraceuticals for cardiovascular disease prevention, skin protection, antidiabetic and anticancer, cosmetic ingredient, natural food colorant, and feed supplement in poultry and aquaculture. Astaxanthin is one of the high-valued microalgae products of the future. However, due to some risks involved or not having adequate research in terms of long-term consumption, it is still yet to be explored by food industries. Although the cost of naturally derived astaxanthin is high, it accounts for only a 1% share in total astaxanthin available in the global market. Therefore, scientists are looking for ways to cut down the cost of natural astaxanthin to be made available to consumers.

KEYWORDS

biological activities, cultivation system, biorefining process

1 | INTRODUCTION

The tetra-terpenoid pigments called carotenoids are naturally produced by higher plants, algae, fungi, and some bacteria; almost 600 of them have been identified and described. Chemically one can divide carotenoids into the carotenes, majorly having carbon and hydrogen in their structure and xanthophylls, which are oxygenated derivatives. Besides providing pigmentation, carotenoids have crucial roles in the biological system, such as scavenging reactive oxygen species (ROS), quenching of chlorophyll triplet state, light-harvesting, and dissipation of excess energy [1,2]. Carotenoids are antioxidants and prevent oxidation of proteins, lipids, DNA, thus protecting chlorophyll by quenching singlet oxygen arising from absorption of sunlight by chromophores [3].

Xanthophyll, the oxygenated derivatives, are categorized under three groups based on the presence of

Abbreviations: ASX, astaxanthin; APGS, astaxanthin polyethylene glycol succinate conjugates; CAT, catalase; CVD, cardiovascular disease; EFSA, European Food Safety Authority; FDA, Food and Drug Administration; GSH-PX, glutathione peroxidase; MDA, malondialdehyde; MED, minimal erythema dose; MMP-1, metalloproteinase-1; ROS, reactive oxygen species; SOD, superoxide dismutase.

functional group: the –OH group (i.e., zeaxanthin), the =O group (i.e., canthaxanthin), or both –OH and =O group (i.e., astaxanthin [ASX] and lutein). A xanthophyll carotenoid that is gaining attention worldwide is ASX. ASX has the molecular formula $C_{40}H_{52}O_4$ and a molecular weight of 596.8 Da [4]. ASX, a ketocarotenoid, belongs to the terpene group and is insoluble in water but soluble in most organic solvents. ASX (3,3′-dihydroxy-β, β′-carotene-4,4′-dione) is a red color, a fat-soluble compound that is very much associated with carotenoids like zeaxanthin, β-carotene, and lutein.

ASX possesses an unusual antioxidant activity, even more, significant than vitamin C, β-carotene, and α-tocopherol [5]; because of this, it has received considerable attention in the prevention and cure of cancer, anti-inflammatory, atherosclerosis, antiaging, eye-related disorders, and cardiovascular disease [6–8]. The present market is chiefly dominated by synthetic ASX and is worth 1000 USD/kg [9]. Chemically synthesized ASX is a combination of different isoforms; thus, it is not pure and is cheap.

It has been approved as a natural food additive for animal and fish feed and as a natural dye for animal and human diet by The US Food and Drug Administration (FDA) and European Commission, respectively [10,11]. A total of 66 tons of algal biomass is produced by 17 countries of the European continent (France, Netherlands, Denmark, and Germany). ASX market value is predicted to reach a worth of 3.4 billion USD by 2030 [12]. The market values depend on a number of factors, including production cost, commercial model, and marketing strategies. Business to business value is in the range of 6.000 and 8.000-euro/kg while for the business to customer range it is between 150- and 300-euro/kg [13]. ASX is used in industries like food and feed [9,14], aquaculture [15], pharmaceutical [13,16], health and personal care [12,17–19]. Due to the wide application of ASX, it is practical and profitable to harvest ASX for commercial purposes.

Two factors that have paced up the synthesis of natural ASX are the higher biological activities of the natural bioactive compound than synthetic ASX and safety for human use [20]. However, there are certain limitations associated with natural ASX production as it is a time-consuming process and involves intricated cultivation techniques. Table 1 shows a comparison between synthetic and natural ASX. Naturally, ASX can be obtained from bacteria, fungus, microalgae, and a few plants. Microorganisms like algae, for instance, *Chlorella zofingiensis, Chlorococcum, Haematococcus,* and yeast-like *Phaffia rhodozyma* are the potential ASX producers [21,22]. *Haematococcus pluvialis* has gained the worldwide attention of researchers. Natural ASX is highly pigmented and is considered safe compared to synthetic ASX. *H. pluvialis* accumulates ASX under stress conditions like high salt, nitrogen deficiency, low light intensity, and high temperature. In 2019, the worth of the global market was assessed to be USD 1 billion by the Grand view research and predicted it to reach up to USD 3398.8 million by 2027 with an annual growth rate of 16.2% from 2019 to 2027, due to rising awareness, and associated health benefits [23]. The present review discusses all the aspects of natural ASX: source, chemistry, bioavailability, safety standards, and current production technology. Also, this paper gives a detailed mechanism for the potential role of ASX as nutraceuticals for cardiovascular disease prevention, skin protection, antidiabetic and anticancer, cosmetic ingredient, natural food colorant, and feed supplement in poultry and aquaculture.

2 | MERITS OF NATURAL ASX

Chemical synthesis of ASX is a low-cost process and achieved via Wittig reaction of 3 methyl-5-(2,6,6-trimethyl-3-oxo-4-hydroxy-1-cyclohexenyl)-2,4-pentadienyltriaryl phosphonium and 2,7-dimethyl-2,4,6-octatrienedial [24,25].

TABLE 1 A comparative summary of natural astaxanthin and synthetic astaxanthin

Type	Merits	Demerits
Synthetic astaxanthin	• Cost-effective • Stereoselectivity intermediates • Better shelf life • High availability • Low antioxidant activity	• Complex synthetic reactions • Use of hazardous reactants, including petrochemicals • Adverse effect on the environment • Carcinogenic in nature • Nonsustainable and nonrenewable
Natural astaxanthin	• Generally regarded as safe for human use • Better antioxidant activity • Negligible impact on the environment • Sustainable and renewable • Eco-friendly	• Expensive biorefinery process • Costly production • Short life span • Low availability • Space and time consuming

On the contrary, ASX production via microorganisms is a high-cost process [26]. Chemical and natural ASX differ in their structure; synthetic ASX is an esterified compound and composed of a 1:2:1 ratio of isomers (3S,3′S), (3R,3S), and (3R,3′R). In contrast, bacterial ASX is unesterified and is an isomer of (3S,3′S), and ASX from yeast is an unesterified isomer (3R,3′R) [9,21,27]. Synthetic ASX contains residues of intermediate, due to which it is not considered safe for human consumption but used for feed in aquaculture [28]. Natural ASX, on the contrary, is used for human consumption and aquaculture by USA, China, Japan, and other European countries [4,20,29]. Natural ASX has better pigmentation and antioxidant activities as compared to synthetic ASX [20,28]. In comparison to synthetic ASX, natural ASX was 50 times stronger in quenching of singlet oxygen and 20 times stronger in neutralizing free radicals. Chemical ASX is not safe for humans and the environment; also, consumers are well aware and want safe and eco-friendly products, which makes it highly favorable to replace synthetic ASX [30].

A significant fraction of the total produced ASX, that is, around 95%, is synthetic ASX and dominates the global market because of low-cost production and cheap prices. In contrast, only a small portion of the market is natural ASX ($5000/kg, not extracted in biomass), usually sold as a cosmetic ingredient, food supplements, and food additives [31]. Chemical ASX is priced at or above $2000/kg, higher than other biologically produced carotenoids, including lutein and zeaxanthin [32]. Using natural ASX instead of synthetic ASX for feed is promising, but it is not cost-effective. One cannot deduce its high cost with the current knowledge and technique of natural ASX production [33]. With the constant improvements in the present method, the cost of natural ASX can be reduced [34]. Apart from the drawbacks of the chemical production of synthetic carotenoids, certain advantages include the production of pigments with desired purity and consistency. Specific isomers of synthetic carotenoids are not found naturally, which could produce different lethal and beneficial activities. The chemical production of ASX is not an energy-intensive process and releases greenhouse gases in a small amount. Due to structural variability, natural ASX shows higher stability, higher oxygen radical absorbance capacity, and assimilability than synthetic ASX. Only natural ASX can be used as dietary supplements for food and feed applications.

3 | ASX SOURCES

As stated above, the organisms for natural ASX include algae, bacteria, yeast, shrimp, trout, crayfish, yeast, and salmon. In contrast, for commercial production of ASX, microalgae (*H. pluvialis*) and *Phaffia* yeast (*Xanthophyllomyces dendrorhous*) are the primary sources (Table 2) [22,35,36]. Plants are also documented for ASX production, but to date, only *Adonis annua* is known to produce 1% ASX of the dry weight (DW) of petals [37]. Though plants are potent, ASX producers still can't be applied for commercial production due to small flowers. *H. pluvialis* is the best and most accepted organism for the synthesis of ASX for human consumption. Among all the microorganisms, under stress conditions, *H. pluvialis* has been reported to amass ASX up to 5% on a DW basis [5,38]. The ASX production life cycle of *H. pluvialis* must be regulated by inductive factors like nitrogen starvation, salinity, high light intensity, extreme temperature, salinity, and pH changes. In contrast, noninductive

TABLE 2 Microbial sources of astaxanthin

Species	Astaxanthin (% dry weight)	Reference
Microalgae		
Haematococcus pluvialis NIES-144	7.72	[40]
H. pluvialis	4	[41]
	3.8	[42]
Botryococcus braunii	0.01	[43]
Coelastrum sp. HA-1	0.63	[44]
Chlorococcum sp.	0.57	[45]
Scenedesmus vacuolatus SAG 211/15	0.27	[46]
Scenedesmus sp.	0.41	[47]
Chlorella sorokiniana	0.12	[48]
Tetraselmis sp.	0.22	[48]
Algae		
Thraustochytrium sp.	0.2	[49]
Catenella rapens	0.02	[50]
Enteromorpha intestinalis	0.02	[50]
Ulva lactuca	0.01	[50]
Yeast		
Xanthophyllomyces dendrorhous ATCC96594 (GM)	0.94	[51]
Bacteria		
Agrobacterium aurantiacum	0.01	[52]
Paracoccus carotinificiens	2.30	[53]
Paracoccus kocurii	1.10	[53]

characteristics are required for the sustenance of cells during the green phase [39]. ASX from *H. pluvialis* is believed to be up to 95% pure, much higher than other microalgae.

Another alga, *Chlorella zofingiensis,* produces natural ASX, approximately 50% of the total carotenoid content. Also, Yuan et al. [54] compared the potential production of monoesters and diesters by *C. zofingiensis* (76.3% of diester and 18% of monoester) with that of *H. pluvialis* (35.5% of diester and 60.9% of monoester). They observed a significant reduction in the production of monoesters.

Wild salmonids are an excellent source of ASX; by eating 165 g salmon daily, one can gain 3.6 mg of ASX. The lowest ASX content was reported in Chum and the highest in *Oncorhynchus* species (26–38 mg/kg flesh). Japanese and European markets extract ASX from large trout [55]. Despite a significant portion of ASX, that is, ~95% synthetically produced, natural sources of ASX (<1% from *H. pluvialis* and rendered from the yeast *X. dendromyces*) are preferred due to consumer awareness about natural and synthetic ASX.

4 | CHEMISTRY OF ASX

Kuhn and Sorenson [56] first identified the chemical structure of ASX. The basic skeleton is made up of two terminal rings bridged by a polyene chain. Two asymmetric carbons bonded with hydroxyl groups occupy the 3,3′ site on the β-ionone ring on either end of the molecule. When any hydroxyl group reacts with a fatty acid, a monoester is produced, while a diester is formed when both hydroxyl groups react with fatty acid. Diester form of ASX is twice more potent than free ASX and is 1.6 times more effective than monoester form. The terminal keto and hydroxyl groups on each ionone ring attribute to the characteristics like polar configuration, capacity to undergo esterification, and potent antioxidant activity of ASX [57]. The red color of ASX is due to the presence of a conjugated double bond. ASX is biologically more effective in protecting the cell because there is a coexistence of hydrophilic and hydrophobic properties imparted by the fatty acid esters. That's why it is used as a stabilizer to sustain the high antioxidant capacities in both conditions [21,58]. The hydrogen atom at C3 methine of a terminal ring of ASX molecule acts as a radical-trapping site [59]. The terminal ring of ASX lies in the hydrophilic layers. At the same time, the polyene chain is positioned in the lipophilic area, spanning the membrane. This links the cell membrane from inside to outside, thus increasing the cell defense [58]. Most antioxidants have a high potential of quenching Reactive oxygen species and free radicals, including hydrogen peroxide, superoxide anion, singlet of the inner and outer side like vitamin E and β carotene or vitamin C, respectively [59]. Due to hydroxyl and keto groups in the same structure, ASX can effectively neutralize ROS. ASX ceases the free radical formation and antioxidation chain reaction by quenching ROS, such as singlet oxygen, hydroxyl, and peroxyl radicals, and transforming them into a stable compound. In addition, ASX functions as a metal chelator and produces harmless molecules from metal prooxidants [60]. ASX is unique in this property as it shows its activity in the outer and inner layers of the cellular membrane.

4.1 | Isomerization of ASX

ASX exists as a geometric isomer, stereoisomers, in both free and esterified forms. There are two geometric isomers (*cis* and *trans*), among which, thermodynamically, *trans* isomers are more stable than *cis* isomers [61]. *Cis* isomer of ASX is believed to have higher bioactivity antioxidant capacity than *trans* isomer, even though the latter is more stable than *cis* isomer [62,63].

There are two asymmetric carbon atoms present at the 3 and 3′ positions of the ASX molecule. Three different enantiomers are 3S,3′S, 3R,3′R, and 3R,3′S. Synthetic ASX is a mixture of 3R,3′R, 3R,3′S, and 3R,3′R in a ratio of 1:2:1 [64]. The *trans* isomers are (3R,3′R), (3R,3′S) and (3S,3′S) and among all-*trans* isomers, (3S,3′S) isomer is the most abundant with high biotechnological value [65]. The 3S,3′S isomeric form is more effective on human health than other isomers.

5 | BIOAVAILABILITY AND SAFETY OF ASX

Bioavailability and absorption of carotenoids depend upon several factors; the former depends explicitly on the structure. For instance, the polar species of carotenoids (e.g., free ASX) are more bioavailable than nonpolar carotenoids (e.g., lycopene and β-carotene) [66]. The *trans* isomeric form has a shorter chain length than the *cis* isomer and less blood plasma. In humans, the absorption of xanthophyll ester and hydrolyzation occurs in the small intestine. Enzymatic esterification of ASX proceeds at a lower rate in intestinal cells and is mediated by enzymes after absorption from the intestine [67]. Absorption depends on the dietary compounds taken along with the carotenoids; for instance, high cholesterol in a diet can increase carotenoid absorption. Along with cholesterol, some dietary oils (e.g., fish oil) also increase the absorption of carotenoids and enhance the hypocholesterolemia/hypolipidemic effect in plasma and amplify the action of activated neutrophils [68].

Comparing the biological effects of fish oil and ASX, the latter is more effective in improving immunological responses and reducing the chances of vascular and infectious diseases. ASX, in combination with fish oil, increases the activity of enzymes such as catalase, glutathione peroxidase (GSH-PX), and superoxide dismutase (SOD), which lower down the production of oxygen ($O_2^{\bullet}$), nitric oxide, and hydrogen peroxide, causing a decrease in T and B-lymphocytes proliferation activity and calcium release in the cytosol [69]. A study conducted by Ranga Rao et al. [70] showed an increase in bioavailability and antioxidant properties when plasma and liver tissues of rats were fed on *H. pluvialis* biomass suspended in olive oil.

Micelles are formed in the intestinal tissue, where they are partially absorbed by mucosal cells after mixing ASX with bile acid. The mucosal cells of the intestine incorporate ASX into chylomicron and get released into the lymphatic fluid. The ASX complex is digested with the enzyme lipoprotein lipase, found primarily on the cells' surface and excreted by the liver and other tissues. Then transported into the tissues in the form of lipoprotein [71]. Many studies were conducted on rats to standardize an optimum dose of ASX for human consumption [72,73]. Earlier, in 2014, the European Food Safety Authority (EFSA) Panel on additives and products or substances used in animal feed recommended 0.034 mg/kg/day and 2.38 mg/kg dose of ASX for rats and humans (weighing 79 kg), respectively. Later, the safe intake dose was updated to 4 mg/day or 0.06 mg/kg/day by the EFSA Panel on nutrition, dietetic products, and allergies. In a study, 19 healthy participants were fed with 6 mg/day of ASX for 8 weeks in their diet to evaluate its effect on blood pressure, cell blood count, metabolic panel, and other biochemical parameters. No harmful impact was produced [74]. FDA has approved dose as 12 mg/day of ASX from *H. pluvialis* for human consumption, but the intake should be less than 30 days [61]. The world has acknowledged the benefits of ASX through the increasing number of clinical studies; the market call is also increasing and putting the market with greater demand than the supply.

6 | BIOSYNTHESIS OF ASX FROM MICROALGAE

ASX production from microalgae requires the cultivation of microalgae, ASX induction, and harvesting biomass. Figure 1 demonstrates the overview of the process in the biosynthesis of natural ASX from *H. pluvialis*. Nowadays, microalgae cultivation is highly automated. Initially, microalgae are inoculated and cultured under suitable temperatures, nutrients, illumination, and pH, leading to biomass accumulation. Once sufficient biomass is accumulated, inducing conditions are provided for ASX synthesis within the cell. For this, several states are simultaneously offered to give carbon supply and nitrogen deficiency to get more ASX [75].

In the one-stage process, media is supplemented with essential nutrients like carbon, phosphorous, and nitrogen to provide a favorable environment for microalgae growth. With microalgae growth, nutrients deplete, and pH changes, leading to adverse conditions [5]. With the onset of the harsh condition, algal cells start to change into resting cysts and initiate the ASX synthesis pathway. Therefore, one stage process is considered a spontaneous process of ASX production with the fluctuations in the growth environment of microalgae. Inducers are also applied along with microalgae, but sometimes inducers can negatively impact the algae growth [76]. There is no manual involvement in one stage production process; therefore, ASX production can be cost-effective. Usually, to alleviate the problem of the one-step process, companies adopt a two-step process for the cultivation of *Haematococcus,* which is based on the green and red stage in the life cycle of *Haematococcus* [76,77]. The green stage is characterized by reproducing cells and biomass accumulation, while the red stage includes loss of motility and reproduction. In the red stage, ASX starts accumulating within the cell, and it can be up to 5% of ASX of dry biomass weight [5,38]. Cells of *Haematococcus* are initially cultivated in enclosed photobioreactors either mixotrophically or phototrophically, under normal growth conditions. After biomass accumulation and cell proliferation, cells are shifted to either raceway ponds or large-scale photobioreactors and stress, and nutrient-deficient conditions are imparted for the ASX accumulation and phototropic encystment [78,79]. Also, in few cases, the red stage is carried out in stainless steel fermenters either mixotrophically or heterotrophically; however, phototrophic induction is more efficacious [40]. ASX induction takes at least 3–5 days, leading to the encystment process, causing the formation of aplanospores (cysts). Once the aplanospores are formed, they are harvested using gravitation settlement first and then ultracentrifugation in later stages. This will break open the cell by disrupting the cell wall and allows access to ASX. The biomass is then spray dried usually as it is a cost-effective method compared to drum drying and freeze-drying, which is then cracked by high-pressure homogenizer or bead millers followed by dehydration by belt or spray dryers [4]. During the 1990s, edible oils or organic solvents like propanol, hexane, and ethanol were applied to extract ASX, but with recent development,

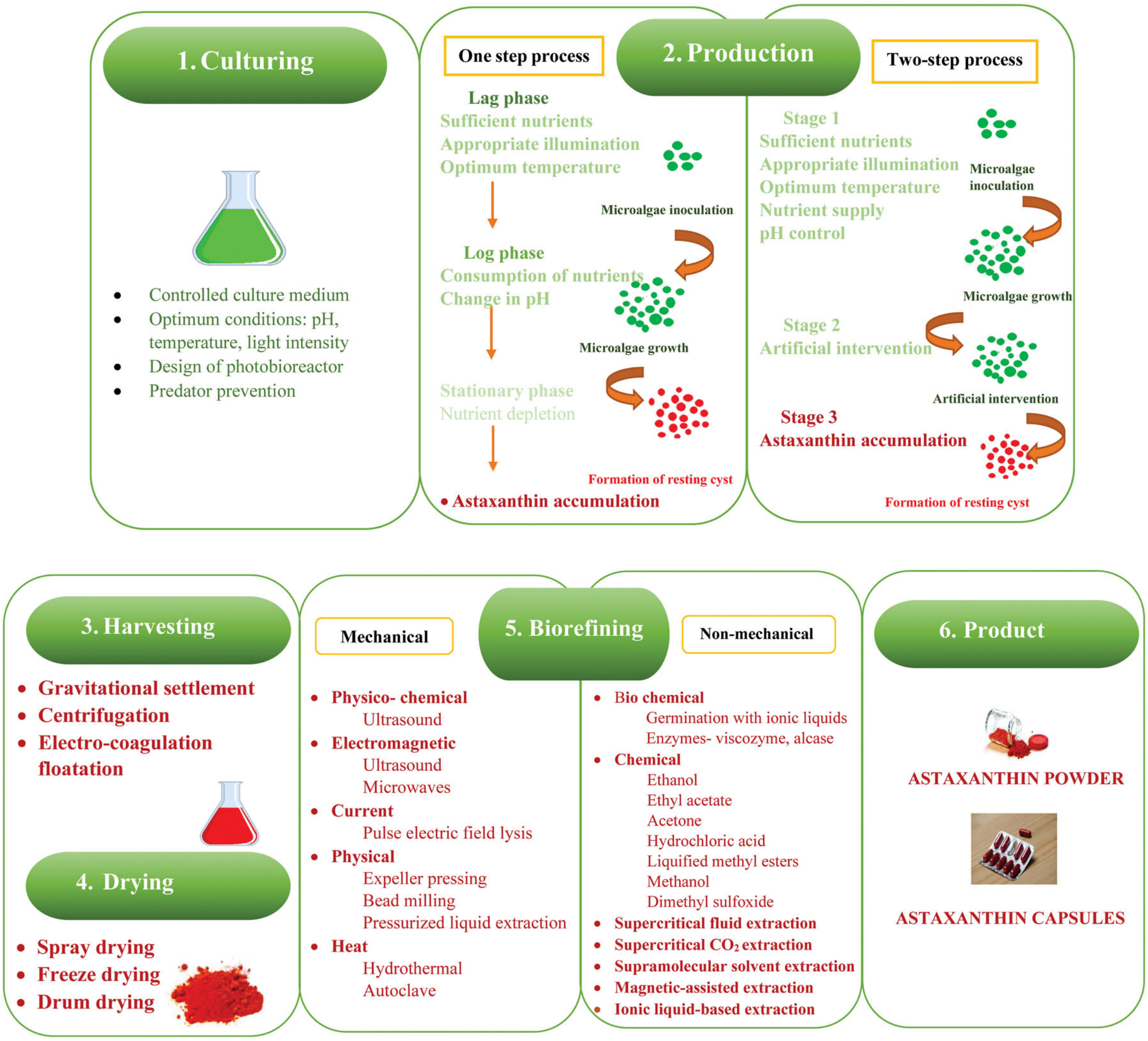

FIGURE 1 Overview of the biosynthesis of natural astaxanthin from *Haematococcus pluvialis*

supercritical fluid extraction with CO_2 is now used [76,80]. Harvesting of aplanospores is crucial as they are resistant to digestion by animals and humans; thus, aplanospores must be broken open to extract ASX. Cost and biomass safety should be taken into consideration during the harvesting process. A 20%–30% of the total cost of ASX accounts for the process of biomass harvesting [81], which, if cut down using cost-effective harvesting techniques, can lower down the price of ASX. Also, the harvesting method should be selected to eliminate toxic compounds contamination in the biomass; for example, the flocculation of microalgae by Al^{3+} can lead to aluminum accumulation in ASX products and food chains and thus should be prevented [82]. The production process must be economical and environmentally sustainable, so to reduce the carbon footprint. This can be achieved by using renewable sources of energy, reducing energy and water consumption. Up till now, only one LCA has been published on *H. lacustris* derived ASX. Electricity is chiefly responsible for environmental degradation, mainly in green stage cultivation [83]. To combat this, producers like AlgaTechnologies use solar power as an energy source (specified at 250 W, 15% conversion efficiency, 1.65 m^2) [84]. Around 1000–1500 tons of freshwater is required per ton production of *H. lacustris* biomass, but simultaneous culturing of the red stage effectively reduces water consumption. Also, only 30% of water is used as an open pond [85]. For the sustainable production process, the biorefinery approach, that is, "high-value product first," can be applied to the combined production of ASX, phytosterols, and polyunsaturated fatty acids. The residual biomass is used as a protein source or a biofertilizer [86]. Waste from soft drinks like carbonates and flue gases like carbon dioxide could pave the way for environmental sustainability. However, for flue gases, a buffering system is essential to conventional and expensive HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid).

7 | PRACTICAL APPLICATION OF ASX

ASX is a super antioxidant and lacks provitamin A activity. It is studied extensively due to its potent biological activities, including anticancer, antidiabetic, anti-inflammatory, antioxidative activities [6,7,87]. At singlet oxygen quenching, ASX is 800 times stronger when compared to coenzyme Q, 550 times than green tea catechins, 6000 times than vitamin C and 11 times than β carotene up to 2.75 times than lutein [33,88]. ASX is considered a "super oxidant" as it spans the biological membranes and protects them, especially the lipids, including phospholipid, against peroxidation by scavenging the free radicals. Due to higher antioxidant activity other than any carotenoids, there is an increasing demand for the use of ASX in the nutraceutical, cosmetic, food, and pharmaceutical industries (Figure 2).

7.1 | Application of ASX in nutraceuticals

7.1.1 | Antidiabetic activity

Diabetes mellitus causes dysfunction and tissue damage of β-pancreatic cells, which induces hyperglycaemic conditions. This results in high oxidative stress in humans. It is linked with excessive production of ROS and lower antioxidant defense capacity. One way ROS is produced in the biological system is through activated neutrophils and macrophages. ROS, so, had led to oxidative stress, causing chronic inflammatory disease [8]. ASX being an antioxidant, can reduce oxidative stress and improve serum insulin and glucose levels in the blood. In a randomized controlled trial (RCT), a total of 54 patients having type 2 diabetes were fed with 12 or 6 mg of placebo and ASX daily for 8 weeks. Continuous intake of ASX reduced the plasma interleukin-6 (IL-6) levels, tumor necrosis factor-α (TNF-α), HbA1c (%), blood glucose levels. A higher dose could lead to lower levels of plasma TG, total cholesterol, LDL, clotting factor-like factor VII, and plasminogen activator inhibitor [89]. Ursoniu et al. [90]. collected and studied the data of 10 RCTs of the recent meta-analysis and revealed a decrease in blood glucose level with ASX supplementation [90]. Chen et al. [91] observed direct transport of ASX liposome altered by the glucose-PEG6-DSPE ligand to the glomerular mesangial cell membrane via overexpressed GLUT1 and attained better drug delivery-targeting kidneys. Yet, there is a scope for more research to recognize the exact mechanism of ASX in diabetes; for instance, immune systems secrete cytokines, carotenoids, and chemokines, but ASX alter the immune system by suppressing the cytokines and chemokines via antioxidant mechanism; there is plenty of evidence to support protective effects of ASX against oxidative damage caused due to cardiovascular diseases, diabetes, neurodegenerative diseases, cancer and obesity [92].

Combined supplementation of mice diets with ASX and astaxanthin polyethylene glycol succinate conjugates (APGS) lowered the serum insulin level and amended lipid accumulation compared to sole supplementation. Sole supplementation of APGS curbed proinflammatory cytokines secretion (CD68, TNF-α, and MCP-1) and increased the number of CD68-positive cells as observed by Liu et al. [93]. Mashhadi et al. [94] conducted a study on humans; a total of 44 participants suffering from two different types of diabetes were fed for 8 weeks with an 8 mg supplement of ASX. They observed increased serum adiponectin concentration and reduced visceral body fat mass ($p < 0.01$), fructosamine concentration ($p < 0.05$), serum triglyceride plasma glucose concentration ($p < 0.057$), along with a decrease in lipoprotein cholesterol concentration and systolic blood pressure ($p < 0.05$). Chan et al. [89] studied the same observations with 54 diabetic patients (with two types of diabetes). They were selected in a double-blind RCT and for 8 weeks were given 6 mg/day of ASX and 12 mg or placebo/day; the former concentration led to a substantial reduction of glucose level in the blood, plasma IL-6, TNF-α, and HbA1c while 12 mg or placebo/day dropped the concentration of cholesterol, clotting factors (plasminogen activator inhibitor and factor VII), and plasma triglycerides.

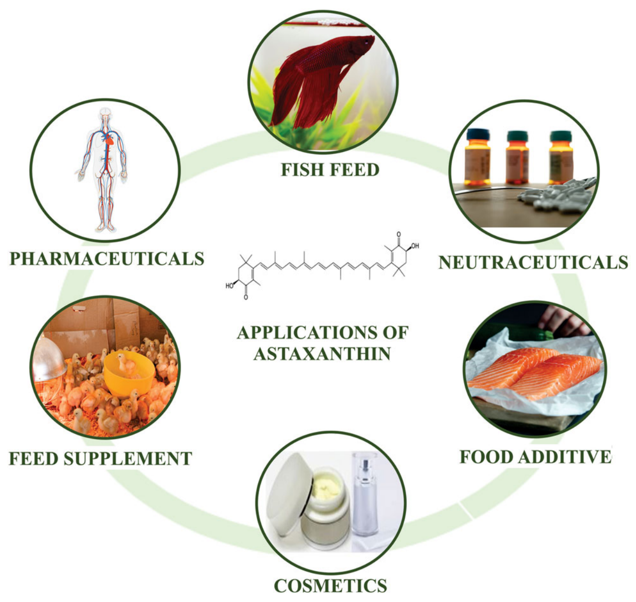

FIGURE 2 Practical application of natural astaxanthin

7.1.2 | Cardiovascular disease prevention

Cardiovascular disease or disorders or CVD are the ailments related to blood vessels and the heart. These include rheumatic heart disease, heart failure, congenital heart disease, peripheral heart disease, coronary heart disease, and cerebrovascular disease [95]. CVD is the primary reason behind morbidity and mortality worldwide, and the expected death by 2030 is 23.3 million people as per reports of the World Health Organization [60,96]. Several cardiovascular disorders are due to reactive oxygen species. ROS are also behind the pathophysiology of cardiomyopathy, ventricular remodeling, cardiac hypertrophy, heart failure, myocardial infarction, and ischemia/reperfusion injury. Carotenoids, antioxidants, polyphenols, vitamin E, and ascorbic acid work effectively to cure and prevent CVD [96]. Carotenoids reduce the proinflammatory cytokines, lower blood pressure, and improve insulin sensitivity in the liver, adipose tissue, and muscle. ASX improves blood lipid profiles and extensively studies CVD treatment and prevention (Figure 3) [97].

ASX has been tested for its effect in animals as well as human subjects. Atherosclerotic cardiovascular disease is related to oxidative stress and inflammation; constant efforts have been put forward to test agents such as β-carotene to treat the illness but always handed over with disappointing results [98]. ASX modulates the blood fluidity and exerts antihypertensive effects, either by normalization sensitivity towards adrenoceptors (a-adrenoceptors) by sympathetic pathway or by refurbishing the vascular tone through attenuation of the angiotensin II and reactive oxygen species-induced vasoconstriction [99]. Kato et al. [100] conducted a pilot clinical study on 16 patients vulnerable to heart failure. They were given a supplement of 12 mg ASX for 3 months. They were assessed for 6 min walk distance, blood pressure, BMI, left ventricular ejection fraction, heart rate, and numerous oxidative stress markers, including urinary 8-hydroxy-2-deoxyguanosine, biological antioxidant potential, and the dacron-reactive oxygen metabolites (d-ROM). They observed increased concentration of ASX in plasma, resulting in lower d-ROM levels followed by low oxidative stress. Also, in left ventricular systolic dysfunction patients, cardiac contractility and exercise tolerance was improved. In adults suffering from milk hyperlipidemia, ASX decreases HDL-cholesterol and serum TG levels and increases adiponectin levels [101]. In the Zucker fatty rats, the ASX's renin-angiotensin system activity and blood pressure could be lowered. A recent study showed increased heart mitochondrial membrane potential and contractility index dose-dependently. It decreased tumor necrosis factor-a, plasma IL-1a, and serum amyloid A concentration in BALB/c mice, supporting the possible effect of ASX in cardiac protection [60].

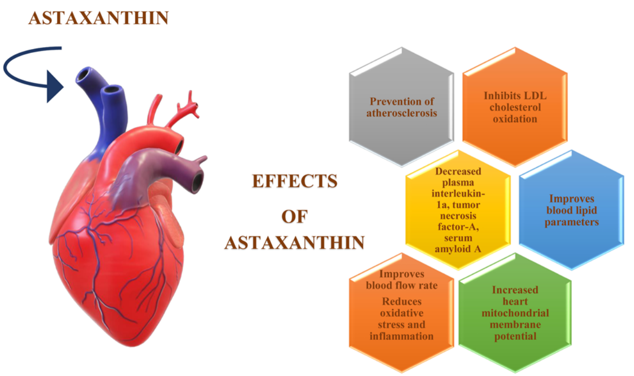

FIGURE 3 Effect of natural astaxanthin on cardiovascular disease prevention

7.1.3 | Anticancer activity

Besides their antioxidant activity and ability to transform into retinoids, Carotenoids are also associated with anticancer activity. Some of the carotenoids are more potent anticarcinogens as compared to β-carotene [102], which can be concluded by a study conducted by Bertram et al. [103] The study revealed the failure of β-carotene in protecting individuals with lung cancer; instead, it induced lung pathology. To combat such a situation, scientists suggested using carotenoids like ASX which lacks provitamin A activity, avoiding the toxicity of retinoids. On equating the effects of canthaxanthin and ASX, on intercellular communication of gap junction, essential for homeostasis, cell development in skin fibroblast, and growth control, ASX was found to be the most potent suppressor and alters the phosphorylation pattern of connexin43, affecting the channel function. At the same time, ASX and some other carotenoids enhance intercellular communication of gap junction and stimulate connexin43 expression [104].

BALB/c mice with a mammary tumor cell line were fed with ASX before tumor initiation, resulting in mammary tumor growth suppression, rise in the levels of plasma interferon-g, and natural killer cells. However, when ASX was given after initiation of a tumor, it resulted in hasty growth of the tumor and enhanced tumor necrosis factor-a and plasma inflammatory cytokines IL-6. Studies showed that when 50 μM of ASX as 5 days treatment is given to mice, a 94% decreased cell viability of epidermal JB6 P + cell line and abortion of neoplastic transformation was observed [105]. Research on melanoma cell lines A375 and A2058 by Chen et al. [106]

resulted in 50% and 80% cell proliferation, respectively, with 168 μM dose of ASX. Moreover, in the case of hepatoma cells (H22), supplemented with low (20 μM) and high-dose (40 μM) of ASX, a more enhanced effect was observed for high dose with time (after 12 and 24 h) while for a lower dose, the efficiency was identical with that of cisplatin. This anticancer drug was used as a control [107]. With the help of in silico molecular docking, Suganya and Anuradha [108] reported that in hepatocellular carcinoma, ASX can show a more pronounced effect than the standard chemotherapy agent sorafenib. Hormozi et al. [109] studied the LS-180 cell line and observed apoptosis and antioxidant activity; an overall increase in the Bax and Caspase3 gene expression, with a decrease in Bcl2 and the growth cells proliferation inhibition, was observed after supplementing with ASX. ASX stimulated catalase, SOD, and GSH-PX, reducing the malondialdehyde (MDA) concentration, indicating enhanced antioxidant activity. It can be concluded that ASX is a potential therapeutic compound. It enhances the apoptotic gene expression and antioxidant enzyme activity, thus inducing apoptosis in LS-180 cells and preventing and curing cancer.

7.1.4 | Skin-protective effect

Oxidative stress so caused by the production of ROS influences skin aging and damage. Oxidative stress is known to cause DNA damage and produces MMPs (metalloproteinase) and inflammatory mediators for collagen degradation. Generally, dermal problems such as skin sagging, wrinkling, dryness, laxity, and pigmentation are due to extended UV-A exposure, which can be cured by topical or oral administration of ASX [110]. ASX suppresses wrinkle formation and skin thickening, increases collagen fibers, capillaries density, and inhibits ROS and epidermal thickening in the skin [111]. Also, photoaging and capillary regeneration are associated events, while a contrary relationship exists between wrinkle formation and the density of capillaries.

In Japan, Tominaga et al. [112] observed the effect of ASX on skin deterioration by assessing anti-inflammatory action for which in vitro ASX treatment was given to fibroblasts (grown in media of UV-B-induced keratinocytes). It decreased the inflammatory response in the fibroblast and matrix of MMP-1. Moreover, 65 healthy female individuals were fed with 6 or 12 mg of ASX or a placebo and were observed for 16-weeks. Results showed reduced deterioration of skin induced by aging and improved skin health. A similar, double-blind, randomized, placebo-controlled study was carried out by Ito et al. [113] in which 23 healthy humans were given a placebo or ASX capsule of 4 mg for 9 weeks, after which the minimal erythema dose (MED) and transepidermal water loss were analyzed at the reference point, along with a subjective visual analog scale for the participants. The outcome was positive effect with alleviated MED and a reduction in skin moisture loss from irradiated areas; also, skin texture was improved. Hence, the studies mentioned above suggest ASX as a protective agent against skin damage by UV rays and helps improve skin health.

7.2 | Application of natural ASX in the cosmetics industry

Of lately, increasing concern and awareness towards mother nature has paved the way for consumers towards a green and sustainable lifestyle. In the past few years, consumers have been making smart and responsible choices, which could dampen the impact on the environment [114]. One of the hazardous commodities in the market is synthetic colors, and with awareness, there is an uprising demand for natural and sustainable replacements. There is a high market value for natural pigments extracted from microalgal biomass, and the need is believed to rise with increasing knowledge about nutritional aspects of microalgae [115]. Apart from food, some microalgae species have application in cosmetics and colorants and are tested to be consumer-friendly. Many scientists have reported the skin health benefits of microalgal pigments, including antiaging, skin protection, color, skin whitening, skin pigmentation, and antioxidants. Pigments extracted from microalgae are an active ingredient in skincare products like emollients, anti-irritants, antiaging, and sunscreen creams [116].

One such primary pigment from *Haematococcus* and *Dunaliella* is carotenoid. It includes lutein, canthaxanthin, ASX, and zeaxanthin; they are effective as they have antioxidant activity and protective characteristics against sun damage [117]. Tominaga et al. [118] reported that the corneocyte condition is reverted to normal when given ASX treatment, thus preventing the keratinocyte differentiation and cornification from oxidative damage. A study on *H. pluvialis* derived ASX showed the protective effect of the ASX against UV-A radiations on filaggrin metabolism and desquamation in the epidermis and the extracellular matrix in the dermis [119]. A diminution of structural ability and physiological functions with aging leads to higher chances of dermatological disorders like wrinkles, xerosis, laxity, dermatitis, and cherry angioma [120]. ASX is the most popular and powerful antioxidant and possesses great potential in skincare, especially antiaging [121].

Daily, human skin has to be exposed to ultraviolet radiations, which produces ROS and degraded skin health; and these oxidative stress cause uneven skin tone, premature aging, and lessens the essential proteins of the skin. With aging, there are many changes in the skin, such as DNA breakdown, low synthesis of antioxidants, inflammatory response, and MMPs, which causes the breakdown of collagen and elastin, along with other oxidative events. These can also cause skin damage by decreasing tensile strength and elasticity, causing age spots, wrinkles, and interruption in the cellular dermal matrix [122]. Therefore, a dynamic equilibrium between Reactive oxygen species and antioxidants acts as a shield against oxidative stress. ASX constrains collagenases, MMP activity, ROS induction, and inflammatory mediators leading to antiwrinkle and antioxidant effects in the skin [123]. There is a demand for natural colorants in cosmetic industries because of their additional health advantage, including antiaging, UV protection, antioxidant, and antibacterial [124].

With the most beneficial properties among all carotenoids, ASX is a potent antioxidant and colorant [125]. Worldwide, particularly in Asia, people are fascinated with skin whitening products as Asian women lean towards fair skin tone, making skin whitener a favorite and best seller product in Asia [126].

7.3 | Application of ASX as a natural food colorant

As emphasized already, there is a shift in demand towards naturally derived products, thus imposing significant innovation [127]. Organoleptic characteristic of any food color impacts the consumer's choice, selection, and acceptance of food. The basic idea behind the food color is to provide an appealing and attractive color to food and beverages, which affects the consumers' food selection decision and preference [128,129]. Exclusively all food products possess a characteristic color and have a defined processing and storage techniques, including air, light, moisture, and temperature, thus affecting the color of the final food product [129]. Also, food colorants can mask unpleasant food attributes. On the basis of the source of extraction, food colorants are bifurcated into natural and synthetic; however, from safety perception, several artificial colors are banned in many countries as some carry lead acetate (cause of nerve damage), allergens, irritants, and even carcinogenic chemicals as well. Therefore, with increasing awareness, people rely more on natural food colorants as they are safer for consumption [130–132].

Colorants extracted from microalgae offer an easily controllable production, extraction, high yield, and depend upon no seasonal variations. ASX is a potential functional food and can effectively work out as a substitute for flour in cookies [133]. However, a practical and commercial approach to food requires more research for stability, preservation, encapsulation, and storage [134].

7.4 | Application of ASX as a feed supplement in aquaculture and poultry industry

Lately, with increasing knowledge about ASX, it is also used as a diet supplement for animal farming [135,136]. ASX is reported to improve immunity, thus reducing the usage of antibiotics and the mortality rate of animals [137]. ASX is a source of pigmentation for ornamental fishes and other aquatic animals like rainbow trout [135]. Market demands for feed supplements are rising, paving the way for industrial applications of naturally derived ASX. Also, microalgae utilize carbon and release oxygen, thus alleviating greenhouse effects; therefore, ASX production can benefit the environment [1,138]. The direct use of microalgae as a diet supplement for aquaculture is a good option due to the expensive extraction process of ASX from microalgae. Notably, the use of natural ASX as a feed supplement for aquaculture is usually safe, and feeding salmon and trout with ASX up to the maximum dietary limit is of no concern and ensures the safety of consumers [139].

Synthetic chemicals and natural compounds have been widely used for pigmentation in the aquaculture and poultry industry. Synthetic ASX has been used for 20 years. Still, natural ASX has tremendous potential to replace the synthetic ones in the aquaculture industry. It imparts color and offers essential supplements for commercial fishes, trouts, and shrimps [4]. ASX intake improves the enzyme activity of catalase (CAT) and superoxide dismutase (SOD), which as a result, lowers the ROS in aquatic animals. Lim et al. [140] observed the consequences of ASX intake in Asian seabass. A rise in phagocytic and lysozyme activity and total immunoglobulin was observed. Natural ASX is used as a supplement to increase bioaccessibility in aquaculture-derived salmon [141]. Recently, Li et al. [142] showed improved growth of sizeable yellow croaker fish when fed with natural ASX than synthetic ASX. While in the poultry industry, *H. pluvialis* derived ASX decreases the mortality in hens and chickens, improves the pigmentation of egg yolk, and production of breeding [143–145] improves breast muscle tissue and increases feed efficiency in broiler chicken [146]. Gao et al. [147] studied the effect of ASX on lipid metabolism, antioxidant capacity, and ASX build-up in the egg yolk of laying hens. Hy-Line Brown layers ($n = 288$, 50-week-old) were randomly grouped into one of the four dietary treatment groups. Each treatment set had six replicates of 12 hens. For 6 weeks, all hens were fed corn-soybean meal supplemented with 0, 25, 50, or 100 mg/kg ASX. In the ASX (ASTA) groups, there was an increase in GSH-PX, SOD levels, and total antioxidant capacity in the plasma, livers, and egg yolks, in the ASX (ASTA) groups as compared to control.

In contrast, the MDA levels were reduced linearly. In ASTA groups, plasma levels of high-density and very-low-density lipoprotein cholesterol were higher as compared to control. Liu et al. [148] suggested ASX as a potential dietary supplement for enhancing nutrient utilization and growth in yellow catfish. A study was conducted by Cheng et al. [149] on pufferfish (*Takifugu obscurus*) to evaluate the effect of ASX (80, 160, and 320 mg/kg supplemented diet) on biochemical and growth parameters, ROS production, and immune gene expression under high-temperature stress. ASX alleviated the levels of plasma alkaline phosphatase activities, CAT, SOD, HSP70 messenger RNA, weight gain (WG), and specific growth rate; on the contrary, it decreased the plasma aspartate aminotransferase and alkaline aminotransferase activities. It enhanced the nonspecific immune responses, defense systems, and resistance against high temperature in butterfish. Feng et al. [150] observed the effects of ASX on the growth parameters, pigmentation, and antioxidant activity in blood parrotfish (*Cichlasoma citrinellum* × *Cichlasoma synspilum*) and followed the enhanced apparent impact. Xie et al. [151] experimented with evaluating antioxidant capacity, anti-inflammation, and growth performance on supplementing the feed of golden pompano (*Trachinotus ovatus*) with synthetic and natural ASX from *H. pluvialis* for 8 weeks. In both the studies, growth performance, that is, final body wet weight, WG, and special growth rate were enhanced. Still, the feed coefficient ratio, SOD, and hepatic MDA were lower in both cases as compared to the control diet of fish. At the same time, the inverse was observed for the hepatic total antioxidant capacity (T-AOC). The acute hypoxia trial of ASX and treatment resulted in higher Nrf2, Keap-1, CAT, SOD, GSH-PX, and HO-1 than the control group. The natural ASX from microalgae used for fish cultivation is the main reason for aquaculture development; it enhances the animal's pigmentation and immunity, preventing antibiotic abuse.

8 | CURRENT PRODUCTION FACILITIES

With increasing time, more and more commercial plants are setting up for the biological production of ASX and many pilot-scale plants are under development. The significant leaders are Alga technologies Inc., Israel, Cyanotech Inc., Fuji chemical industries Co. Ltd., Japan, Mera Pharmaceuticals Inc., and Parry Pharmaceuticals Inc. since the 1990s–early 2000s [152,153]. Table 3 depicts a list of natural ASX-producing companies. The Natural Algae Astaxanthin Association (NAXA) encourages *H. pluvialis* derived ASX and educates people about the health effects of natural ASX.

Beijing Gingko Group (BGG), a producer in China, is a potential leader in the global market of natural ASX [154]. The commercial production of ASX from *H. pluvialis* strains like CCAP 34/12, NIES-144, and SCCAP k-0084, preserved in an artificial condition for an extended period as their primary isolation from nature. However, certain companies, including Cyanotech and MC biotech, utilize strains like H2B and a local Brunei isolate, respectively, isolated directly from nature and are not available in culture collections [155]. Commercial production of *H. pluvialis* is challenging, requiring particular growth conditions during the green stage. In 1987, Microbio Resources Inc. carried out commercial production of ASX in powder form (1% DW) in a 50,000-L bioreactor (4500 m^2) and commercialized it in the name of Algaxan Red [156]. The produced ASX was applied for aquaculture and valued <US $ 20/kg, which was less than synthetic ASX US $2000/kg [156].

Commercial production of ASX is a costly affair and suffers few demerits, including low biomass densities prone to contamination, photobleaching (when transferred for the red stage under high-intensity light), and expensive techniques for the extraction of resistant thick-walled aplanospores. Also, production costs will increase if biofouling occurs, resulting in dead areas with inefficient circulation, leading to poor light penetration and increased downtime. Certain companies like Varicon Aqua Solutions Ltd. (PhycoFlow™) and van de Ven and van de Ven [157] have patented their methodology and techniques for automated self-cleaning systems, reducing biofouling and downtime. Outdoor production of *H. pluvialis* also suffers from contamination chances, slow growth rate, low cell density, and adverse conditions [158].

Commercially, ASX is produced via two-stage culture: a green stage for maximum biomass and a red stage for maximum ASX production [159]; however, Algalif, Iceland employs a three-stage production process (green stage, starvation stage, and red stage). Maximum productivity with the outdoor two-stage production process is 8–10 mg/L/day for a 10-day cycle, including the green stage of 4 days and red stage of 6 days, and 4% DW is obtained provided high-intensity light and nitrate starvation in the red stage [159]. But with the mixed culture of palmelloids and motile macrozooids involving one stage production, two times more ASX productivity

TABLE 3 Major natural astaxanthin-producing companies all over the world

Company	Country	Culturing method	Plant size (ha)	Production capacity (ton/year)	Brand name
Algatech International Sdn. Bhd.	Malaysia	Plastic bag photobioreactor	<5	<10	Premia Health Supplements, AstaTude Skin Care product, Premia Functional Food
Alga Technologies Inc.	Israel	Glass tubular photobioreactor + glass tubular photobioreactor	<10	<50	AstaPure®astaxanthin, AstaPure® Arava
Algalif AS	Iceland	Glass tubular photobioreactor	<5	<10	Astalíf®, Astalíf® Astaxanthin oleoresin
Alimtec S.A.	Chile	Raceway pond	<5	<10	Zanthin
AstaBiotec LLC (BGG)	China	Glass tubular photobioreactor + glass tubular Photobioreactor	<20	<100	AstaZine™
Atacama Bio Natural Product S.A.	Chile	Raceway pond	<10	<50	NatAxtin®, Red Meal
ZionBio Algae Health Co. Ltd.	China	Fermenter + glass tubular photobioreactor	<5	<25	AstaXanthin
Cyanotech Inc.	USA	Covered raceway Pond + raceway Pond	<20	<100	**BioAstin**® Naturose
Kunming Biogenetic Co. Ltd.	China	Bubble column photobioreactor + Column photobioreactor	<10	<1	AstaBio
Mera Pharmaceuticals Inc.	USA	Tubular photobioreactor + raceway Pond	<5	<1	AstaFactor
Parry Nutraceuticals Co., Ltd.	India	Raceway pond	<10	<1	Zanthin
MC Biotech Sdn Bhd	Brunei	Glass tubular photobioreactor + glass tubular photobioreactor	<10	<25	Astabio®, AR10, AR5, AP1/AP2, AW1
Supreme Health NZ Ltd.	New Zealand	Indoor LED photobioreactor + indoor LED photobioreactor	<2	<2	AstaSupreme
Sweden AstaReal AB.	Sweden	Indoor photobioreactor + indoor photobioreactor	<5	<25	Astaxine AstaCaroxe, AstaEquus, Novaasta
U.S.A. AstaReal, Inc.	USA	Indoor photobioreactor + indoor photobioreactor	<10	<50	AstaReal® L10 AstaReal® CWS25 AstaReal® P4AF AstaReal® CLEAR100-C(S) AstaReal® Softgels AstaReal® Biomass

(Continues)

TABLE 3 (Continued)

Company	Country	Culturing method	Plant size (ha)	Production capacity (ton/year)	Brand name
Fuji Chemical Industry Co. Ltd.	Japan, Sweden, USA		<5	<25	AstaREAL, AstaCarox
Yunnan LvA Bio-Tech. Co. Ltd.	China	Flat-panel photobioreactor + raceway Pond	<5	<25	Astaxanthin

(20.8 mg/L/day) is achieved under nitrate-depleted conditions. However, the process is still needed to be explored for commercial scale-up [160]. The majority of the producers are producing ASX outdoors because of the requirement of high light intensities and temperature for the red stage, which will not be feasible indoors. Also, primary production occurs outside the tropics. In indoor cultivation, the red stage holds a 59% share of the total electricity cost [161]. ASX production is limited in temperate zones due to inappropriate climate conditions for outdoor production of ASX. The generalized production process followed for commercial production is shown in Figure 1. Fuji Chemicals and Algalif Fuji Chemicals were two companies carrying out outdoor cultivation using *H. pluvialis* in BioDome™ system in Hawaii but stopped because of the contamination problem and began with indoor production using mixotrophic culture in Washington and Sweden [162].

One of the first global companies is Mera pharmaceuticals Inc. (recognized from Aquasearch Inc.), situated in Kona, Hawaii, USA, and carries out two steps of autotrophic cultivation of *Haematococcus* outdoor as the weather is highly appropriate [163–165]. They opted for enclosed and computerized photobioreactors of 25,000 L capacity for the green phase, while the red phase is achieved in raceway ponds. Cyanotech Inc., established in the late 1990s, also applies a similar strategy and cultivates green stage *H. pluvialis* indoor under controlled culture conditions and later moves the green stage to open ponds, outdoors for red stage cultivation. Another company Alga Technologies Inc., in Eilat, Israel, uses tubular glass photobioreactor for both green and red stages of algae cultivation in outdoor conditions to utilize photovoltaic cells and have maximum advantage of sunlight [162,166]. Another company, Algalif, uses geothermal energy and LEDs (light-emitting diodes) for the indoor production of ASX. Most companies exploit phototrophic cultivation, but some companies like Fuji Chemicals have adopted mixotrophic cultivation in the close fermenter with submerged light conducting tube in media. The company initiated the world's first industrial *Haematococcus* plant, regulated by its subsidiary, AstaReal AB, in Sweden [167]. Parry Pharmaceuticals Co. Ltd., situated in Chennai, India, managed to get a patent for raceway pond cultivation for the green stage by germinating haematocysts of red phase having 10 times higher reproduction potential, which could quickly produce large biomass in a short period [168,169] so that costly photobioreactor production could be avoided. However, their production technique faced failures mainly due to intricated biological system of *Haematococcus* and inefficient methodology. Many companies based on Cyanotech's technique have been set up throughout Asia and South America. Companies like Asta Biotec LLC, located in Shilin, China, and Yunnan Alphy Biotech Co., Ltd., in Chuxiong, China, work on similar techniques, that is, glass tubular outdoor photobioreactors employed by Alga Technologies Inc [170]. Yunnan LvA Bio-Tec, China, cultivated red stage cultivation in raceway ponds, similar to Cyanotech, thus cultivating a minimum of microalgae in small-scale flat panel photobioreactors [171,172].

9 | CONCLUSION

From the present review, it can be concluded that naturally derived ASX is highly effective against several diseases, shows more pronounced effects than other antioxidants, and bears substantial economic importance in almost every aspect of life. However, naturally derived ASX is still in laboratory practice due to the problems associated with production and needs to be tackled for an efficient commercial scale-up. Significant research is required to obtain maximum ASX accumulation and biomass, for this omics approach should be applied to *H. pluvialis* to analyze DNA sequences, gene structure, gene expression, the metabolic pathway for the desired manipulation. This can be a way of obtaining maximum ASX with less biomass under stress conditions. Also, newer production techniques must be developed for the production of cost-effective ASX.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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